UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG

PÓS-GRADUAÇÃO EM CIÊNCIAS FISIOLÓGICAS: FISIOLOGIA ANIMAL COMPARADA

EFEITOS FOTOBIOLÓGICOS DA PRÉ-EXPOSIÇÃO COM RADIAÇÃO INFRAVERMELHA À RADIAÇÃO ULTRAVIOLETA

VINÍCIUS CUNHA GONZALEZ

Tese apresentada ao Programa de Pós-Graduação em Ciências Fisiológicas: Fisiologia Animal Comparada da Universidade Federal do Rio Grande – FURG, como requisito parcial à obtenção do título de DOUTOR.

Orientador: Profª. Drª Gilma Santos Trindade

Co-orientador: Prof. Dr. Antônio Sérgio Varela Junior

RIO GRANDE

Setembro, 2013

AGRADECIMENTOS

Agradeço à Deus por ter me trazido até aqui e me sustentado mesmo quando não podia percebê-lo.

Agradeço à minha esposa, Miriam Bärg Gonzalez, pelo companheirismo, compreensão, orações e dedicação, sem os quais não teria sido possível realizar este trabalho.

Agradeço aos meus pais, Jorge e Rose, pelo apoio incondicional, por acreditarem em mim, apoiarem minhas decisões e orarem por mim.

Agradeço à minha orientadora, Gilma Santos Trindade pela orientação, compreensão, apoio, carinho e às pequenas coisas do dia-a-dia ao longo desta caminhada deste o mestrado.

Agradeço ao meu co-orientador Antônio Sérgio por inventar experimentos de última hora feitos para me levar à loucura, mas sempre presente para participar destas aventuras.

Agradeço aos meus amigos-irmãos, Fernando Tensini, Fábio Mafra, Fabrício Mafra, Felipe Beloto, Diego Fagundes por acreditarem em minhas capacidades, pelos momentos de descontração, pelas conversas sem sentido, mas principalmente por estarem sempre presentes.

Agradeço ao meu amigo-irmão Cleiton Odisi pela sua insistência para que eu terminasse à tese pelos seus motivos próprios e às macros que ele está sempre disposto a fazer.

Agradeço ao meu amigo Bruno Oliveira pelas folhas de resultado desaparecidas, a ajuda sempre presente que não preciso pedir, uma amizade sincera e aquele lanche que sempre nos espera.

Agradeço às amigas Ana Paula Votto e Renata Ottes Vasconcelos pela ajuda nos experimentos, às discussões sobre os resultados e a paciência com minhas piadas e brincadeiras

Agradeço à todos aqueles que de forma direta ou indireta contribuíram para que eu chegasse até a este ponto.

ÍNDICE

Resumo

Divide-se a radiação infravermelha (RIV) em IVA (700-1.400 nm), IVB (1.400-3.000 nm) e IVC (3.000 nm-1 mm). Ela penetra nos tecidos biológicos podendo aumentar a temperatura local (levando a lesões à longo prazo) ou induzir reações fotoquímicas independentes do calor. Reações como o aumento da mitose tecidual e ativação de genes de proliferação celular, reparo de DNA e apoptose. A radiação ultravioleta é dividida em UVC (200-290 nm), UVB (290-320 nm) e UVA (320-400 nm). O UVB causa danos ao DNA que levam à alterações celulares, fotoenvelhecimento, apoptose e necrose. Exposição não térmicas de RIV antes do UVB previne os efeitos danosos deste último. Esta proteção independe de calor ou divisão celular e depende do número de exposições de IVA anteriores ao UVB. Nesta Tese analisou-se a interação entre o IVA e UVB *in vivo* e *in vitro*. Nos experimentos *in vivo* verificou-se que a exposição crônica à RIV aumentou a proliferação celular de forma anormal alterando a pele, sem alterar os órgãos internos ou modificar o peso dos animais estudados. As alterações na pele também aumentaram quando a RIV foi aplicada após a irradiação com UVB, ambos de forma crônica. Em contrapartida, a irradiação crônica com RIV antes do UVB reduziu as alterações na pele originadas pela exposição ao UVB, mas não aos níveis da irradiação apenas com a RIV. Nos experimentos *in vitro*, a irradiação com RIV antes do UVB reduziu o número de células apoptóticas e necróticas e esta proteção não dependeu da redução da taxa mitótica ou da expressão do gene p53. Além disso, revisou-se a importância de que experimentos com RIV sem um controle adequado da temperatura poderem induzir proteínas de choque térmico ou alterar tecidos de uma forma que independe dos comprimentos de onda da RIV, portanto, a melhor forma de evitar o aumento da temperatura nestes experimentos é através do uso de LEDS de RIV. Conclui-se que a irradiação crônica com a RIV causa proliferação anormal das células da pele, reduz as lesões por exposição crônica com UVB quando exposta antes do UVB e aumenta estas lesões quando exposta após o UVB e é capaz de prevenir a morte celular causada pela radiação UVB.

Palavras-chaves: IV, UVB, calor, histopatológico, crônico, B16F10, interação, viabilidade celular

Abstract

Infrared radiation (IR) is divided into IRA (700-1,400 nm), IRB (1,400-3,000 nm) and IRC (3,000 nm-1 mm). It penetrates into biological tissues and can increase local temperature (causing lesions in the long term) or induce photochemical reactions independent from heat. Among these, stands out the increase of tissue mitosis, activation of genes related to cellular proliferation, DNA repair and apoptosis. Ultraviolet radiation (UV) is divided into UVC (200-290 nm), UVB (290-320 nm) and UVA (320-400 nm). UVB damages DNA, which can lead to cellular alterations, photoaging, apoptosis and necrosis. Non thermal IR irradiation before UVB irradiation prevents the deleterious effects of the last one. This protection is independent of heat or cellular division and it is dependent of the number of irradiations with IR before exposition to UVB. In this Thesis, it was analyzed the interaction between the IR and UVB radiations *in vivo* and *in vitro*. In the *in vivo* experiments it was verified that chronic IR exposition altered the skin increasing cellular proliferation in an abnormal way, without altering the visceral organs or modifying the weight of the studied animals. Skin alteration also increased when IR was applied after irradiation with UVB. In contrast, chronic IR irradiation before UVB irradiation created a protector effect reducing skin alterations originated from UVB exposition, but not into the same levels of IR irradiation alone. In the *in vitro* experiments, it was verified that IR irradiation before UVB reduced the number of apoptotic and necrotic cells and these protection was independent from a reduction in mitotic rate or p53 gene expression. Finally, it was reviewed the importance that IR experiments being made without an adequate temperature control can induce heat shock proteins or alter tissues in a manner that is independent from IR wavelengths. The best way to avoid the temperature increase in an experiment is through IR LEDs. We concludes that chronic IR irradiation causes abnormal skin cellular proliferation, reduces the lesions of chronic UVB exposition while used before UVB and increases these lesions when exposed after UVB, also IR is capable to prevents cellular death caused by UVB exposition.

Key-words: IR, UVB, heat, histopathological, chronic, B16F10, interaction, cellular viability

1. Introdução

Das radiações solares eletromagnéticas que chegam à Terra, a radiação infravermelha (RIV) representa 54,3% da energia recebida quando comparada com as radiações ultravioleta (UV) e visível (Schieke et al., 2003). Assim, os seres vivos são expostos a uma quantidade significativa de infravermelho (IV) com doses ambientais variando entre 20 a 48 mW/cm² (λ = 700 a 1400 nm) (Piazena et al., 2004; Piazena et al., 2002 apud Gebbers et al.; 2007).

A CIE (Comissão Internacional de Iluminação) divide o IV em IVA (700 - 1.400 nm), IVB (1.400 - 3.000 nm) e IVC (3.000 nm - 1 mm). Alternativamente o IV pode ser dividido em IV próximo (760 - 3.000 nm), médio (3.000 - 30.000 nm) e distante (30.000 nm - 1 mm) (Schieke et al. 2003). A maior parte da energia do IV situa-se no IVA, totalizando 30% da energia solar quando comparado as radiações UV e visíveis (Schroeder et al, 2007).

A penetrabilidade do IV na pele e no tecido subcutâneo diminui com o aumento do comprimento de onda (Schieke et al., 2003), mas ainda não há clareza sobre quão profunda é esta penetração, visto que diferentes autores apresentam valores diferentes. Estes valores variam de 0,7 a 30 mm (Eelss et al., 2004) a até 230 mm (λ = 630 – 800 nm) (Beauvoit et al., 1994).

O IV é absorvido pela água e pelo tecido através de ligações covalentes e pontes de hidrogênio (Walther et al., 2002; Maeda et al., 1999) induzindo vibrações moleculares (Laurent-Applegate & Roques, 2002) e gradualmente transferindo energia ao tecido profundo por um mecanismo de ressonância-absorção que pode aumentar a temperatura local com efeitos similares aos da hipertermia (IRC) (Schieke et al., 2003) ou induzir reações fotoquímicas (800-900 nm) (Laurent-Applegate, & Roques, 2002).

Dentre estas reações fotoquímicas destaca-se o aumento da atividade mitocondrial (citocromo c-oxidase) (Wong-Riley et al. 2005), da taxa de mitose dos fibroblastos e queratinócitos, da gênese de colágeno (Morita et al. 2006), da regeneração do colágeno, da infiltração de fibroblastos no tecido subcutâneo (Toyokawa et al. 2003), e do aumento da força tensil do epitélio em regeneração (Schramn et al. 2003).

A RIV também aumenta a expressão de genes como TGF-ß1 (atua na apoptose, crescimento, proliferação e diferenciação celular), MMP-2 (atua na degradação de

colágenos e gelatina) (Danno et al.; 2001; Toyokawa et al. 2003), HIST (expressão do aminoácido histidina) e genes ligados a proliferação celular como TUGCP3, KIF11, TTK e KPNA5; aumenta a deleção de MMP-10 (degrada colágeno IV, laminina, fibronectina e elastina) e genes relacionados à apoptose como CASP2 e TRPM2 (Morita et al. 2006).

Devido ao IV ser percebido como calor pela maioria dos vertebrados, o aumento da temperatura foi considerado o fator primário dos seus efeitos biológicos. Contudo, estudos demonstraram respostas, como as acima, causados por IV sem calor (Danno et al., 2001; Karu, 1999), assim como um aumento da microcirculação epitelial de uma forma não térmica e não autonômica através de um mecanismo ligado a rota metabólica da L-arginina / Óxido nítrico (Yu et al. 2006).

Até o presente momento, os efeitos negativos do IV estão ligados à exposição crônica ao calor moderado em mamíferos, resultando em alterações epiteliais como o eritema *ab igne*, uma dermatose macular de pigmentação reticular. Na maioria dos casos ela ocorre através do contato direto com a fonte de calor (condução ao invés de irradiação) (Walder & Hargis, 2002).

A radiação ultravioleta (UV) é outra radiação solar eletromagnética que atinge a Terra. Ela é dividida em UVC (200-290 nm), UVB (290-320 nm) e UVA (320-400 nm). O UVB e o UVA alcançam a superfície da Terra, enquanto o UVC normalmente não penetra a atmosfera (Chapman et al. 1995).

Os danos do UV podem ser no DNA (UVB e UVC) ou na membrana celular (UVA). Os danos causados no DNA pela radiação UV levam à alterações celulares, fotoenvelhecimento, apoptose e necrose (Aufiero, et al., 2006; Berneburg et al., 2000), tornando a radiação UV um dos principais fatores ambientais responsáveis pelo cancêr da pele (Bachelor et al., 2004), como o melanoma, que é o câncer da pele originado nos melanócitos.

A incidência do UVB varia ao longo do dia com um pico próximo ao meio-dia e valores mínimos antes das dez da manhã e após as quatro da tarde (Kullavanijaya et al. 2005). Além disso, o UVB é parcialmente bloqueado pela camada de ozônio, transformando moléculas de ozônio em moléclas de oxigênio (Kerr & McElroy, 1993). A diminuição da camada de ozônio em latitudes altas e médias latitudes (Manney et al.,

1994) tem levado a um aumento na pesquisa sobre os efeitos ambientais do UVB e os danos que ele causa em diferentes seres vivos (Gouveia et al., 2004; Gouveia et al., 2005). Sabe-se que doses não térmicas de IV previnem os efeitos citotóxicos do UVB em *Eschericcia coli* e do UVA e do UVB em fibroblastos humanos (Menezes et al., 1998). Esta proteção não está relacionada à inibição da peroxidação lipídica, ativação de proteínas de choque térmico (HSP70) ou divisão celular. Além do mais, o efeito protetor do IV contra o UV não foi dose dependente, mas dependente do número de vezes ao qual as células foram pré-expostas ao IV (Continenza et al., 1993).

Experimentos *in vitro* com fibroblastos humanos demonstraram que este efeito protetor em relação ao UVB ocorreu por modulação dos mecanismos de equilíbrio dos genes Bcl2/Bax, acúmulo de p53 com estabilização e fosforilação de serina (Ser15 e Ser 20) e aumento na expressão de p21 e GADD45. Esta pre-exposição também diminuiu a formação de dímeros de timina induzidos por UVB (Frank et al., 2006). Ainda assim, não existe um consenso se o IV pode induzir carcinogênese atuando em conjunto com a radiação UV (Jantschitsch et al., 2009).

1.1 Hipóteses

Os antecedentes descritos acima permitiram formular as seguintes hipóteses que foram investigadas nesta Tese:

- Exposição crônica com radiação infravermelha sem aumento de temperatura não causa alterações teciduais;

- Pré-exposição crônica com radiação infravermelha sem aumento de temperatura protege de lesões teciduais causadas pela radiação ultravioleta;

- Pré-exposição com radiação infravermelha sem aumento de temperatura previne morte celular causada pela radiação ultravioleta *in vitro*;

- A radiação infravermelha possui efeitos distintos causados pelo calor e pelo comprimento de onda.

1.2 Objetivos

Objetivo Geral

Avaliar os efeitos da radiação UVB após pre-exposição com à radiação IV.

Objetivos Específicos:

1 – Estudar se a RIV crônica causa alteração de peso em animais;

2 – Avaliar possíveis alterações teciduais causadas pela exposição crônica à RIV;

3 – Determinar se a exposição crônica à RIV antes de exposição crônica ao UVB previne lesões teciduais;

4 – Avaliar se a exposição à RIV antes da exposição ao UVB altera a viabilidade de células B16F10;

5 – Analisar a expressão do gene p53 em células B16F10 expostas à radiação infravermelha e a radiação ultravioleta;

6 – Revisar os efeitos térmicos e não térmicos da RIV e sua distinção de efeitos puramente térmicos.

1.3 Estrutura da Tese

Os objetivos específicos desta Tese são abordados em estudos independentes que se encontram no capítulo 5 sob forma de trabalhos que serão submetidos para publicação. Os objetivos 1 e 2 fazem parte do trabalho intitulado “Chronic infrared irradiation causes skin alteration without altering other organs” (Artigo 5.1). O objetivo 3 faz parte do trabalho intitulado “Histopathological analyze of UVB and infrared radiations interaction” (Artigo 5.2). Os objetivos 4 e 5 fazem parte do trabalho intitulado “Effect of Infrared and UVB radiations interaction in B16F10 cells” (Artigo 5.3). Por último, o objetivo 6 faz parte do trabalho intitulado “Infrared radiation in biological research – moving away from heat” (Artigo 5.4). Nestes trabalhos, encontram-se os detalhes metodológicos, resultados sucintos e discussão de cada objetivo.

Para cada um dos artigos citados acima, uma síntese dos principais aspectos metodológicos, resultados, discussão e conclusão é apresentadao a seguir em português como corpo principal desta Tese.

2. Síntese do Estudo

2.1 – IRRADIAÇÃO CRÔNICA COM INFRAVERMELHO CAUSA ALTERAÇÃO DA PELE SEM ALTERAR OUTROS ÓRGÃOS

Rattus norvegicus machos de 2 e 8 meses de idade foram expostos a radiação infravermelha usando uma lâmpada Phillips Infraphil (13379F/479, 150W, 600 - 1500 nm, com pico em 1000 nm) à 30 cm de distância. Os animais com 2 meses de idade foram expostos diariamente por 32 dias, por 10 min (360 J/cm²) e os animais com 8 meses de idade foram expostos diariamente por 48 dias, por 30 min (1.080 J/cm²). A temperatura foi controlada e monitorada para evitar valores acima de 37ºC. Os animais foram pesados durante o experimento. No dia seguinte ao último dia de irradiação, os animais foram sacrificados e extraíram-se a aorta, baço, coração, encéfalo, estômago, fígado, pâncreas, pele, pulmões, rins e testículos para análise histopatológica dos animais de 2 meses de idade.

Não houve diferença de peso dos animais tratados em relação ao controle em nenhuma das idades analisadas. As amostras de pele irradiadas com infravermelho apresentaram acantose e hiperqueratose ortoqueratótica. Os outros órgãos analisados não tiveram alterações histológicas.

Möckel et al., (2006) demonstraram que a exposição com infravermelho aliada ao exercício aeróbico reduziu o peso de voluntários, mas os autores concluíram que o efeito foi devido ao aumento do metabolismo devido ao calor fornecido pela radiação. Diferentemente, em nossos experimentos não houve aumento da temperatura; o que pode explicar a diferença entre os resultados. A presença de acantose e hiperqueratose ortoqueratótica em experimentos com temperatura controlada, descarta a possibilidade de fotoenvelhecimento por calor (Seo & Chung, 2006). Como um dos efeitos da RIV é alterar o balanço mitótico tecidual, pode ter ocorrido uma expressiva mitose no tecido levando ao espessamento do epitélio.

A ausência de alteração na aorta, baço, coração, encéfalo, estômago, fígado, pâncreas, pulmões, rins e testículos indica que a RIV foi absorvida pela pele e pelos músculos, não chegando até estes órgãos e assim, não produziu efeitos biológicos capazes de alterações histológicas.

Concluí-se que exposição crônica à radiação infravermelha causa alteração no tecido epitelial com aumento da proliferação celular, sem alterar órgãos mais profundos ou modificar o peso dos animais estudados.

2.2 – ANÁLISE HISTOPATOLÓGICA DA INTERAÇÃO DAS RADIAÇÕES INFRAVERMELHA E UVB

Rattus norvegicus machos foram expostos à radiação UVB (pico de emissão em 313 nm) durante 90 min (55,08 J/cm²), à radiação infravermelha (IV) (600 - 1500 nm, com um pico em 1000 nm) por 30 min (1080 J/cm²) e depois de 90 min à radiação UVB (IVUVB) ou à radiação UVB e depois de 90 min à radiação IV (UVBIV) durante 15 dias. No 16º dia os animais foram sacrificados e amostras de pele retiradas para avaliação histopatológica.

Animais tratados apenas com UVB ou com UVB seguido de IV apresentaram hiperqueratose paraqueratótica e ortoqueratótica, acantose com pustulas intraepidérmicas, pérolas de queratina, separação da epiderme da derme com vacuolização das células basais, degeneração da derme superficial, infiltrado inflamatório, vasodilatação e necrose do colágeno. Animais tratados com IV antes do UVB apresentaram as mesmas características descritas acima com exceção de hiperqueratose paraqueratótica, pérolas de queratina e degeneração da derme superficial.

Na maioria das análises, a porcentagem de amostras com as características descritas foi menor nos animais irradiados primeiro com infravermelho e depois com UVB. Animais irradiados primeiro com UVB e depois com IV apresentaram porcentagens variando entre abaixo do grupo tratado apenas com UVB (mas não menor do que os tratados primeiro com IV) à acima dos tratados com UVB.

Alguns autores (Frank et al., 2006) demonstraram que a radiação IV pode proteger células dos efeitos deletérios da radiação UVB, incluindo protegendo da formação de tumores. Contudo, em relação aos tumores, após formados pela exposição ao UVB, a radiação infravermelha aumentou sua taxa de replicação (Jantschitsch et al.; 2011). Estes efeitos podem estar relacionados à capacidade do gene p53 em lidar com as lesões ocasionadas pelo UVB.

Em todos os tratamentos com infravermelho, os valores de colágeno estiveram maiores do que no tratamento apenas com UVB. Neste sentido, ambas as radiações são capazes de alterar a produção de colágeno ou degradar as proteínas já existentes (Brenneisen et al., 2002; Schroeder, et al.; 2008) e parecem atuar de forma sinérgica na lesão tecidual. Apesar de algumas destas alterações serem semelhantes ao processo de

fotoenvelhecimento, outros autores (Lee et al. 2006) demonstraram efeitos de fotorejuvenescimento ao utilizar a radiação IV.

Conclui-se que a exposição à radiação infravermelha após exposição à radiação ultravioleta B aumentou a taxa de lesões de algumas variáveis histopatológicas sem proteger o tecido, enquanto exposição à radiação infravermelha antes de exposição à radiação ultravioleta B ocasionou um efeito protetor na maioria das variáveis.

2.3- EFEITO DA INTERAÇÃO DAS RADIAÇÕES INFRAVERMELHA E UVB EM CÉLULAS B16F10

Células B16F10 foram expostas à radiação infravermelha por 30 min (1080 J/cm², 600-1500nm, com pico em 1000 nm), à radiação UVB por 35 min (8,19 J/cm², pico de emissão em 313 nm) ou à radiação IV 12 h antes da radiação UVB. Após as exposições, os tratamentos foram analisados em 0 horas, 24 horas, 48 horas e 72 horas para viabilidade celular com os testes de MTT, Vermelho Neutro, coloração com brometo de etídio/laranja de acridina (EB/AO), contagem total de células e para a expressão do gene p53.

Em 48 e 72 horas, tanto o controle quanto o tratamento apenas com IV apresentou maior viabilidade por MTT do que as células irradiadas por UVB com ou sem pré-exposição ao IV. Enquanto que para o teste do Vermelho Neutro, células expostas ao IV com ou sem UVB apresentaram valor muito maiores do que o controle e aquelas tratadas só com UVB em 24 horas, modificando este padrão em 48 horas para uma redução na viabilidade por Vermelho Neutro para às células irradiadas com UVB com ou sem a presença de IV em relação ao controle negativo e às células tratadas apenas com IV.

A coloração por EB/AO demonstrou uma redução na viabilidade das células tratadas somente com UVB, exceto no tempo de 24 horas; aumento da quantidade de células apoptóticas quando tratadas com UVB em todos os tempos em relação aquelas tratadas somente com IV e em 48 e 72 horas em relação ao controle e às tratadas com IV antes do UVB, e aumento das células necróticas quando tratadas com UVB em relação ao infravermelho em todos os tempos, assim como para infravermelho antes do UVB em 0 e 48 horas. Apesar de ocorrer um aumento no número total de células do tempo 0 para o tempo de 72 horas, o grupo tratado somente com infravermelho apresentou menos células (menor crescimento) nos tempos de 48 e 72 horas. Já para a expressão do gene p53 não houve diferença entre os tratamentos em nenhum dos tempos analisados.

A exposição ao infravermelho não danificou nem protegeu as células em relação à viabilidade por MTT, provavelmente devido às células expostas não estarem em um estado de falta de ATP (Karu et al., 2004). Ao mesmo tempo a exposição ao infravermelho causou um aumento momentâneo na atividade dos lisosomos, mas que

não se manteve. Ambos os resultados não demonstram um efeito protetor ou prejudicial do infravermelho em relação ao ultravioleta.

Contudo, a coloração por EB/AO demonstrou o efeito protetor do infravermelho ao aumentar a viabilidade das células, reduzindo as células apoptóticas e necróticas. Ao mesmo tempo a exposição ao infravermelho reduziu o número total de células, mas sem ter relação com a proteção ao ultravioleta. O que elimina a redução da frequência mitótica como a causa da proteção ao UVB. Esta proteção também não foi causada por expressão do gene p53.

Em conclusão, a exposição ao infravermelho com controle de temperatura não alterou a viabilidade celular, protegeu às células expostas ao UVB reduzindo o número de células apoptóticas e necróticas e esta proteção não foi dependente da redução da taxa mitótica ou da expressão do gene p53.

2.4 – RADIAÇÃO INFRAVERMELHA NA PESQUISA BIOLÓGICA – SE AFASTANDO DO CALOR

Durante muito tempo calor radiante e radiação infravermelha foram considerados equivalentes e usados como sinônimos, devido a hipertermia (Song et al., 1982) ser uma das propriedades da radiação infravermelha. Mas com a demonstração de que o infravermelho (IV) tem efeitos biológicos sem ação do calor (Fuchs et al, 2004), faz-se necessário algumas distinções.

Trabalhos com IV sem controle de temperatura ou usando o calor como medida para o IV (Pujol and Lecha 1992) resultam na indução de proteínas de choque térmico (HSPs) (Lee et al 2006, Maytin et al., 1993) que podem levar a conclusões sobre calor em vez de conclusões sobre o efeito do comprimento de onda.

Destes conflitos entre calor e IV destacam-se o fotoenvelhecimento (Shin et al 2012), o fotorejuvenescimento (Weiss et al, 2003), alterações na expressão de matrix metaloproteínases (Kim et al, 2005) e na expressão de colágeno (Kim et al, 2006). Novamente, a indução das HSPs pode estar envolvida nestes dados conflitantes.

Outro motivo para evitar o uso de calor ou o aumento da temperatura em experimentos reside no fato de que incrementos baixos de temperatura incapazes de ativar as HSPs podem causar alterações histológicas a longo prazo (Kibbi & Tannous, 1998) como dano de DNA ou eritema *ab igne*.

A melhor forma de se evitar o aumento da temperatura nos experimentos é através do uso de LEDs de infravermelho (Barolet, 2008), capazes de emitir uma ampla gama de comprimentos de onda de forma não coerente e até o momento sem efeitos colaterais.

Em conclusão, experimentos com infravermelho sem um controle adequado da temperatura podem resultar em indução de HSPs ou alterações teciduais que não dependem dos comprimentos de onda do infravermelho. A melhor forma de evitar o aumento da temperatura nestes experimentos é através do uso de LEDS de infravermelho.

3. Manuscritos na forma original de submissão para a revista Photochemistry and Photobiology

3.1 ARTIGO 1

Chronic Infrared Irradiation Causes Skin Alteration Without Altering Other Organs

Gonzalez, V.C[1].; Beheregaray, A.C.M[2].; Peres, B.M[3].; Sallis, E.S.V[4].; Varela Junior, A.S[3].; Trindade, G.S[1].;

[1]Programa de Pós-Graduação em Ciências Fisiológicas – FURG; [2]Faculdade de Medicina – FURG; [3]Instituto de Ciências Biológicas – FURG; [4]Faculdade de Veterinária – UFPEL;

Abstract

To determine if chronic infrared irradiation altered the histology of skin and internal organs male *Rattus norvegicus* were exposed to infrared irradiation (λ = 600 - 1500 nm, with peak at 1000 nm). 2 months old animals were exposed for 10 min (360 J/cm²) during 32 days and 8 months old animals were exposed for 30 min (1080 J/cm²) during 48 days. Animals were weighted and organs were extracted for histopathological analysis. No weight alteration was found, neither were found histological changes for brain, aorta, heart, stomach, lungs, liver, spleen, pancreas, kidneys and testicles. Infrared irradiate skin sample showed acanthosis and orthokeratotic hyperkeratosis. In conclusion, chronic non thermal infrared irradiation alters epithelial tissue with increased abnormal cellular mitosis, without altering visceral organs or modifying animal weight.

1. Introduction

Infrared radiation (IR) represents 54.3% of the solar electromagnetic radiations that reach Earth every day, when compared to ultraviolet (UV) and visible radiations (Kochevar et al. 1999 apud Schiekel et al., 2003). Thus, living beings are exposed to a significant amount of IR, varying from 20 to 48 mW / cm² (Piazena et al., 2002; 2004 apud Gebbers et al.; 2007). These environmental values are influenced by cloud cover, relative humidity, latitude, longitude and zenith.

CIE (International Commission on Illumination) divides IR into: IRA (700 - 1,400 nm), IRB (1,400 - 3,000 nm) and IRC (3,000 nm - 1 mm), or alternatively IR can be divided as near IR (760 - 3,000 nm), medium IR (3,000 - 30,000 nm) and far IR (30,000 nm - 1 mm) (Schieke et al. 2003). All IR bands have biological effects through interaction with water and biomolecules *in vitro*. Besides, covalent and hydrogen bonds absorb low frequency vibrations (Walther et al. 2002; Maeda et al. 1999).

Far IR reaches skin tissue and gradually transfers energy to deep tissue by a mechanism of resonance-absorption of organic molecules and water (Inoue et al. 1989). Penetrability in skin and subcutaneous tissue decreases while IR increases its wavelengths. Shorter wavelengths (IRA) reach subcutaneous tissue without increasing significantly the skin temperature. Longer wavelengths (IRC) are absorbed completely by the epithelial layers, increasing skin temperature and resulting in thermal sensation (Schieke et al. 2003). (This increase is caused by induction of molecular vibrations and rotations).

Also IR is absorbed by and increases the activity of mitochondrial complex IV (cytochrome c-oxidase) (Karu et al. 1999), which results in increased ATP output. Also, IR is responsible for skin regeneration by increasing: fibroblasts and keratinocytes mitosis rate, collagen genesis (Morita et al. 2006) and collagen regeneration, fibroblast infiltration in subcutaneous tissue (Toyokawa et al. 2003) and increasing the tensile strength of the regenerating epithelium (Schramn et al. 2003).

Due to IR be perceived as heat by most vertebrates, temperature rise was deemed the primary reason for their biological effects. However, others studies showed IR responses caused by non-thermal infrared radiation (Danno et al., 2001), but how this occurs have not yet been explained.

IR exposition increases expression of TGF-ß1, MMP-2 (Danno et al.; 2001; Toyokawa et al. 2003), HIST genes and genes connected to cell proliferation, as TUGCP3, KIF11, TTK and KPNA5, deletion of MMP-10 and apoptosis-related genes such as, CASP2 and TRPM2 (Morita et al. 2006). Furthermore, it causes a non-thermal non-autonomic increase in epithelial microcirculation through a mechanism related to L-arginine/NO metabolic route (Yu et al. 2006).

Moreover, the negative effects of IR, to date, are linked to chronic exposure to moderate heat which can result, e.g. erythema *ab igne*, a macular dermatosis, and reticular pigment (Walder & Hargis, 2002).

Infrared therapy has become more and more usual, mostly in physiotherapeutic treatments for muscle injuries, but little is known about the effects of whole body irradiation with chronic IR in healthy tissue. What we known (erythema *ab igne*) only occurs after months or years of constant direct contact, a time range far greater than those used for treatment. So, the objective of this study is to determine if whole body chronic IR irradiation has any biological effect.

2. Material and Methods:

2.1. Animal Model

Male *Rattus norvegicus* (Wistar) from Central Animal Facility of Universidade Federal do Rio Grande – FURG, were kept at Instituto de Ciências Biológicas' bioterium.

Animals were held for two weeks and maintained in polypropylene boxes in groups of up to four (2 months old) or up to 3 animals (8 months old) in each cage at 21° C ± 2 ° C, photoperiod of 12 hours light/dark, with food and water *ad libitum* before and during experiments. At the end of experiments, animals were sacrificed in a CO_2 chamber and their bodies were collected by Aborgama do Brasil for proper biological destination. All experiments were approved by CEUA (Ethics Committee on Animal Use) of FURG by Opinion P062/2011.

2.2. Infrared Exposition

Animals were irradiated in groups of four while inside polypropylene boxes similar to those used for maintenance, using a Phillips Infraphil 13379F/479, 150W lamp, ranging from 600 - 1500 nm, with a peak at 1000 nm, and distant 30 cm from animals. Two doses were used, 360 J / cm² (10 min irradiation) and 1080 J / cm² (30 min irradiation). Times used were determined in previous experiments (data not shown).

2.3. Effects of Chronic Infrared Radiation:

2.3.1 Effects of Chronic Infrared Radiation in 2 month old animals

Animals were divided into control (n = 10) and IR (n = 10) groups. During 32 days, IR group was irradiated for 10 minutes (360 J / cm²). Temperature was controlled to prevent heating by indoors air-conditioning with plenty ventilation and measured constantly at animal level using a mercury thermometer. Control group were exposed only to indoors light. At initial (day 1) and at final (day 33) weight were measured. On 33th day, animals were sacrificed. Aorta, brain, heart, kidneys, liver, lungs, pancreas, skin, spleen, stomach and testicles were removed for analysis. All organs were weighted. After weighing, organs underwent histological processing as described in 2.4

2.3.2 Effects of Chronic Infrared Radiation in 8 month old animals

Animals were divided into control (n = 7) and IR (n = 8) groups. During 48 days IR groups were irradiated for 30 minutes (1080 J / cm²). Temperature was controlled to prevent heating as described in 2.3.1. Control group were exposed only to indoors light. Weight was measured before the first irradiation (Day 1) and throughout all experiment, when animals were sacrificed. At this analysis, organs were not collected.

2.4 - Histological Analysis:

Organs were cut into 3 – 5 mm segments and stored in 50 ml “Falcon” tubes filled with paraformaldehyde at 1:20 ratio with at least 20 ml of solution per tube. Paraformaldehyde was prepared no later than 24 hours prior to use and kept cooled at 5° C. Organs were fixed for 24h, and then placed in an alcoholic solution at 70° GL. Three segments from each organ were dehydrated, diafanized and embedded in Paraplast Xtra (Sigma P3808). From every piece, 3 - 4 slices were cut with 4 microns of thickness with a rotatory microtome (Leica – RM 2255). Slides were stained with HEs and send to a professional blinded to the experiment conditions, which carried out the histopathological analysis at Departamento de Patologia Animal of Faculdade de Veterinária – Universidade Federal de Pelotas.

2.5 Statistical analysis:

Initial and final body weight and the weight of organs were analyzed using T test. A regression curve was created for daily body weight gain for each animal, and the beta of each regression curve were pooled and tested with T test. All data were analyzed with Statistica 7.0 and p values < 0.05 were considered statistically significant.

3. Results

3.1 Effects of Chronic Infrared Radiation in 2 month old animals

3.1.1 Weight analysis

Body weight: Neither initial nor final weight was statistically significant ($p>0.05$, T-test) between and within groups.

Organs weight: There was no statistically difference between groups ($p > 0.05$, T-test) for the weight of all organs analyzed. Although, brain ($p = 0.08$) and spleen ($p = 0.07$) weight were smaller in the IR-treated group, but this was not statistically significantly.

3.1.2 Histopathological results:

No organs analyzed except for the skin samples had any pathological alteration. Results for skin tissue are showed in Table 1 (Appendix I). Percentage means how many slides had the feature, with + meaning mild intensity and ++ meaning moderate intensity. No slide had +++ severe intensity. All skin samples treated with IR developed acanthosis and orthokeratotic hyperkeratosis. Figure 1 (Appendix II) shows acanthosis in a 20x increase.

3.2 Effects of Chronic Infrared Radiation in 8 month old animals

3.2.1 Weight analysis

Body weight: As for young animals, there was no statistical difference ($p>0.05$, T-test) between initial and final weight either between or within groups.

Growth analysis: Each animal was weighted during 48 days. A regression curve of weight and days was plotted for each animal. After that, the Beta value of the regression curve was pooled and a T-Test between control and infrared group were made. Pooled beta T-Test was not statistically significant ($p = 0.13$, T-Test), although control group gained body mass faster than infrared group as showed in Figure 2 (Appendix III) graphic of pooled betas.

4. Discussion

4.1 Effects of Chronic Infrared Radiation in 2 and 8 month old animals

Although there was no significant body weight alteration, there was less visceral fat and body mass in the IR group. This can indicate that although overall final weight did not change in the IR group but some alterations between fat and muscle mass may be occurring, as showed by Möckel et al, (2006) with the fat tissue and the fact that muscle can absorbing IR through myoglobin and through it, activates an increase in muscle fiber diameter. Also, the same increased ATP output (Karu et al, 1999) could be demanding a higher use of fat tissue reserves. With the older animals a higher dose of IR was used and this time daily weight was noted and a regression curve was calculated. Again, IR-treated animals had less body mass and a less pronounced regression curve showed by a lower pooled beta box than in control animals, but neither was statistically significant as illustrate in Appendix III graph.

Differently from us, Möckel et al (2006) found out that three 45 min IR (500 – 1400 nm) sessions during four weeks during aerobics exercise with a bicycle had a lipolytic effect. But at their experiment, IR was accompanied with heat and exercises, which they deemed the major factor to reduce fat mass in the IR tested group.

It is interesting to note that brain and spleen weight were smaller in the IR-treated group, although this was not significantly ($p = 0.08$; 0.07). This data, need more tests, as it is known that IR can have an antidepressant and anxiolytic effects with increase BrdU-positive cells at CA1 hippocampal region (Tanaka et al., 2001) as well as causing memory and, learning improvements.

4.2 Histopathological results:

Infrared radiation is mainly absorbed by the skin and the muscle adjacent to it. This absorption leads to all skin samples analyzed from IR-treated animals showed mild to moderate acanthosis as showed in Appendix II. The thickening of the epidermis occurred in 100% of those treated with IR as mild to moderate. This can indicate that chronic IR exposition damages the skin tissue (through an unknown process) and as a result skin thickens, similar to what happens with UV skin damage. But, one must also consider an over signaling of mitosis, as it is already known that IR activates mitotic genes and increase ATP output needed for a high mitotic rate (Karu et al, 1999; Morita et al. 2006). Either way, long-term IR exposition of healthy tissue (i.e. not damaged tissue) could result in mitosis deregulation.

The fact that all samples had mild orthokeratotic hyperkeratosis corroborates the idea of mitosis deregulation, as it involves a change to epidermopoiesis. As no other histopathological feature was displayed in the samples it is difficult to discern if it is an inflammatory or neoplastic alteration.

We disregard this histopathological finding as heat photoaging, as temperatures were controlled and never reach any threshold that could activate a heat shock protein chain reaction, as reviewed by others (Schroeder et al, 2008). Also in heat photoaging, solar elastosis would be found, with accumulation of elastotic distrophic material (Warren et al.; 1991), severe hyperplasia of elastic fibers and degeneration of the collagen fibers of the dermis, which happens in erythema ab igne (Kibbi & Tannous, 1998). More recently, Shin et al (2012) reported that chronic heat treatment (43ºC) causes wrinkle formation.

All these reports of histological alteration used some form of heat, and with tissue temperature above physiological parameters, but it’s interesting to notice that

Gebbers et al (2007) have irradiated human fibroblasts with single IR dose as high as 4080 – 10880 J / cm² and only have a 10 to 20% mortality rate; while using chronic doses (1 session / week; 3 - 10 weeks) ranging from 300 to 1220 J / cm² without any alteration in cell viability. Both testes were made with heat control and never reached a temperature that would induce a heat stress response.

These differences regarding histological alterations caused by heat, and the fact that IR doses much higher than ours, but without heat, could not induce any noticeable alteration, lead us to disregard our findings as a heat photoaging process.

From the skin and muscle IR absorption, a mechanism of resonance-absorbance could be in effect. This mechanism can transfer IR energy to internal organs, the same way it delivers heat to internal tissue, but for the organs analyzed (aorta, brain, heart, kidneys, liver, lungs, pancreas, spleen, stomach and testicles), no histopathological changes were found. This can be because mostly of IR is absorbed at skin by cytochrome c oxidase of the mitochondria and partly at muscles by myoglobin. Although, the minimal IR dose that reaches internal organs after being absorbed by skin and muscle was not sufficient to alter organs histology, it can be enough to alter brain and spleen development as the almost significant weight difference need further studies.

5. Conclusion

We conclude that chronic whole body IR exposition causes skin tissue alteration with increased abnormal cell proliferation without causing any pathology to visceral organs and the brain.

6. REFERENCES

Danno, K.; Mori, N.; Toda, K-I.; Kobayashi, T.; Utani, A. 2001: Near-infrared irradiation stimulates cutaneous wound repair: laboratory experiments on possible mechanisms. Photodermatol. Photoimmunol. Photomed. 17: 261-265;

Gebbers, N.; Hirt-Burri, N.; Scaletta, C.; Hoffman, G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. GMS Ger Med Sci 2007;5:Doc8

Inoue, S.; Kabaya, M. 1989. Biological activities caused by far-infrared radiation. *Int. J. Biometeorol*. 33: 145-150;

Karu, T. 1999. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B. Biol. 49(1): 1-17;

Kibbi, A.G. & Tannous, Z. Skin Diseases Caused by Heat and Cold Clinics in Dermatology. 1998;16:91-98

Kochevar IE, Pathak MA, Parrish JA. Photophysics, photochemistry and photobiology. In:Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA.,editors.Fitzpatrick's Dermatology in General Medicine. New York: McGraw-Hill; 1999.pp.220-229 apud: Schiekel, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol. Photoimmunol. Photomed.* 19: 228-234;

Maeda, Y.; Ide, M.; Kitano, H. 1999. Vibrational spectroscopic study on the structure of water in polymer systems. *J. Mol. Liq.* 80: 149–163;

Möckel, F.; Hoffman,G.; Obermüller, R.; Drobnik, W.; Schmitz,G.2006. Influence of water-filtered infrared-A (wIRA) on reduction of local fat and body weight by physical exercise. German Medical Science 4,Doc05

Morita, S.; Narita, M.; Kitatani, N,; Terauchi, T. 2006. Effect of light-emitting diode irradiation on skin. J. Dermatol. *Science*, 42: 133-198;

Piazena H, Meffert H. Dosis-Wirkungs-Beziehungen bei Infrarot- Bestrahlung der Haut und die Extrapolation der Daten von in vitro-Messungen auf die Bedingungen in vivo - Erfordernisse und Fehler [Dosis related effects during infrared irradiation of

the skin and the extrapolation of the data from in vitro measurements to in vivo conditions - requirements and errors]. Lecture presented at the symposium entitled "Water-filtered infrared-A (wIRA) in dermatology" of the Dr. med. h.c. Erwin Braun Foundation in Liestal/Basel, Switzerland, November 20, 2004. Apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Piazena H, Meffert H. Therapie mit Infrarotstrahlen – Physikalische Grundlagen und Anwendung in der Sauna und in Infrarotgeräten. In: Bühring M, Kemper FH, Hrsg. Naturheilverfahren [Loseblattsammlung]. Berlin, Heidelberg, New York: Springer; 2002. Bd. 1, 03.09. S.1-22 apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Schieke, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol. Photoimmunol. Photomed.* 19: 228-234;

Schramm, J.M; Warner, D.; Hardesty, R.A.; Oberg, K.C. 2003. A unique combination of infrared and microwave radiation accelerates wound healing. *Plast. Reconstr. Surg*. 111(1): 258-266;

Schroeder,P.; Haendeler, J.; Krutmann, J. 2008. The role of near infrared radiation in photoaging of the skin. Experimental gerontology 43:629-632.

Shin, M. H., Seo, J.E.; Kim, y.K.; Kim, K.H.; Chung, J.H. 2012. Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mechanisms of Ageing and Development 133: 92–98

Tanaka,Y.; Akiyoshi,J.; Kawahara,Y.; Ishitobi,Y.; Hatano,K.; Hoaki,N.; Mori,A.; Goto,S.; Tsuru,J.; Matsushita,H.; Hanada,H.; Kodama,K.; Isogawa,K.; Kitamura,H.; Fujikura,Y.2011.Infrared radiation has potential antidepressant and anxilolytic effects in animal model of depression and anxiety. Brain stimulation 4:71-76

Toyokawa, H.; Matsui, Y.; Uhara, J.; Tsuchiya,H.; Teshima, S.; Nakanishi, H.; Kwon, A.; Azuma, Y.; Nagaoka, T.; Ogawa, T.; Kamiyama, Y. 2003. Promotive effects of

far-infrared ray on full-thickness skin wound healing in rats. *Exp Biol Med.* 228: 724–729;

Walder, E.Y.J. e Hargis, A.M. 2002. Chronic moderate heat dermatitis (erythema ab igne) in five dogs, three cats and one silvered langur. *Vet. Dermatol*. 13; 283-292;

Walther, M.; Plochocka, P.; Fischer, B.; Helm, H.; Jepsen, P. U. 2002. Collective vibrational modes in biological molecules investigated by terahertz time-domain spectroscopy. *Biopolymers* 67, 310–313;

Warren R, Gartstein V, KligmanAM,MontagnaW, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. J Am Acad Dermatol 1991;25:751—60.

Yu, S.; Chiu, J.; Yang, S.; Hsu, Y.; Lui, W.; Wu, C. 2006. Biological effect of far-infrared therapy on increasing skin microcirculation in rats. *Photodermatol. Photoimmunol. Photomed.* 22: 78–86;

APPENDIX I – Histopathological results table

	Control	IR
Acanthosis	0%	100% (+ to ++)
Orthokeratotic Hyperkeratosis	0%	100% (+)

Table 1. Skin histopathological results. Two month old male wistar rats (n = 10, both groups) irradiated with IRA during 32 days for 10 minutes (360 J / cm^2) (IR) or exposed only to indoor lights (Control). Data are expressed of a percentage of slides with the histopathological feature. + meaning mild intensity and ++ meaning moderate intensity.

APPENDIX II – Acanthosis 20x magnification photo

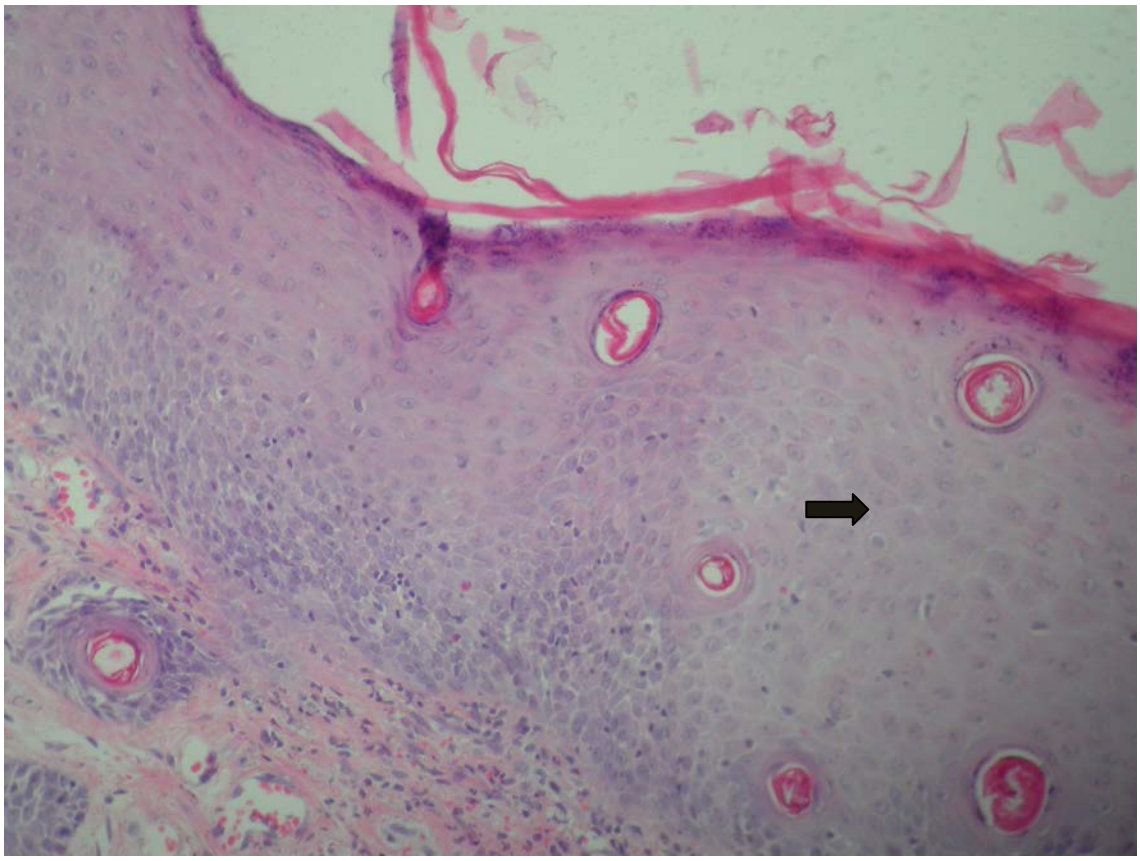

Figure 1. Acanthosis in infrared treated animals' skin. Two month old male wistar rats (n = 10) irradiated with IRA during 32 days for 10 minutes (360 J / cm²) (IR). Arrow indicates the acanthosis. Picture was taken in an optical microscope at 20 x magnification.

Appendix III – Graphic of pooled beta daily weight measurement

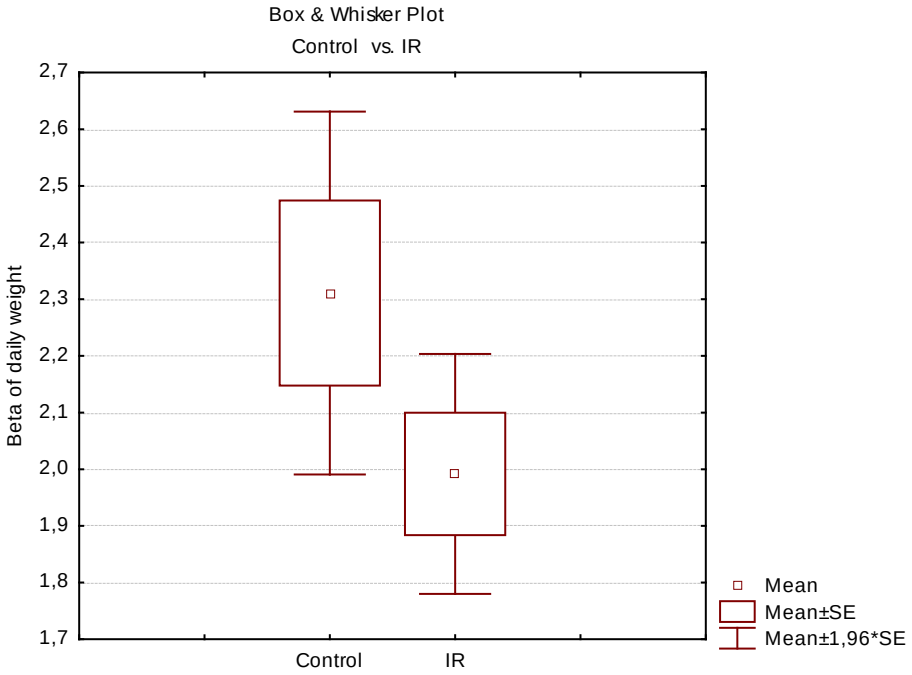

Figure 2. Pooled betas from the regression curve of daily weight measurement. Eigth month old male wistar rats (Control, n = 7, IR, n = 8) irradiated with IRA during 48 days for 30 minutes (1080 J / cm²) (IR) or exposed only to indoor lights (Control). Data are expressed as a box plot with mean, standard error and 1.96 * standard error.

3.2 ARTIGO 2

Histopathological analyze of UVB and Infrared radiations interaction

Gonzalez, V.C[1].; Beheregaray, A.C.M[2].; Peres, B.M[3].; Sallis, E.S.V[4].; Varela Junior, A.S[3].; Trindade, G.S[1].

[1]Programa de Pós-Graduação em Ciências Fisiológicas – FURG; [2]Faculdade de Medicina – FURG; [3]Instituto de Ciências Biológicas – FURG; [4]Faculdade de Veterinária – UFPEL;

Abstract

Male *Rattus norvergicus* were exposed to UVB radiation (peak emission at 313 nm) during 90 min (55.08 J/cm²), to infrared radiation (IR) (600 - 1,500 nm, peak at 1000 nm) during 30 min (1080 J/cm²) followed by 90 min UVB irradiation (IRUVB), or irradiated first with UVB radiation and after 90 min with IR (UVBIR) during 15 days. Skin histopathological analyzes showed the presence of acanthosis, parakeratotic and orthokeratotic hyperkeratosis, intraepidermal pustules, keratin pearls, detachment of epidermis, collagen necrosis, inflammatory infiltrate, vasodilation, basal cell vacuolization and superficial dermis degeneration in both UVB alone and UVBIR treatments. Animals within IRUVB group showed the same characteristics above except for parakeratotic hyperkeratosis, keratin pearls and superficial dermis degeneration. In conclusion, IR exposition after UVB irradiation increases the rate of damage for some histopathological variables, above UVB irradiation only, without protecting the tissue; while IR exposition before UVB irradiation showed a protective effect in most of the variables.

1. Introduction

Infrared radiation (IR) represents 54.3% of the solar electromagnetic radiations that reach Earth when compared to ultraviolet (UV) and visible radiations (Kochevar et al. 1999 apud Schiekel et al., 2003). Thus, living beings are exposed to a significant amount of IR, with an environmental values ranging from 20 to 48 mW/cm² (Piazena et

al., 2004; Piazena et al., 2002 apud Gebbers et al.; 2007). These environmental values are influenced by cloud cover, relative humidity, latitude, longitude and zenith.

CIE (International Commission on Illumination) divides IR into IRA (700 - 1,400 nm), IRB (1,400 - 3,000 nm) and IRC (3,000 nm - 1 mm), or alternatively IR can be divided as near IR (760 - 3,000 nm), medium IR (3,000 - 30,000 nm) and far IR (30,000 nm - 1 mm) (Schieke et al. 2003). All IR bands have biological effects through interaction with water and biomolecules *in vitro*, since covalent and hydrogen bonds absorb low frequency vibrations (Walther et al. 2002; Maeda et al. 1999).

IR reaches deeper in the epithelial tissue of terrestrial organisms. Far IR reaches epithelial tissue and gradually transfers energy to deep tissue by a mechanism of resonance-absorption of organic molecules and water (Inoue et al. 1989). Depth of penetration in skin and subcutaneous tissue decreases while IR increases its wavelengths. Shorter wavelengths (IRA) reach subcutaneous tissue without increasing significantly the skin temperature. Longer wavelengths (IRC) are absorbed completely by the epithelial layers, increasing skin temperature and resulting in thermal sensation (Schieke et al. 2003) (This increase is caused by induction of molecular vibrations and rotations).

Also, IR is absorbed by the mitochondrial complex IV (cytochrome c-oxidase) (Karu et al, 1999), and from it, IR is responsible for epithelial regeneration by increasing: fibroblasts and keratinocytes mitosis rate, collagen genesis (Morita et al. 2006) and collagen regeneration, fibroblast infiltration in subcutaneous tissue (Toyokawa et al. 2003) and increasing the tensile strength of the regenerating epithelium (Schramn et al. 2003).

Due to IR be perceived as heat by most vertebrates, temperature rise was deemed the primary reason for their biological effects. However, others studies showed effects caused by non-thermal IR (Danno et al., 2001; Karu, 1999), but how this occurs have not yet been explained.

IR increases the expression of TGF-ß1, MMP-2 (Danno et al.; 2001; Toyokawa et al. 2003), HIST genes and genes connected to cell proliferation, as TUGCP3, KIF11, TTK and KPNA5, deletion of MMP-10 and apoptosis-related genes such as, CASP2 and TRPM2 (Morita et al. 2006). Furthermore, it causes a non-thermal non-autonomic

increase in epithelial microcirculation through a mechanism related to L-arginine/NO metabolic route (Yu et al. 2006).

Moreover, the negative effects of IR, to date, are linked to chronic exposure to moderate heat in mammals. This exposure results in erythema *ab igne*, a macular dermatosis, and reticular pigment in general, and in most reported cases, occurs through direct contact with the heat source (conduction instead of irradiation) (Walder & Hargis, 2002).

Besides IR, ultraviolet radiation (UV) is another electromagnetic solar radiation that reaches Earth. UV radiation is divided didactically and biologically into UVC radiation (200 - 290 nm), UVB (290 - 320 nm) and UVA (320 - 400 nm). UVB and UVA reaches Earth's surface, while UVC generally doesn't penetrate the atmosphere (Chapman et al. 1995).

UV damages can be directly to DNA (UVB and UVC) or to the cell membrane (UVA). Those damages make UV radiation one of the environmental factors most responsible for skin carcinogenesis (Bachelor et al., 2004), as melanoma, which is a skin cancer originated from melanocytes.

As for IR, latitude, altitude, clouds, ozone layer thickness, seasons and period of day determine the amount of UV that reach Earth's surface. With UVB incidence shows a peak near midday with minimums before 10 a.m. and after 4 p.m. (Kullavanijaya et al. 2005). Likewise, UVB is partially blocked by the ozone layer, transforming ozone molecules into oxygen molecules (Kerr & McElroy, 1993).

As the ozone layer is diminishing at high and mid-latitudes (Manney et al., 1994), UVB incidence increases, leading to increased exposure and tissue damage of living beings at those latitudes (Gouveia et al., 2005), generally by interacting with other substances (Filgueira et al., 2007).

We have an extensive body of knowledge of UV effects, mainly due to its prejudicial effects. Living beings, whether terrestrial or aquatic, prokaryotes or eukaryotes, possess endogenous chromophores capable of interacting with UV. For UVB, DNA is the preferred chromophore (Godar et al., 1993).

Although scientific data in relation to UV is vast and in relation to IR is increasing, little is known about their interaction. *In vivo* findings shows that IR reduces UV induced apoptosis in mice keratinocytes *in vivo* and *in vitro* (Jantschitsch, et al.; 2009), alters the type of UV-induced tumors with fewer mutated p53 clones and have a tendency to slow UV tumor formation in mice (Jantschitsch, 2011). IR interacting with UVB also regulates mast cell numbers and tryptase expression in vivo (Kim et al.; 2009) and protect fibroblast from the cytotoxic effects of UV radiation (Menezes et al, 1998).

As described above, IR and UV can alter tumor formation and this leaded Jantschitsch, et al. (2009) to postulate that IR has a role in UV induced carcinogenesis. In the actual state of knowledge, neither of those hypotheses can be denied or confirmed. So, our aim was to determine if chronic IR irradiation can prevent chronic UVB irradiation damage.

2. Material and Methods:

2.1. Animal Model

Male *Rattus norvegicus* (Wistar) from Central Animal Facility of Universidade Federal do Rio Grande – FURG, were kept at Instituto de Ciências Biológicas' bioterium. Animals were held for two weeks and maintained in polypropylene boxes in groups of up to four (2 months old) in each cage at 21° C ± 2 ° C, photoperiod of 12 hours light/dark, with food and water *ad libitum* before and during experiments. At the end of experiments, animals were sacrificed in a CO_2 chamber and their bodies were collected by Aborgama do Brasil for proper biological destination. All experiments were approved by CEUA (Ethics Committee on Animal Use) of FURG by Opinion P062/2011.

2.2. Infrared Exposition

Animals were irradiated in groups of four while inside polypropylene boxes similar to those used for maintenance, using a Phillips Infraphil 13379F/479, 150W lamp, ranging from 600 - 1500 nm, with a peak at 1000 nm, and distant 30 cm from

animals. Irradiation times were 30 minutes (1080 J / cm²). This time was determined in previous experiments to obtain the best results (data not shown).

2.3 Ultraviolet B Exposition

Animals were irradiated of up to four while inside polypropylene boxes for mice and irradiated during 90min (55.08 J/cm²) (time of exposition was determined in previous studies, data not shown) with UVB radiation using a VL: 115, 30 W (peak emission at 313 nm) UVB lamp.

2.3. Effects of Infrared Radiation and Ultraviolet B interaction:

2.3.1 Experimental Model

Animals were divided into UVB (control) (n = 9), IRUVB (n = 6) and UVBIR (n = 9). They were sedated by pentrane (methoxyflurane) inhalation in a dose not larger than 50 ml / kg and had their back (exposure area) depilated before irradiation procedures begin. Irradiation started 3 days after depilation and was performed during 15 days. UVB group was irradiated only with UVB, IRUVB group was irradiated first with IR and after a 90 minutes interval was irradiated with UVB, finally UVBIR group was irradiated first with UVB, than after 90 minutes interval was irradiated with IR. To prevent IR heating, temperature was controlled by indoors air-conditioning with plenty ventilation and measured constantly at animal level using a mercury thermometer. At 16th day, animals were sacrificed. Skin samples were removed for histopathological analysis and underwent histological.

2.3.2 - Histological Analysis:

Skin samples were cut into 3 - 5mm segments and stored in 50 ml Falcon tubes filled with paraformaldehyde at 1:20 ratio with at least 20 ml of solution per tube. Paraformaldehyde was prepared no later than 24 hours prior to use and kept cooled at 5° C. Samples were fixed for 24h, and then placed in an alcoholic solution at 70° GL. Three segments from each sample were dehydrated, diafanized and embedded in

Paraplast Xtra (Sigma P3808). From each piece, 3 - 4 slices were cut to 4 microns thick with a rotatory microtome (Leica – RM 2255). Slides were stained with HEs and send to a professional blinded to the experiment conditions, which carried out the histopathological analysis at Departamento de Patologia Animal of Faculdade de Veterinária – Universidade Federal de Pelotas.

3. Results

3.1 Histopathological results:

Results for skin tissue are showed in Table 1 (Appendix I). Percentage means how many slides had the feature, with + meaning mild intensity, ++ moderate intensity and +++ severe intensity.

When compared to UVB alone, IRUVB treatment reduced the incidences of parakeratotic hyperkeratosis, intraepidermal pustules, keratin pearls, detachment of epidermis (Figure 1, Appendix II), basal cell vacuolization and superficial dermis degeneration, while increasing acanthosis, orthokeratotic hyperkeratosis, collagen necrosis (Figure 2, Appendix III) and vasodilation.

When compared to UVB alone, UVBIR treatment reduced the incidences of parakeratotic hyperkeratosis, basal cell vacuolization and superficial dermis degeneration, while increasing the incidences of acanthosis, orthokeratotic hyperkeratosis, intraepidermal pustules, keratin pearls, collagen necrosis (Figure 2, Appendix III), inflammatory infiltrate and vasodilatation.

4. Discussion

4.1 IR pretreatment

Some authors have demonstrated that pretreatment with IR can protect cells from UVB damaging effects through p53 induction (Menezes et al, 1998). We corroborate these findings with our histopathological results. Protective effects were seen by a reduction in the severity and incidence of parakeratotic hyperkeratosis; reduction in the incidences of intraepidermal pustules, keratin pearls, detachment of

epidermis, and superficial dermis degeneration; increased incidence of vasodilation and inflammatory infiltrate, albeit, this last one was almost similar to UVB treated animals. Protective effects against UV radiation were also found in relation to tumor formation with fewer mutated p53 cells (Jantschitsch et al.; 2011).

These findings could be a result of infrared radiation activating and increasing p53 activity. Previous p53 activation could lead to a higher repair rate of damaged cells as indicated by basal cell vacuolization and these leading to vasodilation and inflammatory infiltrate (Menezes et al, 1998). Others have found a similar IRA reduction of UV-induced apoptotic cell death *in vitro* and *in vivo* and that this reduction may be due to induction of repair mechanisms (Jantschitsch et al.; 2009), since it is known that IR can modulate the mitochondrial pathway of apoptosis (Frank et al, 2004).

Although, the majority of effects can be seen as positives, pretreatment with IR enhanced some deleterious effects, as can be seen by increased incidence and severity of orthokeratotic hyperkeratosis and collagen necrosis. Also, there was an increase in overall acanthosis rate, but with a higher severity variance.

It's known that IR modulates collagen production and degradation during a scarification process and can over degrade the collagen matrix in healthy tissue (Schroeder, et al.; 2008). The same excessive degradation through matrix metalloproteinase (MMP)-1 happens by means of UVB radiations (Brenneisen et al., 2002). Also, UV causes mechanical degradation of collagen (Rabotyagova et al, 2008). These could indicate that although IR is protecting cells from UVB damage, the extracellular matrix, mainly composed of proteins, don't have the mechanisms to couple with UVB lesions and the intensified collagenase activity. In the long run, could these lead to increased cell damage?

Differently from us Lee et al. (2006) found that a 6-month IR treatment (900 – 1000 nm, 35 mW / cm^2, 15 - 20 minutes, 5 days / week) improved skin condition, reducing wrinkles by increasing collagen and elastin contents from stimulated fibroblasts.

4.2 IR post treatment

As IR irradiation can promote wound healing, we needed to discern if IR was preventing tissue alteration or healing skin damage. To achieve that, animals were irradiated with IR after UVB irradiation. If skin healing was happening, IR pretreatment and IR post treatment should have no differences, but if treatments showed different results, it could indicate that one was protecting the tissue while the other was healing UVB damage.

It was interesting to found that IR post treatment seems to had lower protective effect than IR pretreatment. For most characteristics, it even enhanced UVB deleterious effects.

Positive effects were lower incidence and severity of parakeratotic hyperkeratosis, lower incidence of detachment of epidermis, basal cell vacuolization and superficial dermis degeneration; also a higher incidence of inflammatory infiltrate and vasodilation can be seen as a positive effect.

Parakeratotic hyperkeratosis, basal cell vacuolization and superficial dermis degeneration were closer to UVB control than IR-UVB group and detachment of epidermis was closer to IR-UVB group than to UVB control group. These could indicate that although the regenerative properties of IR are in effect, they're much less efficient in dealing with UVB effects than they are to prepare cells to receive UVB radiation. The increase in basal cell vacuolization can indicate that more cells are damaged beyond repair and IR, through P53, leads to apoptosis. This data disagree with Jantschitsch et al. (2009) findings, but they only pre-treated animals with IR before UV exposition.

Differently from IR pretreatment, post-treatment enhanced many UVB deleterious effects. Findings were grouped as increased incidence and severity of acanthosis, orthokeratotic hyperkeratosis and collagen necrosis; increased incidence of keratin pearls and intraepidermal pustules. IR post-treated group showed interesting findings, as differently from pre-treated groups, most results were close to or worse than UVB group. These could indicate that IR concomitantly with UVB exposure lead to more damage than we previously supposed.

This increased damage can come from an excessive ROS production of oxidants mediated by IR induction of cytochrome IV (Schroeder et al., 2007).

One could argue that these synergic damage is a heat product (as part of IR is heat), but heat photoaging is characterized by solar elastosis with accumulation of elastotic distrophic material, severe hyperplasia of elastic fibers and degeneration of collagen fibers from the dermis. All of these are characteristic of erythema ab igne (Kibbi & Tannous, 1998). But as our findings showed different skin alterations and IR exposition was controlled to prevent heating, we disregard heating damage as the cause for UVB-IR lesions. Besides, as groups were exposed to same irradiation protocols, damaged should be seen in IRUVB and UVBIR groups, which wasn't the case.

As it is known, good Sun time is before 10 a.m. and after 4 p.m. This is the period when UVB radiation incidence is lower. Although our data need further replications from other researchers, we can say that sun bath took in between those hours can be more dangerous than were previously known. Someone that exposes himself to sun light at that time interval would receive high UVB radiation without an infrared previous protective signal and this concomitantly exposition to IR would enhance the UVB damages. We can also reiterates that exposition before 10 a.m. leads to increase protection from UVB damages as the body received first infrared radiation, which can prepares the cells to handle with the UVB effects.

5. Conclusion

In conclusion, our work showed that IR exposition before UVB irradiation reduce the incidence of parakeratotic hyperkeratosis, intraepidermal pustules, keratin pearls, detachment of epidermis, basal cell vacuolization and superficial dermis degeneration, while increasing acanthosis, orthokeratotic hyperkeratosis, collagen necrosis and vasodilation. In contrast, IR exposition after UVB irradiation enhances most of those prejudicial histological characteristics.

6. REFERENCES

Bachelor, M. A., Bowden, G. T. 2004. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. Seminars in Cancer Biology. 14: 131-138,

Brenneisen P, Sies H, Scharffetter-Kochanek K (2002) Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. Ann N Y Acad Sci 973:31–43

Chapman, R.S.; Cooper, K.D.; Fabo, E.C.; Frederick, J.E.; Gelatt, K.N.; Hammond, S.P.; Hersey, P.; Koren, H.S.; Ley, R.D.; Noonan, F.; Rusch, G.M.; Sarasin, A.; Selgrade, M.J.; Sobolev, I.; Ward, B.J. e Werkema, T.E. 1995. Solar Ultraviolet Radiation and the risk of infectious disease: sumary of a workshop. Photochem. Photobiol. 61(3): 223-247;

Danno, K.; Mori, N.; Toda, K-I.; Kobayashi, T.; Utani, A. 2001: Near-infrared irradiation stimulates cutaneous wound repair: laboratory experiments on possible mechanisms. Photodermatol. Photoimmunol. Photomed. 17: 261-265;

Filgueira, D.M.V.B.; Freitas, D.P.S.; Votto, A.P.S.; Fillmann, G.; Monserrat, J.M.; Geracitano, L.A.; Trindade, G.S. 2007. Photodynamic Action of Benzo[a]pyrene in K562 Cells. Photochem. Photobiol. 83: 1358–1363;

Frank, S.; Oliver, L.; Coster,C.L.; Moreau, C.; Lecabellec, M.T.; Michel, L.; Vallette, F.M.; Dubertret, L.; Coulomb, B. 2004. Infrared radiation affects the mitochondrial pathway of apoptosis in human fibroblasts. J Invest Dermatol 123:823-831

Godar, D.E., D.P. Thomas, A.S. Miller, W. Lee .1993. Long-wavelength UVA radiation induces oxidative stress, cytoskeletal damage and hemolysis. Photochem. Photobiol. 57: 1018-1026;

Gouveia, G.R.; Marques, D.S.; Cruz, B.P.; Geracitano, L.A.; Nery, L.E.M.; Trindade, G.S. 2005. Antioxidant defenses and DNA damage induced by UV-A and UV-B radiation in the crab chasmagnathus granulata (Decapoda, Brachyura). Photochem. Photobiol. 81: 398-403;

Inoue, S.; Kabaya, M. 1989. Biological activities caused by far-infrared radiation. Int. J. Biometeorol. 33: 145-150;

Jantschitsch C, Majewski S, Maeda A., Schwarz,T., Schwarz, A. 2009. Infrared radiation confers resistance to UV-induced apoptosis via reduction of DNA damage and upregulation of antiapoptotic proteins. J Invest Dermatol 2009: 129: 1271–1279.

Jantschitsch,C.; Weichenthal, M.; Maeda, A.; Proksch, E.; Schwarz, T.; Schwarz, A. 2011. Infrared radiation does not enhance the frequency of ultraviolet radiation-induced skin tumors, but their growth behavior in mice. Experimental Dermatology 20, 346-350

Karu, T. 1999. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B. Biol. 49(1): 1-17;

Kerr, J.B. e McElroy, C.T. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science 262: 1032-1034;

Kibbi, A.G. & Tannous, Z. Skin Diseases Caused by Heat and Cold Clinics in Dermatology. 1998;16:91-98

Kim,M-S; Kim, Y.K.; Lee. D.H.; Seo, J.E.; Cho, K.H.; Eun, H.C.; Chung, J.H.2009. Acute exposure of human skin to ultraviolet or infrared radiation or heat stimuli increases mast cell numbers and tryptase expression in human skin in vivo. British Journal of Demartoly 160: 393-402

Kochevar, I.E.; Pathak, M.A.; Parrish, J.A. Photophysics, photochemistry, and photobiology. In: Freedberg IM, Eisen AZ, Wolff K, et al., eds. Fitzpatrick's Dermatology in General Medicine. New York: McGraw-Hill, 1999; 220–229. Apud: Schiekel, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. Photodermatol. Photoimmunol. Photomed. 19: 228-234;

Kullavanijaya, P.; Lim, H. W. 2005. Photoprotection. J. Am. Acad. Dermat. 52: 937-958;

Lee, J.H.; Roh, M.R.; Lee, K.H. 2006. Effects of infrared radiation on skin photo-aging and pigmentation. Yonsei Med J 47 (4):485-490

Maeda, Y.; Ide, M.; Kitano, H. 1999. Vibrational spectroscopic study on the structure of water in polymer systems. J. Mol. Liq. 80: 149–163;

Manney, G.L.; Froidevaux, L.; Waters, J.W.; Zurek, R.W.; Read, W.G.; Elson, L.S.; Kumer, J.B.; Mergenthaler, J.L.; Roche, A.E.; O'Niell, A.; Harwood, R.S.; MacKenzie, I. e Swinbank, R. 1994. Chemical depletion of ozone in the Arctic lower stratosphere during winter 1992-93. Nature 370: 429-434;

Menezes, S.; Coulomb, B.; Lebreton, C. and Dubertret, L. Non-coherent near infra-red radiation protects normal human dermal fibroblasts from the solar ultraviolet toxicity. J. Invest Dermatol 111:629-633, 1998

Morita, S.; Narita, M.; Kitatani, N,; Terauchi, T. 2006. Effect of light-emitting diode irradiation on skin. J. Dermatol. Science, 42: 133-198;

Piazena H, Meffert H. Dosis-Wirkungs-Beziehungen bei Infrarot- Bestrahlung der Haut und die Extrapolation der Daten von in vitro-Messungen auf die Bedingungen in vivo - Erfordernisse und Fehler [Dosis related effects during infrared irradiation of the skin and the extrapolation of the data from in vitro measurements to in vivo conditions - requirements and errors]. Lecture presented at the symposium entitled "Water-filtered infrared-A (wIRA) in dermatology" of the Dr. med. h.c. Erwin Braun Foundation in Liestal/Basel, Switzerland, November 20, 2004. Apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Piazena H, Meffert H. Therapie mit Infrarotstrahlen – Physikalische Grundlagen und Anwendung in der Sauna und in Infrarotgeräten. In: Bühring M, Kemper FH, Hrsg. Naturheilverfahren [Loseblattsammlung]. Berlin, Heidelberg, New York: Springer; 2002. Bd. 1, 03.09. S.1-22 apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Rabotyagova,O.S.; Cebe, P.; Kaplan, D.L. 2008. Collagen structural hierarchy and susceptibility to degradation by ultraviolet radiation. Materials Science and Engineering C 28 : 1420-1429

Schieke, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. Photodermatol. Photoimmunol. Photomed. 19: 228-234;

Schramm, J.M; Warner, D.; Hardesty, R.A.; Oberg, K.C. 2003. A unique combination of infrared and microwave radiation accelerates wound healing. Plast. Reconstr. Surg. 111(1): 258-266;

Schroeder P, Pohl C, Calles C, Marks C, Wild S, Krutmann J (2007) Cellular response to infrared radiation involves retrograde mitochondrial signaling. Free Radic Biol Med 43:128–35

Schroeder,P.; Lademann, J.; Darvin,M.E.; Stege,H.; Marks, C.; Bruhnke, S.; Krutmann,J. 2008. Infrared radiation-induced matrix metalloproteinase in human skin: implications for protection. Journal of Investigative Dermatology 128: 2491-2497

Toyokawa, H.; Matsui, Y.; Uhara, J.; Tsuchiya,H.; Teshima, S.; Nakanishi, H.; Kwon, A.; Azuma, Y.; Nagaoka, T.; Ogawa, T.; Kamiyama, Y. 2003. Promotive effects of far-infrared ray on full-thickness skin wound healing in rats. Exp Biol Med. 228: 724–729;

Walder, E.Y.J. e Hargis, A.M. 2002. Chronic moderate heat dermatitis (erythema ab igne) in five dogs, three cats and one silvered langur. Vet. Dermatol. 13; 283-292;

Walther, M.; Plochocka, P.; Fischer, B.; Helm, H.; Jepsen, P. U. 2002. Collective vibrational modes in biological molecules investigated by terahertz time-domain spectroscopy. Biopolymers 67, 310–313;

Yu, S.; Chiu, J.; Yang, S.; Hsu, Y.; Lui, W.; Wu, C. 2006. Biological effect of far-infrared therapy on increasing skin microcirculation in rats. Photodermatol. Photoimmunol. Photomed. 22: 78–86;

APPENDIX I - Histopathological table results

	UVB	IRUVB	UVBIR
Acanthosis	38% (++ to +++)	15% (+++), 54 % (+ to ++)	46% (+++)
Parakeratotic Hyperkeratosis	50% (++ to +++)	0%	26%(+)
Orthokeratotic Hyperkeratosis	59% (+)	92% (+ to ++)	80% (++to +++)
Intraepidermal pustules	13%	8%	40%
Keratin pearls	8%	0%	20%
Detachment of epidermis	46%	15%	13%
Collagen necrosis	4% (+ to ++)	15% (+) 31% (+++)	40%(+++)
Inflammatory infiltrate	13%	15%	40%
Vasodilatation	4%	15%	33%
Basal cell vacuolization	50%	8%	40%
Follicles and epidermis attachments	0%	0%	13% (+++)
Superficial dermis degeneration	34%	0%	20%

Table 1. Histopathological results of chronic IR and UVB irradiations in mice skin. Male wistar rats were irradiated with UVB (n = 9, 90min, D = 55.08 J / cm²), with IR and after 90 min irradiated with UVB (IRUVB, n = 6, IR = 30 min, 1080 J / cm², UVB = same as above) and with UVB and after 90 min irradiated with IR (UVB and IR parameters as above). Data are expressed of a percentage of slides with the

histopathological feature. + meaning mild intensity, ++ meaning moderate intensity and +++ meaning severe intensity.

APPENDIX II –Detachment of epidermis 20x magnification photo

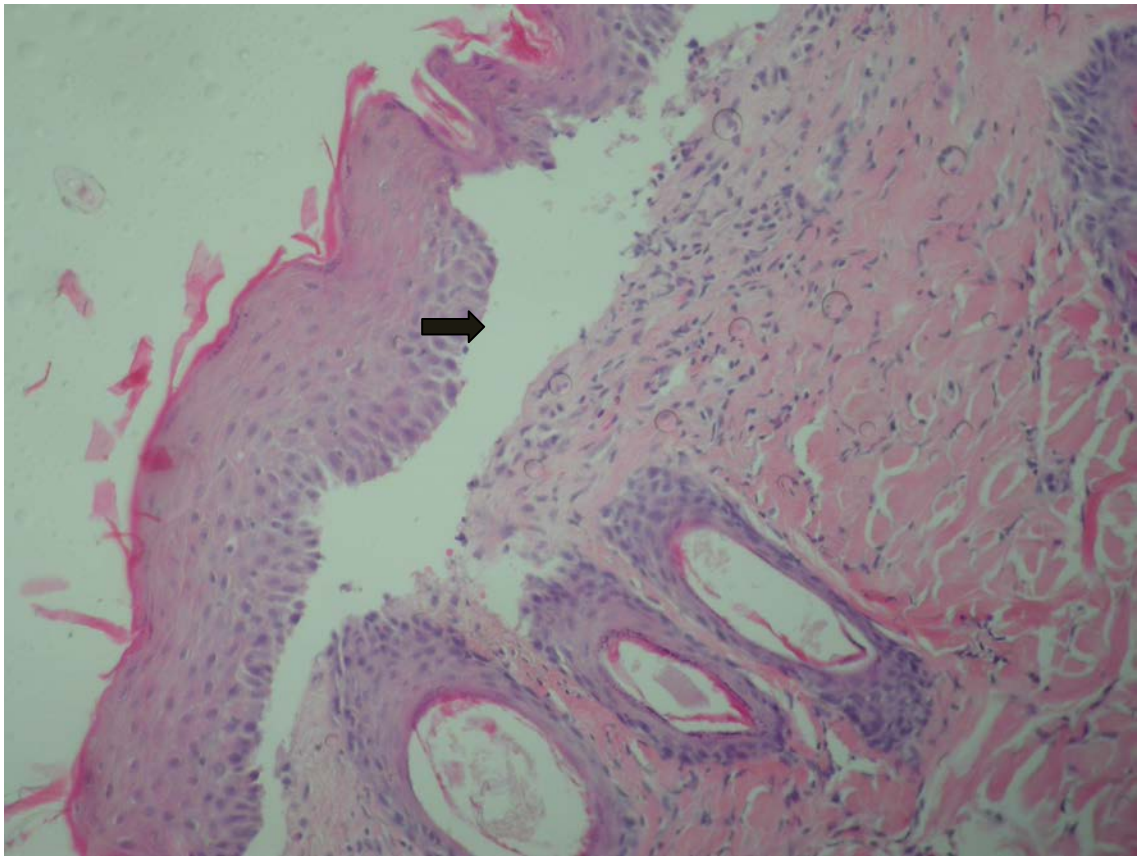

Figure 1. Detachment of epidermis in UVB exposed mice. Male wistar rats were irradiated with UVB (n = 9, 90 min, D = 55.08 J / cm²), with IR and after 90 min irradiated with UVB (IRUVB, n = 6, IR = 30 min, 1080 J / cm², UVB = same as above) and with UVB and after 90 min irradiated with IR (UVBIR, UVB and IR parameters as above). Arrow indicates detachment of epidermis. Picture was taken in an optical microscope at 20 x magnification.

APPENDIX III –Collagen necrosis 40xmagnification photo

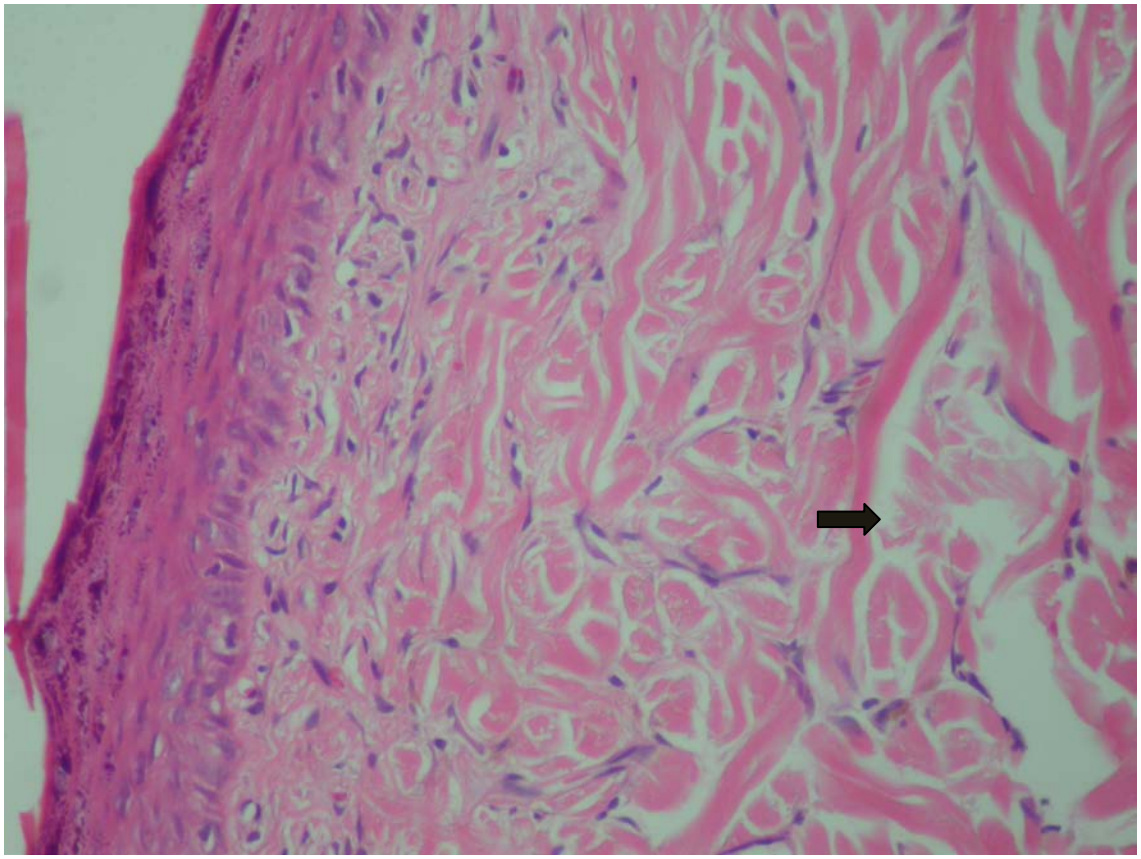

Figure 2. Collagen necrosis in UVB exposed mice. Male wistar rats were irradiated with UVB (n = 9, 90min, D = 55.08 J / cm²), with IR and after 90 min irradiated with UVB (IRUVB, n = 6, IR = 30 min, 1080 J / cm², UVB = same as above) and with UVB and after 90 min irradiated with IR (UVBIR, UVB and IR parameters as above). Arrow indicates detachment of epidermis. Picture was taken in an optical microscope at 20 x magnification.

3.3 ARTIGO 3

Effect of Infrared and UVB radiations interaction in B16F10 cells

Gonzalez, V.C.[1]; Oliveira, B.R.[1]; Vasconcelos, R.O.[1]; Podewils, T.[2], Votto, A.P.S.[1]; Marins,L.F.F.[1]; Varela Junior, A.S[2].; Trindade, G.S[1].

[1]Programa de Pós-Graduação em Ciências Fisiológicas – FURG; [2]Instituto de Ciências Biológica - FURG

Abstract

To determine the cell viability effects of infrared irradiation prior to UVB irradiation, B16F10 melanoma cells were exposed to infrared radiation (IR, 1080 J/cm²), UVB radiation (8.19 J/cm²) and IR 12 hours before UVB. Cells were analyzed at 0, 24, 48, and 72 hours after exposition by MTT, Neutral Red, EB/AO staining assays, total cell count and p53 gene expression. MTT and Neutral Red assays showed that IR did not protect the cells from UVB effects, while EB/AO showed that IR protected cells from UVB irradiation, without affecting p53 gene expression. Also, IR alone reduced the total cell count. In conclusion, IR irradiation did not altered cellular viability, but protected cells from UVB deleterious effects reducing the number of apoptotic and necrotic cells without dependence from a reduction in mitosis rate or altering the p53 gene expression.

1. Introduction

Infrared radiation (IR) is a solar electromagnetic radiation that compared to ultraviolet (UV) and visible radiations represents 54.3% of the Sol irradiated energy (Kochevar et al. 1999 apud Schiekel et al., 2003). Livings beings are exposed to IR values varying from 20 to 48 mW / cm² (Piazena et al., 2002; 2004 apud Gebbers et al.; 2007).

IR is divided into: IRA (700 - 1,400 nm), IRB (1,400 - 3,000 nm) and IRC (3,000 nm - 1 mm). Most of IR energy is within IRA, totaling 30% of solar energy when compared to UV and visible radiation (Schroeder et al, 2007).

IR is absorbed by water and tissues through covalent and hydrogen bonds (Walther et al., 2002; Maeda et al., 1999) inducing molecular vibrations (Laurent-Applegate & Roques, 2002) and gradually transferring energy to deep tissue by a mechanism of resonance-absorption that may increase the local temperature with effects similar to hyperthermia (IRC) (Schieke et al., 2003) or induce photochemical reactions (800-900 nm) (Laurent-Applegate, & Roques, 2002).

One of those photochemical reactions induced by IR irradiation is mitochondrial increased activity (Wong-Riley et al., 2005), which increases: mitosis rate in fibroblasts and keratinocytes cells, deletion of MMP-10 and apoptosis-related genes such as CASP2 and TRPM2 (Morita et al., 2006). Still, there is no consensus if IR can induce carcinogenesis while acting together with UV radiation (Jantschitsch et al., 2009).

UV radiation ranges from 200 to 400 nm wavelengths. It is divided didactically and biologically into UVC radiation (200 - 290 nm), UVB (290 - 320 nm) and UVA (320 - 400 nm). The solar UV comprises UVB and UVA, while UVC does not pass through the atmosphere (Chapman et al., 1995).

Another non thermal IR photochemical reaction is the prevention of UVB cytotoxic effects in *Eschericcia coli* and of UVA and UVB in human fibroblast (Menezes et al., 1998). This was not related to inhibition of lipid peroxidation, activation of heat shock protein (HSP70) or cell division. Besides, the protective IR effect against UV was not dose-dependent, but dependent on the number of times the cells were pre-exposed to IR (Continenza et al., 1993).

In vitro experiments with human fibroblast showed that the IR protective effect against UVB occurs by modulating Bcl2/Bax balance mechanisms, accumulation of p53 with stabilization and phosphorylation of serine (Ser15) and (Ser20) and increased expression of p21 and GADD45. Also, it decreased the formation of UVB induced thymine dimers (Frank et al., 2006).

UV damages can be directly to DNA (UVB and UVC) or to the cell membrane (UVA). Those damages make UV radiation one of the major environmental factors responsible for skin carcinogenesis (Bachelor et al., 2004), as melanoma, which is a skin cancer originated from melanocytes. Theoretical calculations for melanoma cancer incidence postulate a much higher number of cases than the real ones, but the main

probabilistic analyzes are based on data from studies using only the UV radiation as an oncostarter factor. A possibility for lower cases than theoretical prediction is the interaction between the various non-ionizing electromagnetic radiation, as well as their interactions with biological tissues.

To clarify the above points, B16F10 melanoma cells were exposed to infrared and UVB radiations to determine their interaction effects in cell viability

2. Material and Methods:

2.1 Cells and culture conditions:

B16F10 melanoma cells were maintained in DMEM supplemented with sodium bicarbonate (0.37 g / L), Hepes (25 mM) supplemented with 10% fetal bovine serum, 1% penicillin (100 U / ml) and streptomycin (100 mg / mL) at 37° C. Cells were obtained from Banco de Células do Rio de Janeiro (BCRJ).

2.2 Irradiation protocol

5 x 10^5 cells.ml^{-1} cells were incubated in culture flasks at 37° C. Upon reaching log phase, maintenance medium was removed; flasks were washed two times with Phosphate Buffered Saline (PBS) and suspended again in PBS for irradiation. 5 x 10^5 cells.ml^{-1} (unless otherwise noted) were seeded in 96 dish plates, divided into the following irradiation protocols: Control (no irradiation), 30 min IR irradiation (1080 J / cm^2, Phillips Infraphil 13379F/479, 150 W, ranging from 600 - 1500 nm, with a peak at 1000 nm), 35min UVB irradiation (8.19 J / cm^2, VL: 115, 30 W, with peak emission at 313 nm) and irradiated with IR 12 h before UVB irradiation (IRUVB). Irradiation times were determined in previous experiments (data not shown). After irradiation, PBS was removed and cells received maintenance medium. To preventing heating from the IR light bulb, a water layer was interposed between the plates and the IR lamp. Temperature was measured and never passed 37° C.

2.3 Cell viability

2.3.1 MTT assay (mitochondrial viability assay)

Cells were prepared as described in irradiation protocol and cell viability was tested by 3 - (4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT, 0.5 mg / mL) at 0, 24, 48 and 72 h. Briefly, after irradiation 20 µl of MTT was added to each dish. After 3 hours, PBS with MTT was removed and 200 µl dimethyl sulfoxide (DMSO) was added. Readings were made in a spectrophotometer at 590 nm and absorbance values were used as indicators of cell viability (Trindade et al, 1999). For each group n was: Control 55 (0 h), 35 (24 h), 62 (48 h), 64 (72 h), UVB 21 (0 h), 20 (24 h), 23 (48 h), 32 (72 h), IRUVB 20 (0 h), 20 (24 h), 23 (48 h), 29 (72 h), IR 52 (0 h), 35 (24 h), 60 (48 h) and 63 (72 h).

2.3.2 Neutral Red assay (lysosomal activity viability assay)

Cells were prepared as described in irradiation protocol and cell viability tested by 3-amino-7-dimethylamino-2-methylphenazine hydrochloride (40 ug / mL, neutral red) at 0, 24, 48 and 72 h. Briefly, cells were incubated for 3 h with 200 uL of neutral red dye dissolved in serum free medium. The pH of the neutral red solution was adjusted in all experiments to 6.35 with the addition of KH2PO4 (1 M). Cells were then washed with PBS and was added 1 ml of elution medium (EtOH / AcCOOH, 50% / 1%). Readings were made in a spectrophotometer at 540 nm and absorbance values were used as indicators of cell viability (Fotakis et al., 2006). For each group n was: Control 10 (0 h), 7 (24 h), 10 (48 h), 10 (72 h), UVB 10 (0 h), 7 (24 h), 11 (48 h), 10 (72 h), IRUVB 11 (0 h), 6 (24 h), 11 (48 h), 11 (72 h), IR 11 (0 h), 8 (24 h), 9 (48 h) and 10 (72 h).

2.4. EB/AO Chromatin Structure assay (DNA integrity viability assay)

Cells were prepared as described in irradiation protocol, but seeding was 1 x 10^5 cells.ml^{-1}. According to Ribble et al. (2005) at reading time, medium was removed, cells were washed two times with PBS, after washing was added to each well 20 µL of PBS and 5 µl of EB/AO (ethidium bromide and acridine orange) work solution, composed of

100 µg / mL of ethidium bromide (EB) and 100 µg / mL of acridine orange (AO) diluted in PBS (Coligan, et al.; 1995). 200 cells were counted at an Olympus IX81 fluorescence microscope. Cells with normal size nucleus and emitting green fluorescence were considered normal (viable). Other fluorescence cells with pyknotic nucleus were considered to have fragmented nucleus, with orange fluorescence being apoptotic and red being necrotic cells.

2.4.1 Total Cell Count Assay

After irradiation protocol all wells of all times (0, 24, 48 and 72 h) were photographed with an Olympus DP72 camera at 10x magnification within an Olympus IX81 fluorescence microscope. Visible light was used to illuminate the wells. All cells within each well were counted using ImageJ software. Number of wells (n) used for cell count for each irradiation group and time were: Control 12 (0 h), 11 (24 h), 13 (48 h), 12 (72 h), UVB 10 (0 h), 13 (24 h), 11 (48 h), 14 (72 h), IRUVB 11 (0 h), 14 (24 h), 13 (48 h), 13 (72 h), IR 11 (0 h), 12 (24 h), 12 (48 h) and 12 (72 h).

2.5 p53 expression

After irradiation protocol, p53 gene expression was analyzed by quantitative real time PCR. Total cellular RNA was isolated with Trizol LS Reagent (Invitrogen, Brazil) (n = 4 for each treatment). RNA samples were quantified using Quanti-iT RNA assay kit in a Qubit fluorometer (Invitrogen, Brazil). Then, RNA was treated with Dnase1 (Invitrogen, Brazil) and reverse transcripted (complementary DNA synthesis) using 2 µg of total RNA, following the manufacturer's instructions of reverse transcriptase (RT High capacity, Invitrogen, Brazil). Gene expression analysis was realized using a Real-time PCR System 7500 (Applied Biosystems, Brazil). Each PCR reaction was composed by a 10 µL mix (5 µL of SYBR Green PCR Master Mix (Applied Biosystems, Brazil), 0.2 µL of ROX, 0.25 µL of each primer, 2.7 µL of ultrapure water (Invitrogen, Brazil) and 1.6 µL of diluted cDNA from the B16F10 cell lineage). Primers were designed using the Primer-Blast tool from NCBI website (http://www.ncbi.nlm.nih.gov). Actin gene was used as for data normalization.

2.6 Statistical Analysis

Cell viability tests and total cell count data is presented as means ± standard error and was analyzed with Statistica 7.0 using ANOVA and Tukey post-test. p53 gene expression data was analyzed by comparison of median and standard deviation using Data Assist software with the 2-ΔΔCt-Method (Livak & Schmittgen, 2008; Mestdagh et al., 2009; Vandesompele et al., 2002). p values < 0.05 were considered statistically significant.

3. Results

3.1. Cell viability

3.1.1 MTT assay

At 0 and 24 hours, all treatments were not statistically significant. At 48 and 72 hours, control was not different from IR treatment and both were statistically different from UVB and from IRUVB treatments (Figure 1). Control and IR had readings 3 times higher than UVB and IRUVB treatments at 48 h, which was reduced to 2 times higher at 72 h. At the same time (48 and 72 h) UVB and IRUVB treatments were not statistically significant.

3.1.2. Neutral Red assay

At 0 hours all treatments were not statistically significant (Figure 2). At 24 hours, IR and IRUVB treatments were not statistically significant, and both were statistically higher from Control and UVB treatments. At 24 hours, Control and UVB were not statistically different.

A different pattern was showed by neutral red assay at 48 hours. UVB and IRUVB were not statistically significant, and both were different from Control and IR, while Control and IR were not statistically significant. At 72 hours all treatments were not statistically different.

3.2 EB/AO Chromatin Structure assay (DNA integrity viability assay)

3.2.1 Viable Cells (normal nucleus)

UVB and IR treatment were different at all times tested, with fewer UBV viable cells than IR viable cells (Figure 3). Also, UVB-treated cells were different from Control in all times except at 24 hours, with fewer UVB-treated cells than Control cells.

IRUVB were not different from Control, IR and UVB at 0 and 24 hours, being different from UVB at 48 and 72 hours.

3.2.2 Non-Viable Cells

When apoptotic and necrotic cells were pooled together, UVB-treated cells had the higher non-viability rate. In contrast to IR-treated cells that had the lowest count of non-viable cells at all times analyzed, although it is not statistically significant. UVB treatment was different from IR in all times analyzed, different from control at 0, 48 and 72 hours (Figure 4). IRUVB were different from UVB treatment from 48 and 72 hours.

3.2.3 Apoptotic cells / fragmented DNA

At all times analyzed the number of apoptotic cells was consistent for UVB and IR treatments, with UVB treatment having the highest number of cells and IR having the lowest number of cells. UVB were different from IR in all times, and IR was equal to Control in all times. IRUVB were only different from UVB treatment at 48 and 72 hours (Figure 5).

3.2.4 Necrotic cells / fragmented DNA

Although the maximum number of necrotic cells was low, UVB treatment had the highest cell count and were different from all other treatments, except as follow: at 24 hours when all treatments were not statistically significant, at this time UVB had a lower cell count than IRUVB treatment; and at 72 hours when UVB and IRUVB treatments were not significant (Figure 6). IR had the lowest cell count in all times

except at 24 hours, when Control had fewer cells than IR, but this was not statistically significant.

3.2.5 Total Cell Count Assay

At 0 and 24 hours all treatments were equal. At 48 and 72 hours, IR treated cells had a low cell count than the other treatments and were different from control cell count. All other treatments were equal to IR and to control (Figure 7).

3.3 P53 expression

There was no statistical difference between treatments within the same time of analysis (Figure 8).

4. Discussion

As infrared radiation is mainly absorbed by cytochrome c oxidase it would be expect that IR treatment had higher MTT values than Control, but Control and IR treatments were not statistically different. The dependence of IR to the cell state may explain this. Healthy cells will not show the same increase in mitochondrial activity than cells in poor state (Karu et al., 2004). Even B16F10 cells being a tumoral lineage, during experiments cells were at their optimum state, which can explain no increase in mitochondrial activity.

Damage from UVB exposition could be responsible for lower mitochondrial activity at all times, although only statistically different at 48 and 72 hours, with a small recovery at 72 hours. This UVB induced mitochondrial damage was not prevented by pretreatment with IR 12 hours prior to UVB.

Interesting, IR irradiation seemed to increase lysosomal activity within 24 hours after exposition as indicate by the neutral red assay. Since UVB and Control are statistically equal at this time, and lower than the activity of IR-treated cells, the increased activity showed by IRUVB treated cells does not configure a protective effect, but the same stimulatory effect from IR only treated cells.

After 48 hours of exposition, neutral red assay showed a similar pattern observed in the MTT assay, with Control and IR treated cells equal and UVB and IRUVB treated cells equal. After 72 hours, the effects of IR or UVB exposition ceased to impact the lysosomal activity, as all treatments were not statistically significant.

When both assays are analyzed together, although IR pretreatment did not protect UVB exposed cells it did not harm them, as there was no reduction in viability in IR treated cells. This is interesting, especially because all experiments were heat controlled as experiments without heat control can activate heat shock proteins, which through mitochondria induce deleterious ROS generation effects (Shin et al., 2008, 2012).

Differently from mitochondrial and lysosomal activity assays which did not demonstrate any protective effect from IR irradiance prior to UVB, fluorescent staining with ethidium bromide and acridine orange (EB/AO) showed that at DNA level, treatments result in different effects.

At 48 and 72 hours, viable IR pretreatment prior to UVB irradiation increased the number of viable cells, while UVB only could not recover the number of viable cells. The same pattern appears when total non-viable cells and apoptotic only cells were analyzed.

UVB induced cellular damage that result in non-viable cells, mostly leading to an apoptotic state, which is similar to Jantschitsch et al (2009) findings in mice keratinocytes *in vivo* and *in vitro*. Also, pretreatment with IR, after 48 hours, had the same pattern found by those researchers. They concluded that these reductions in apoptotic cells may lead to carcinogenesis. We cannot totally agree with their conclusion, as when analyzing the cell count results, IR alone had fewer cells than Control treatment. This can indicate that at the same time that IR protects from UVB damage it also reduces the mitotic rate of B16F10 cells. Also, it is known that IR irradiation reduces the formation of UVB induced thymine dimers (Frank et al, 2006), which can explain the reduction in non-viable cells

IR protection against UVB was also demonstrated within necrotic cells, but was dependent from the period of analysis. 24 hours after the last irradiation seem to be a transitional period, in which cells are dealing with the UVB-induced damage.

Different from other authors (Frank et al., 2004) that found p53 levels increased by IR exposition; we found that neither treatment affected p53 gene expression. This data also conflicts with Frank et al (2006), whereas they found that p53-deficient SaOs (sarcoma osteogenic) cells were not protected from UVB cytotoxicity by IR pretreatment, whereas non-deficient cells were protected.

Overall, it is interesting to see that although IR had a protective effect against UVB effects, it diminish the total cell count. Our findings agreed with Menezes et al. (1998) where at 72 h readings they find an IR protective effect against UVB irradiation, except that we found a decrease in cell numbers at 72 h. This may be because in their experiment UVB was applied immediately after IR irradiation, and that they were using non-tumoral fibroblast cells.

5. Conclusion

In this paper we showed that heat-controlled IR exposition did not alter mitochondrial and lysosomal cell viability. At the same time, pretreating cells with IR 12 hours prior to UVB exposition enhanced DNA stability with fewer apoptotic and necrotic cells than at UVB treated ones. Different from other authors we found that neither treatment induced p53 gene expression.

6. References

Bachelor, M. A., Bowden, G. T. 2004. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. Seminars in Cancer Biology. 14: 131-138,

Beauvoit, B.; Kitai, T.; Chance, B. 1994. Contribution of the mitochondrial compartment to the optical properties of the rat liver: a theoretical and practical approach. *Biophys. J*. 67(6): 2501-2510;

Chapman, R.S.; Cooper, K.D.; Fabo, E.C.; Frederick, J.E.; Gelatt, K.N.; Hammond, S.P.; Hersey, P.; Koren, H.S.; Ley, R.D.; Noonan, F.; Rusch, G.M.; Sarasin, A.; Selgrade, M.J.; Sobolev, I.; Ward, B.J. e Werkema, T.E. 1995. Solar Ultraviolet Radiation and the risk of infectious disease: sumary of a workshop. *Photochem. Photobiol*. 61(3): 223-247;

Coligan, J.E., Kruisbeek, A.M., Margulies, D.H., Shevach, E.M., Strober, W. (1995) Current protocols in immunology. In related isolation procedures and functional assays. Volume 1. Edited by: Coico R John Wiley & Sons, Inc. 3.17.1;

Continenza, M.A.; Ricciardi, G.; Franchitto, A. 1993. Effects of low power 904 nm radiation on rat fibroblasts explanted and in vitro cultured. *J. Photochem. Photobiol. B: Biol.* 19(3): 231-234;

Eells, J.T.; Wong-Riley, M.T.T.; Verhoeve, J.; Henryd, M.; Buchman, E.V.; Kane, M.P.; Gould,L.J.; Das,R.; Jett, M.; Hodgson, B.D.; Margolis, D.; Whelan, H.T.; 2004. Mitochondrial signal transduction in accelerated wound and retinal healing by near infrared light therapy. Mitochondrion 4:559-567;

Fotakis, G.; Timbrell, J.A. In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology Letters 160 (2006) 171 – 177;

Frank, S.; Oliver, L.; Coster,C.L.; Moreau, C.; Lecabellec, M.T.; Michel, L.; Vallette, F.M.; Dubertret, L.; Coulomb, B. 2004. Infrared radiation affects the mitochondrial pathway of apoptosis in human fibroblasts. J Invest Dermatol 123:823-831

Frank, S.; Menezes, S.; Lebreton-De Coster, C.; Oster, M.; Dubertret, L.; Coulomb, B. 2006. Infrared radiation induces the p53 signaling pathway role in infrared prevention of ultraviolet B toxicity. *Experimental Dermatology* 15(2): 130-137;

Gouveia, G.R; Lopes, T.M.; Neves, C.A.; Nery, L.E.M.; Trindade, G.S. 2004. Ultraviolet radiation induces dose-dependent pigment dispersion in crustacean chromatophores. *Pigment Cell Res* 17: 545-548;

Gouveia, G.R.; Marques, D.S.; Cruz, B.P.; Geracitano, L.A.; Nery, L.E.M.; Trindade, G.S. 2005. Antioxidant defenses and DNA damage induced by UV-A and UV-B radiation in the crab chasmagnathus granulata (Decapoda, Brachyura). *Photochem. Photobiol*. 81: 398-403;

Jantschitsch C, Majewski S, Maeda A., Schwarz,T., Schwarz, A. 2009. Infrared radiation confers resistance to UV-induced apoptosis via reduction of DNA damage and upregulation of antiapoptotic proteins. J Invest Dermatol 2009: 129: 1271–1279.

Karu, T.I.; Pyatibrat, L.V.; Afanasyeva, N.I. 2004. A novel mitochondrial signaling pathway activated by visible-to-near infrared radiation. Photochemistry and photobiology 80:366-372,

Kerr, J.B. e McElroy, C.T. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science* 262: 1032-1034;

Kochevar, I.E.; Pathak, M.A.; Parrish, J.A. Photophysics, photochemistry, and photobiology. In: Freedberg IM, Eisen AZ, Wolff K, et al., eds. *Fitzpatrick's Dermatology in General Medicine*. New York: McGraw-Hill, 1999; 220–229. *Apud*: Schiekel, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol. Photoimmunol. Photomed.* 19: 228-234;

Kuerbitz, S.J.; Plunkett, B.S.; Walsh, W.V.; Kastan, M.B. 1992. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc. Natl. Acad. Sci. USA* 89: 7491-7495;

Lane, D.P.; Lu, X.; Hall, P.A. 1994. The role of p53 protein in the apoptotic response. *Philos. Trans. R. Soc. Lond.* B 345: 277-280

Laurent-Applegate, L. & Roques, S. 2002. CHAPTER 16 Biological Actions of Infrared Radiation, in Sensing, Signaling and Cell Adaptation. Edited by K.B. Storey and J.M. Storey

Livak, K.J. and Schmittgen, T.D. Analyzing real-time PCR data by the comparative CT method. Nature Protocols 3, 1101-1108 (2008).

Maeda, Y.; Ide, M.; Kitano, H. 1999. Vibrational spectroscopic study on the structure of water in polymer systems. *J. Mol. Liq.* 80: 149–163;

Manney, G.L.; Froidevaux, L.; Waters, J.W.; Zurek, R.W.; Read, W.G.; Elson, L.S.; Kumer, J.B.; Mergenthaler, J.L.; Roche, A.E.; O'Niell, A.; Harwood, R.S.; MacKenzie, I. e Swinbank, R. 1994. Chemical depletion of ozone in the Arctic lower stratosphere during winter 1992-93. *Nature* 370: 429-434;

McNutt, N.S.; Saenz-Santamaria, C.; Volkenandt, M. 1994. Abnormalities of p53 protein expression in cutaneous disorders. *Arch. Dermatol*. 130: 225-232;

Menezes, S.; Coulomb, B.; Lebreton, C. and Dubertret, L. Non-coherent near infra-red radiation protects normal human dermal fibroblasts from the solar ultraviolet toxicity. J. Invest Dermatol 111:629-633, 1998

Mestdagh P.,Van Vlierberghe P., De Weer A., Muth D., Westermann F., Spelemean F., Vandesompele J. A novel and universal method for microRNA RT-qPCR data normalization. Genome Biology 10,R64 (2009).

Morita, S.; Narita, M.; Kitatani, N,; Terauchi, T. 2006. Effect of light-emitting diode irradiation on skin. J. Dermatol. *Science*, 42: 133-198;

Piazena H, Meffert H. Dosis-Wirkungs-Beziehungen bei Infrarot- Bestrahlung der Haut und die Extrapolation der Daten von in vitro-Messungen auf die Bedingungen in vivo - Erfordernisse und Fehler [Dosis related effects during infrared irradiation of the skin and the extrapolation of the data from in vitro measurements to in vivo conditions - requirements and errors]. Lecture presented at the symposium entitled "Water-filtered infrared-A (wIRA) in dermatology" of the Dr. med. h.c. Erwin Braun Foundation in Liestal/Basel, Switzerland, November 20, 2004. Apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered

Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Piazena H, Meffert H. Therapie mit Infrarotstrahlen – Physikalische Grundlagen und Anwendung in der Sauna und in Infrarotgeräten. In: Bühring M, Kemper FH, Hrsg. Naturheilverfahren [Loseblattsammlung]. Berlin, Heidelberg, New York: Springer; 2002. Bd. 1, 03.09. S.1-22 apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8;

Ribble, D., Goldstein, N.B. Norris, D. A., Shellman, Y.G. (2005) A simple technique for quantifying apoptosis in 96-well plates. BMC Biotechnology.5;

Schieke, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol. Photoimmunol. Photomed.* 19: 228-234;

Schroeder P, Pohl C, Calles C, Marks C, Wild S, Krutmann J (2007) Cellular response to infrared radiation involves retrograde mitochondrial signaling. Free Radic Biol Med 43:128–35

Shin, M. H., Seo, J.E.; Kim, y.K.; Kim, K.H.; Chung, J.H. 2012. Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mechanisms of Ageing and Development 133: 92–98

Shin, M.H., Moon, Y.J., Seo, J.E., Lee, Y., Kim, K.H., Chung, J.H., 2008. Reactive oxygen species produced by NADPH oxidase, xanthine oxidase, and mitochondrial electron transport system mediate heat shock-induced MMP-1 and MMP-9 expression. Free Radic. Biol. Med. 44, 635–645.

Trindade, G. S., Rumjanek, V.; Capella, M.A.M.; Mitidieri, O.R.A.; Capella, L.S. (1999) Differences in Sensitivity to UVC, UVB and UVA Radiation of a Multidrug Resistant Cell Line Overexpressing P-glycoprotein. Photochem. Photobiol. 69, 694-699.

Vandesompele J., De Preter K., Pattyn F., Poppe B., Van Roy N, De Paepe A., Speleman F. Accurate normalization of real-time quantitative RT-PCR data by

geometric averaging of multiple internal control genes. Genome Biology 3, research0034 (2002)

Walther, M.; Plochocka, P.; Fischer, B.; Helm, H.; Jepsen, P. U. 2002. Collective vibrational modes in biological molecules investigated by terahertz time-domain spectroscopy. *Biopolymers* 67, 310–313;

Wong-Riley, M.T.T.; Liang, H.L.; Eells, J.T.; Chancel, B.; Henry, M.M.; Buchmann, E.; Kane, M.; Whelan, H.T. 2005. Photobiomodulation Directly Benefits Primary Neurons Functionally Inactivated by Toxins – Role of Cytochrome c Oxidase. *J. Biol. Chem*. 280 (6): 4761-4771;

APPENDIX 1 – MTT assay graphic and table

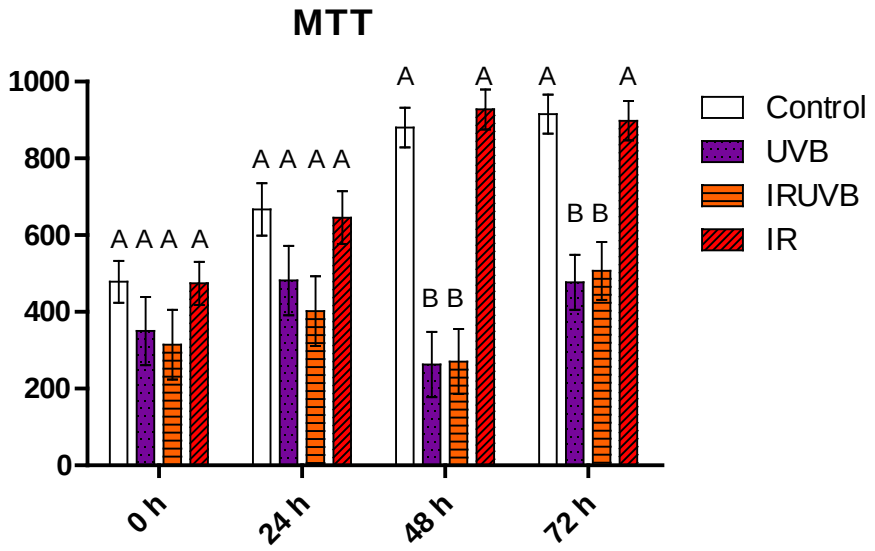

Figure 1. MTT viability assay graphic. 5 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). Heat was prevented by a water layer between IR lamp and dish plates. Data showed as means ± standard error of absorbance at 590 nm. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	478,359	54,7347	55	350,212	88,5798	21	314,322	90,7673	20	474,351	56,2915	52
24 h	666,977	68,6136	35	481,688	90,7673	20	402,385	90,7673	20	645,815	68,6136	35
48 h	880,331	51,5523	62	262,887	84,6409	23	270,754	84,6409	23	927,540	52,4045	60
72 h	915,116	50,7404	64	476,954	71,7578	32	506,883	75,3781	29	898,054	51,1416	63

Table 1. MTT viability assay table. 5 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). Data showed as means ± standard error of absorbance at 590 nm. Heat was prevented by a water layer between IR lamp and dish plates.

APPENDIX 2 – Neutral red assay graphic and table

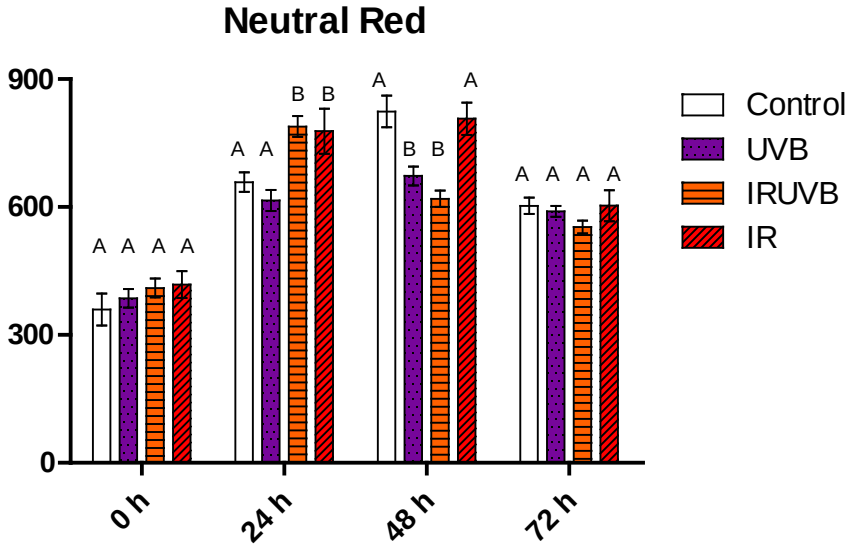

Figure 2. Neutral Red viability assay graphic. 5 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). Data showed as means ± standard error of absorbance at 560 nm. Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	359,500	37,5165	10	386,100	21,6689	10	410,181	22,1149	11	418,090	31,4563	11
24 h	658,428	22,8909	7	615,428	25,0112	7	788,666	24,3812	6	777,875	52,9600	8
48 h	824,000	37,0917	10	672,818	22,0844	11	619,363	18,9080	11	807,000	37,7727	9
72 h	602,900	19,2379	10	589,900	12,6125	10	553,000	14,9830	11	603,000	36,0237	10

Table 2. Neutral Red viability assay table. 5 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). Heat was prevented by a water layer between IR lamp and dish plates.

APPENDIX 3 – Viable cells graphic and table

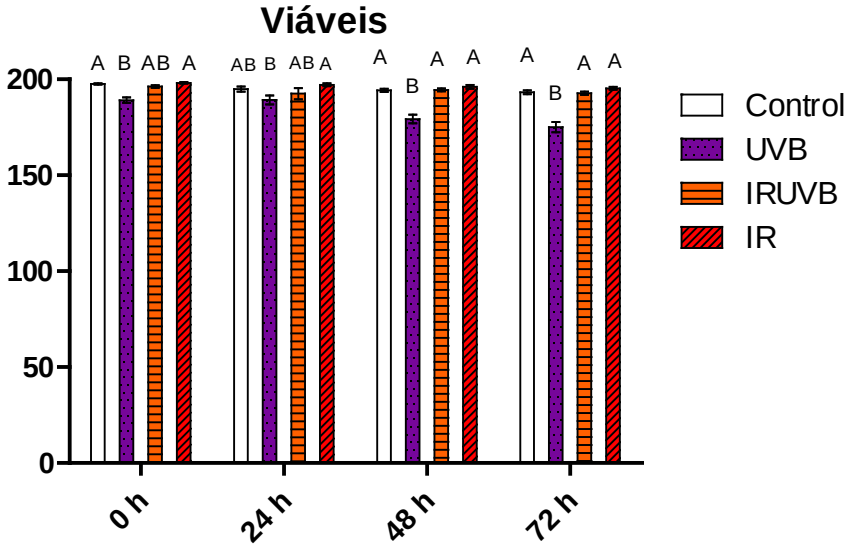

Figure 3. EB / AO Chromatin Structure Assay Viable Cells Graphic. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81 fluorescence microscope. Graphic represents only normal (viable) cells. Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	197,642	0,34141	14	189,142	1,38618	14	196,214	0,67269	14	198,142	0,37588	14
24 h	194,916	1,29368	12	189,285	2,36603	14	192,500	2,89704	14	197,142	0,79736	14
48 h	194,285	0,86079	14	179,285	2,27366	14	194,461	0,76473	13	196,071	0,94033	14
72 h	193,285	0,99132	14	175,071	2,73452	14	192,785	0,84631	14	195,333	0,81958	12

Table 3. EB / AO Chromatin Structure Assay Viable Cells Table. 1 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81

fluorescence microscope. Graphic represents only normal (viable) cells. Heat was prevented by a water layer between IR lamp and dish plates.

APPENDIX 4 - Non-viable cells graphic and table

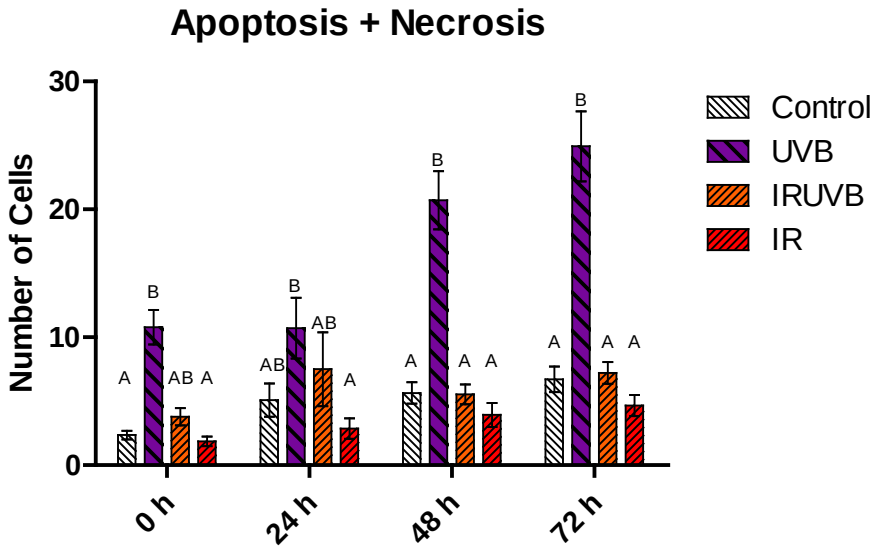

Figure 4. EB / AO Chromatin Structure Assay Non-Viable Cells Graphic. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm^2 (UVB), 30 min of IR, 1080 J / cm^2 (IR) or to 30 min of IR, 1080 J / cm^2 and after 12 hours to 35 min of UVB, 8.19 J / cm^2 (IRUVB). 200 hundred cells were counted in an Olympus IX81 fluorescence microscope. Graphic represents only apoptotic and necrotic cells (non-viable cells). Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	2,3571	0,34141	14	10,7857	1,34727	14	3,7857	0,67269	14	1,8571	0,37588	14
24 h	5,0833	1,29368	12	10,7142	2,36603	14	7,5000	2,89704	14	2,8571	0,79736	14
48 h	5,6428	0,84259	14	20,7142	2,27366	14	5,5384	0,76473	13	3,9285	0,94033	14
72 h	6,7142	0,99132	14	24,9285	2,73452	14	7,2142	0,84631	14	4,6666	0,81958	12

Table 4. EB / AO Chromatin Structure Assay Non-Viable Cells Table. 1 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm^2 (UVB), 30 min of IR, 1080 J / cm^2 (IR) or to 30 min of IR, 1080 J / cm^2 and after 12 hours to 35 min of UVB, 8.19 J / cm^2 (IRUVB). 200 hundred cells were counted in an Olympus IX81

fluorescence microscope. Graphic represents only apoptotic and necrotic cells (non-viable cells). Heat was prevented by a water layer between IR lamp and dish plates.

APPENDIX 5 - Apoptotic cells graphic and table

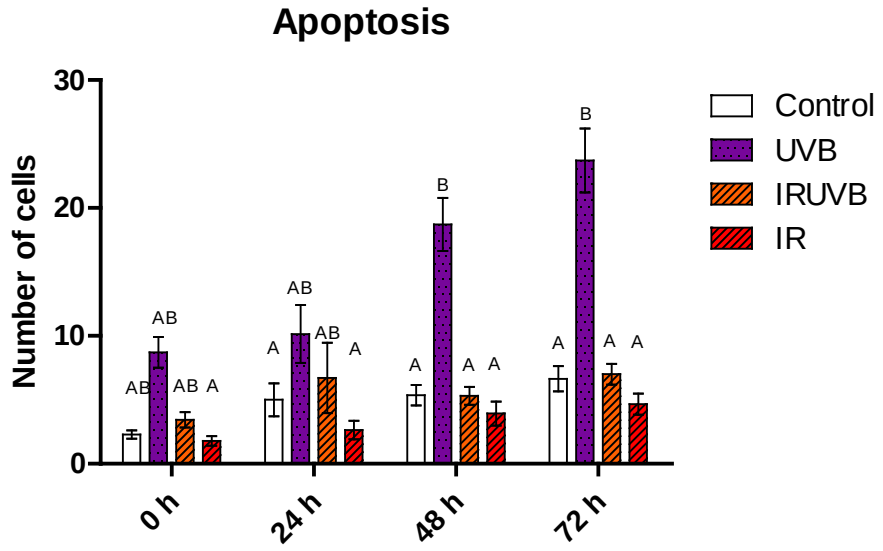

Figure 5. EB / AO Chromatin Structure Assay - Apoptotic Cells Graphic. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81 fluorescence microscope. Graphic represents only apoptotic cells (non-viable cells). Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	2,2857	0,32188	14	8,7142	1,20634	14	3,4285	0,59958	14	1,7857	0,38055	14
24 h	5,0000	1,27920	12	10,1428	2,26258	14	6,7142	2,74247	14	2,6428	0,73085	14
48 h	5,3571	0,79564	14	18,7142	2,07133	14	5,3076	0,70150	13	3,9285	0,94033	14
72 h	6,6428	0,98676	14	23,7142	2,50807	14	7,0000	0,81873	14	4,6666	0,81958	12

Table 5. EB / AO Chromatin Structure Assay - Apoptotic Cells Table. 1 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81 fluorescence microscope. Graphic represents only apoptotic cells (non-viable cells).

Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

APPENDIX 6 - Necrotic cells graphic and table

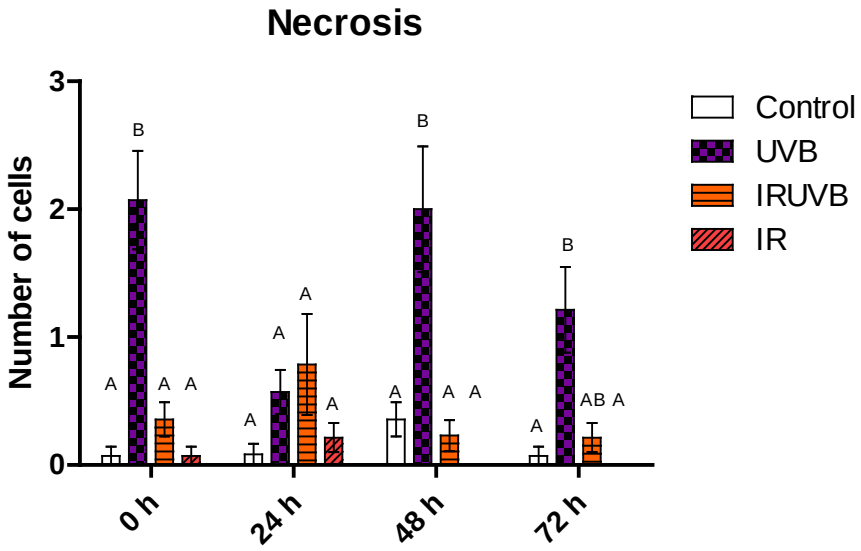

Figure 6. EB / AO Chromatin Structure Assay Necrotic Cells Graphic. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81 fluorescence microscope. Graphic represents only necrotic cells (non-viable cells). Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
0 h	0,07142	0,07142	14	2,07142	0,38465	14	0,35714	0,13289	14	0,07142	0,07142	14
24 h	0,08333	0,08333	12	0,57142	0,17270	14	0,78571	0,39472	14	0,21428	0,11380	14
48 h	0,35714	0,13289	14	2,00000	0,49168	14	0,23076	0,12162	13	0,00000	0,00000	14
72 h	0,07142	0,07142	14	1,21428	0,33444	14	0,21428	0,11380	14	0,00000	0,00000	12

Table 6. EB / AO Chromatin Structure Assay Necrotic Cells Table. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). 200 hundred cells were counted in an Olympus IX81

fluorescence microscope. Graphic represents only necrotic cells (non-viable cells). Heat was prevented by a water layer between IR lamp and dish plates.

APPENDIX 7 – Total Cell Count Assay graphic and table

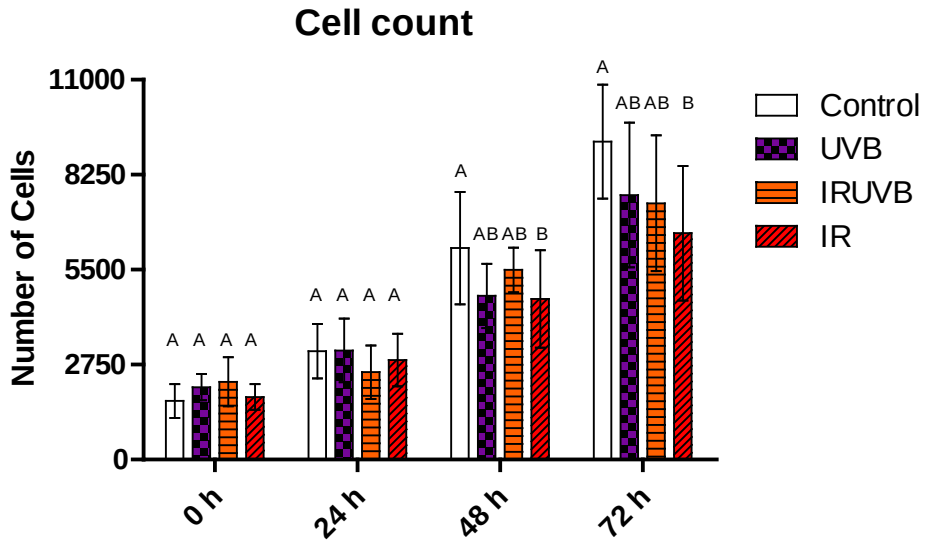

Figure 7. Total Cell Count Graphic. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). All wells were photographed with an Olympus DP72 camera at 10x magnification within an Olympus IX81 fluorescence microscope using visible light. All cells from within each well were counted using ImageJ software. Graphic represents mean and standard error of cells per treatment and time. Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

	Control			UVB			IRUVB			IR		
	Mean	SE	n	Mean	SE	N	Mean	SE	n	Mean	SE	n
0 h	1696,2	488,31	12	2098,4	380,86	10	2253,4	707,98	11	1814,4	371,36	11
24 h	3137,3	788,29	11	3161,3	921,71	13	2532,8	775,72	14	2884,2	761,07	12
48 h	6124,0	1629,73	13	4739,4	931,19	11	5492,0	648,83	13	4651,3	1409,90	12
72 h	9207,0	1646,98	12	7662,2	2095,01	14	7423,3	1965,164	13	6555,0	1949,40	12

Table 7. Total Cell Count Table. 1 x 10^5 cells.ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm² (UVB), 30 min of IR, 1080 J / cm² (IR) or to 30 min of IR, 1080 J / cm² and after 12 hours to 35 min of UVB, 8.19 J / cm² (IRUVB). All wells were photographed with an Olympus DP72 camera at 10x magnification within an

Olympus IX81 fluorescent microscope using visible light. All cells from within each well were counted using ImageJ software. Graphic represents mean and standard error of cells per treatment and time. Heat was prevented by a water layer between IR lamp and dish plates. Different letters means statistically significance.

APPENDIX 8 - p53 expression graphic

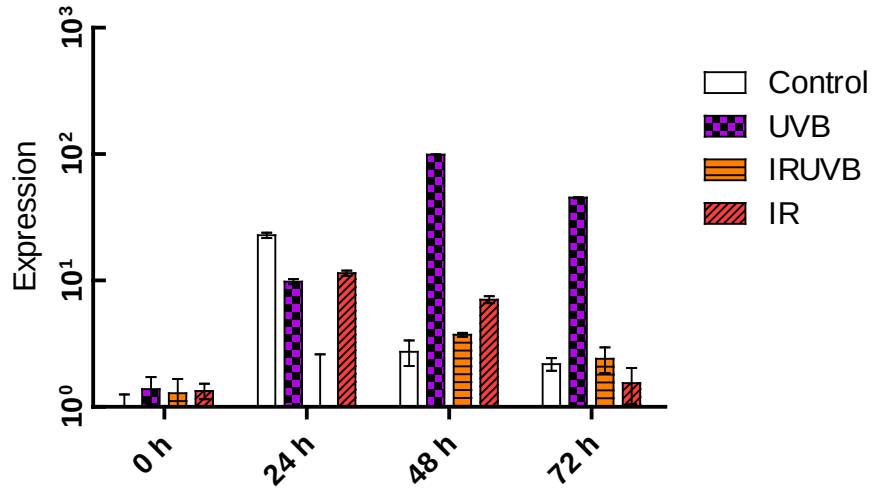

Figure 8. p53 quantitative real time PCR graphic. 5 x 10^5 cells .ml^{-1} were exposed to: no radiation (Control), 35 min of UVB, 8.19 J / cm^2 (UVB), 30 min of IR, 1080 J / cm^2 (IR) or to 30 min of IR, 1080 J / cm^2 and after 12 hours to 35 min of UVB, 8.19 J / cm^2 (IRUVB). Graphic represents mean and standard error by 2-ΔΔCt-Method using Data Assist software. Heat was prevented by a water layer between IR lamp and dish plates.

3.4 ARTIGO 4

Infrared Radiation in Biological Research – Moving away from heat

Gonzalez, V.C[1].; Varela Junior, A.S[2].; Trindade, G.S[1].

[1]Programa de Pós-Graduação em Ciências Fisiológicas – FURG; [2]Instituto de Ciências Biológicas - FURG

Abstract

For a long time, radiant heat and infrared radiation (IR) were considered equivalent due to hyperthermia being an IR property. But with the demonstration that IR has biological effects without heating, some distinctions need to be made. Heat can induce heat shock proteins (HSPs) expression, which may mislead conclusions about IR experiments. Some conflicts can be found at photoaging, photorejuvenation, MMPs and collagen expression papers. Also, moderate chronic heat exposition can cause tissue alterations. From the IR emitter devices, IR LEDs can be used without the risk of heating the tissue. In conclusion, IR experiments without heat control can induce HSPs or tissue alteration; to avoid heating we recommend the use of IR LEDs.

1. Introduction

Infrared radiation (IR) represents 54.3% of the solar electromagnetic radiations that reach Earth every day when compared to ultraviolet (UV) and visible radiations (Kochevar et al. 1999 apud Schiekel et al., 2003). Environmental IR values range from 20-48 mW / cm² (Piazena et al 2002, 2004 apud Gebbers et al.; 2007).

CIE (International Commission on Illumination) divides IR into: IRA (700 - 1,400 nm), IRB (1,400 - 3,000 nm) and IRC (3,000 nm - 1 mm). Alternatively IR can be divided as near IR (760 - 3,000 nm), medium IR (3,000 - 30,000 nm) and far IR (30,000 nm - 1 mm) (Schieke et al. 2003). IRA comprises 30% of solar energy when compared to UV and visible radiation (Schroeder et al, 2007).

Since IR discovery, heat and IR have been described as one and the same. Early biological experiments used heaters to delivery IR in directly contact or as radiant energy. Today, it is known that IR can have biological effects without head.

In this article will discuss why IR and heat need to be separated in biological experiments.

2. IR characteristics

2.1 Penetrability

IR penetrability into skin and subcutaneous tissue decreases with increasing wavelengths (Schieke et al, 2003), but its penetrability into living tissue still needs clarification as researches report different penetration values. This values range from 0.7 - 30 mm (Eelss et al 2004) to 230 mm (λ = 630 - 800nm) (Beauvoit et al., 1994). One explanation to these might be that into living tissues water absorbs some bands of IRA, all of IRB and part of IRC within the first 0.25 - 0.5 mm. Those bands of IRA not absorbed are called wIRA (780 - 1400 nm) and can penetrate 20 - 30 mm without irritating or overheating of the skin (Laurent-Applegate & Roques, 2002). Other authors disregard wIRA and states that IRA as a whole deeply penetrates human skin (Schroeder, et al 2007).

Penetrability also depends if irradiation happens in ultra-short pulses (lasers, LEDs) or in a continuous wave ([CW] lamps, LEDs) (Barolet, 2008). Ultra-short pulses can travel deeper into tissues than CW (Pogue et al, 1997) because the first pulse interact with all the upper tissue layer chromophores opening a road for the following pulses, until those chromophores leave their excited state (Barolet, 2008).

2.2 Energy transfer mechanism

As IR penetrates, it is absorbed by water and tissues through covalent and hydrogen bonds (Walther et al. 2002; Maeda et al. 1999) inducing molecular vibrations (Laurent-Applegate & Roques, 2002) and gradually transferring energy to deep tissue by a mechanism of resonance-absorption that may, increase the local temperature with

effects similar to hyperthermia (IRC) (Schieke et al. 2003), or induce photochemical reactions (800 - 900 nm) (Laurent-Applegate, & Roques, 2002).

2.3 Dose

Dose (or fluence) is equal to the intensity (irradiance) x time (J/cm^2). Therefore, the same exposure should result from reducing duration and increasing light intensity, and vice versa (reciprocity law). Dose reciprocity studies showed that varying irradiance and exposure time to achieve a constant specified energy density affects outcomes (Lanzafame et al, 2007). If intensity (irradiance) is lower than the physiological threshold value, it does not produce photostimulatory effects even when irradiation time is extended. Moreover, photoinhibitory effects may occur at higher fluencies. A minimal exposure time is necessary (minutes) to achieve tissue response (Barolet, 2008).

2.4 Cell state and effect

Besides dose and its intensity x time dependency, the biostimulation effect depends on the physiological condition of exposed area at the irradiation time (Karu et al, 2004). Compromised areas respond readier than healthy ones to IR energy transfers. For instance, light only stimulate cell proliferation if cells are growing poorly at the irradiation moment, as if IR is energizing the cells to its own maximal biological potential. This may explain the different studies results.

3 IR and Heat

Heat is an energy that can be transmitted by conduction (direct collision with neighboring atoms), convection (motion of liquid/gas due to changes in density as thermal energy is absorbed) or as radiant heat (infrared electromagnetic energy that is released into space as atomic particles decelerate, it originates from one solid object, passes through space or air, and is absorbed by another solid object) (Dover et al, 1989).

Radiant heat has been described as equivalent to IR and many studies use heat and IR as synonyms, due to the thermal capacity and hyperthermia being dominant properties of IR. But as wIRA has thermal and non-thermal effects (Fuchs et al, 2004) some distinction need to be made. IR non thermic biological effects have been highly neglected due to the lack of appropriate IR and heat source filtration.

Many studies with biological effects triggered by IR have serious increases in tissue temperature caused by infrared light bulbs, hot water baths, heated air and even heating coils (Laurent-Applegate & Roques, 2002). This can lead to reports of heat shock protein inductions by IR that are most likely associated with the heat generated rather than with the IR itself (Maytin et al., 1993). Also, temperature increase does not mean that the same biological effect will happen.

Heat can act by (1) increasing tissue temperature which accelerates metabolic reactions non-specifically, or (2) initiating specific responses to heat stimuli that are dependent on the type, intensity, duration and region of the body to which the stimulus is applied (Laurent-Applegate & Roques, 2002), mainly through heat shock proteins.

As heat is one of the properties of IR, IRA can transfer heat to the bloodstream affecting distant tissues from the site of irradiation (Vaupel et al., 1991). IRB and IRC can increase skin temperature (Schieke et al., 2003). wIRA thermal effects result from penetration (20-30 mm) into the tissue and vasodilatation of capillaries, followed by better transport (conduction) of heat into deeper layers (Fuchs et al., 2004) (Rendell et al., 1997).

3.1 Heat as an IR measurement

Within heat being a dominant property of IR some authors attempted to use heat as a biological measure for IR, proposing the Minimal Response Dose (MRD) (Pujol and Lecha, 1992) and the Minimal Heating Dose (MHD) (Lee et al., 2006 A). MRD use the time needed for a heat erythema to appear and MHD use the irradiation dose needed to obtain a stable skin temperature, when it ceases to increase.

As both measures leads to tissue or body temperature increase, we consider that they create misunderstanding when one tries to determine the source of biological response: heat or non-thermic IR.

3.2 Heat treatments

Early experiments with heat, determined three types of heat treatments:

Diathermy and photocoagulation (>65°C) with destructive effects caused by a direct cytotoxic effect with an ischemic effect (Davidorf et al., 1970), thermotherapy (45–65°C) with an irreversible cytotoxic effect (Weenink et al., 1998) (Journée-de-Korver et al., 1995) and hyperthermia.

Hyperthermia (42–44°C) temperatures can be reached by infrared light sources and inactivates damage repair proteins (Song et al., 1982), induces UVB-resistance (murine keratinocytes) (Maytin et al., 1993), increases cell body, dendricity, p53 and p21 expressions and tyrosinase (dopaoxidase) activity (melanocytes monolayer) (Nakazawa et al., 1998), slows down DNA repair of UV or x-ray induced damage, further reduce the latent period for UV-induced carcinogenesis in mice as opposed to those exposed to room temperature (Corry et al., 1977) and, used prior or after UVB exposition, reduces UVB-induced erythema (Park et al., 1984). Most of these effects are mediate through heat shock proteins and can be obtained with different heat sources, not only IR sources.

4. Conflicting results

As heat an IR have been used as one and the same, not all results in literature are similar. In some ones, it is difficult to say if biological responses are a result of IR irradiation or of heating, since many researches did not measure the temperature of *in vivo* or *in vitro* experiments. Below there are some results that need clarifying into this heat issue.

4.1 Photoaging and photorejuvenation

Photoaging is the premature aging of skin by chronic photodamage. It is characterized by wrinkling, pigmentary changes, solar elastosis (Warren et al, 1991), decreased procollagen synthesis, connective tissue damage and matrix metalloproteinase (MMP) overexpression (Shin et al 2012).

Photorejuvenation uses light sources to reverse or repair sun-induced aging or skin damage (Weiss et al, 2003 a), generally by thermal injury (heating the dermis or blood vessels) (Fatemi et al, 2002). IR wavelengths used in photorejuvenation include 1,064, 1,320, 1,450, and 1,540 nm (Weiss et al, 2003 b).

Chronic IRA lamp treatment produced wrinkling and augment UV-wrinkle formation (hairless mice) but there was no heat measurement in the experiment (Kim et al 2005), whereas a heat-controlled (< 37° C skin temperature) chronic IRA lamp treatment improved skin condition, reducing wrinkles by increasing collagen and elastin contents from stimulated fibroblasts (Lee et al, 2006 b).

Also, mild heat shock chronic treatment (hyperthermic) provided anti-aging benefits such as increased collagen production (human fibroblasts) (Mayes and Holyoak, 2008).

4.2 MMP expression

Since MMPs are involved in photoaging and photorejuvenation, which have conflicting results, it is interesting to analyze the effects of heat and IR into MMP expression.

MMPs and TIMPs (tissue inhibitors of MMPs) are part of a coordinated network. Increased MMP activity results in skin aging, rheumatic diseases, hepatic cirrhosis, tumor invasion, metastasis (Westermarck and Kahari, 1999), degradation of collagen and elastic fibers (Seo et al 2006).

MMP-1 expression was increased both by heat (human dermis in vivo) (Seo et al, 2006) (HaCat cells) (Shin et al., 2008) (cultured dermal human fibroblasts) (Park et al, 2004) and by heat controlled wIRA (cultured human dermal fibroblasts) (Schieke et

al, 2002). But IRA LED was showed to downregulated MMP-1 (fibroblasts cultures, skin biopsies) (McDaniel et al, 2002).

MMP-1 degrades collagen-1, -3 and elastic fibers. It plays a key role in human skin photoaging (Rittie et al., 2006) but also accelerates wound remodeling with IR (Barolet, 2008). wIRA MMP-1 increase seems to be mediated by oxidative stress response (Schroeder et al 2008).

MMP-3 expression was increased by heat (cultured dermal human fibroblasts) (Park et al, 2004) and by IR (hairless mice skin) but there was no measurement of skin temperature (Kim et al, 2005).

MMP- 13 expression was increased by heat (hairless mice skin) (Shin et al 2012) and by IR (hairless mice skin) but there was no measurement of skin temperature (Kim et al, 2005).

Heat also overexpressed MMP-9 (human dermis in vivo) (Seo et al, 2006), (HaCat cells) (Shin et al., 2008) and MMP-12 (human skin in vivo) (Chen et al, 2005).

IRA increased MMP-2 expression (Danno et al.; 2001) and MMP-10 deletion (Morita et al. 2006).

4.3 Collagen expression

As MMP-1 (collagenase) can be regulated both by heat and IR, it is interesting to review heat and IR effects in the collagen expression.

A single dose of IR increased procollagen-1 expression within 24 h, while repeated exposure to IR decreases procollagen-1 expression (human skin), but authors used MHD as their IR parameter Kim et al, (2006) which can reach hyperthermic values, while heat from natural sunlight did not alter the expression of procollagen and IR and visible light from natural sunlight decreased procollagen-1 expression (human skin in vivo) (Cho et al, 2008).

Also IR LED and NIR LED irradiation upregulated collagen expression (McDaniel et al, 2002; Morita et al. 2006) and IRC improved collagen regeneration (Toyokawa et al. 2003), all within controlled temperature experiments.

5. Explaining conflicting results

5.1 HSP (Heat Shock Protein)

One possible explanation to the conflicting results found in photoaging, photorejuvenation, MMP and collagen experiments is due to HSP induction. HSPs are produced when cells are under stress, such as heat shock (Welch, 1993). Without appropriate heat control, many effects that are said to be IR effects can originate from HSP.

Heat shock induces MMP-1 expression, calcium influx (human keratinocytes) (Li et al., 2007), upregulates procollagen type I and III (human dermal fibroblasts) (Dams et al., 2010), causes oxidative DNA damage, which (Shin et al., 2008) induces expression of MMP-1 and MMP-3 but not MMP-2, (cultured human fibroblasts) (Park et al, 2004). All of these results are in one way or another found in IR experiments that *in vivo* or *in vitro* temperature rose above 38°C.

Although, IR induces molecular vibrations that may increase cell temperature and might cause a heat shock response, heat controlled experiments are able to avoid this response, as IRA irradiation does not induces Hsp70 expression (Schieke et al, 2002).

6. Heat adverse effects

Apart from leading to HSP expression and interfering with non-thermic IR effects, there are a couple of adverse effects derived from heat. These adverse effects can be present in IR chronic experiments since some are caused by chronic low heat exposure.

Adverse effects of heat were reviewed (Kibbi & Tannous, 1998) and can be summarized as acute and chronic effects.

Acute adverse effects are: increasing the minimum UVA phototoxic dose (Juhlin et al, 1983), erythema and DNA damage (Dewey et al, 1977).

Chronic adverse effects are: heat induced urticaria, cholinergic urticaria, erythemalgia, giant cell arteritis, mudi-chood, acne mechanica, polymyalgia rheumatic (Kibbi & Tannous, 1998); solar elastosis (Warren et al.; 1991), and erythema ab igne (EAI) (Markandeya & Shenoi, 2000), cutaneous changes similar to chronically sun exposed skin (Benedetto, 1998), thermal photoaging (Seo & Ching 2006) and cancer (Schieke et al 2003) (Boukamp et al, 1999).

Besides, some IR sources, mostly the ones that produce IR from a hot body, can produce burns *in vitro* and *in vivo*. Ventral abdominal skin of rats exposed constantly to water (35ºC, 7 days) did not suffer first-degree burns, but it suffered first-degree burns at 5 days of constant exposure of water at 36° C. Also, 3 min of exposure to 60° C water caused deep third-degree burns (Suzuki et al, 1991). As pigs and humans have thicker skin than rats 6 hours of constant exposure to water at 44°C was the lowest temperature resulting in second-degree burns (Moritz & Henriques, 1947).

7. IR sources and avoiding heat

There are three common IR sources today: Light bulbs, LEDs and lasers. Each has different characteristics.

7. 1 Light bulbs

Light bulbs are the oldest IR source between those three. They emit noncoherent light within an ample spectrum with peak wavelength around IRA. Generally, light bulbs emit IR with contamination of red wavelengths. This contamination can be avoided with the use of specific filters.

IR light bulbs are broadly used within heat treatments since radiant heat has advantages for local warming compared with other heat sources, such as heating bandage systems or hot packs whose heat is absorbed in the epidermal layers and may cause burns of the skin (Toyokawa,et al. 2003).

As it is, the major problem when using light bulbs is the heat generation, since discussed into the Dose section, some time and irradiance are needed to achieve

biological response. As often, the time needed to achieve a specific dose is more than enough to increase tissue or cell temperature above hyperthermic values.

7.2 Lasers

Laser (Light Amplification by Stimulated Emission of Radiation) devices emit coherent light within a specific wavelength ranging from 157 to 27,000 nm. Radiation can be emitted in continuous or in short pulse waves. IR lasers penetrate the skin and can damage basal keratinocytes, melanocytes and blood vessels (Parrish et al, 1983).

The major problem of lasers is that the beam of light produces heat that can damage tissues (Eells et al, 2004). This "problem" is used in photothermolysis.

Specific wavelengths and pulse durations subjects the target structure to a thermal effect with as little damage as possible to surrounding tissues. Chromophores absorb the energy, transforming it into heat, sufficient to alter the physical properties of the tissue (Handley, 2006).

Lasers can be used in non-thermic phototherapy, generally in the IR spectrum. It is effective in the treatment of impaired microcirculation, delayed wound healing, pain syndromes (Schindl et al., 2000).

7.3 LEDs

LEDs (light-emitting diodes) emit noncoherent light in an ample array of wavelengths. They are safe, nonthermic, nontoxic and noninvasive, and to date, no side effects have been reported in published literature (Barolet, 2008). NASA developed LEDs as an effective alternative to lasers. These diodes can be arranged in large, flat arrays and produce no heat. They convert electrical current into incoherent narrow spectrum light, emitting light in wavelengths varying from 247 to 1300 nm (Barolet, 2008).

LEDs and lasers have significant differences. LEDs provide a gentler delivery of the same wavelengths of light compared to lasers and at a substantially lower energy output (milliwats instead of watts). LEDs do not deliver enough power to damage

tissues and do not have the same risk of accidental eye damage that lasers do. It is possible to combine wavelengths with an array of various sizes and LEDs can be used within large areas (Barolet, 2008).

LED photomodulation is a novel category of nonthermic light-based treatment designed to regulate the activity of cells rather than to invoke thermal wound healing mechanisms (McDaniel et al, 2002). It has an effect on human skin that is nonthermic increasing cellular metabolic activity by targeted cells, such as increased collagen synthesis by fibroblasts (McDaniel et al, 2002) (Weiss, 2003 b).

From the three IR sources, LEDs should be strongly considered by researchers when trying to distinguish IR effects from heat, since the only heat produced would be the vibrational state induced by the IR irradiation. Because of their small size, LEDs can be arranged in a variety of displays that light bulbs and lasers cannot and it is easier to avoid red wavelengths contamination.

8. Conclusion

Heat and IR are generally described as one and the same. But, recently LED experiments lead to IR effects that are distinct from heat. Also, IR experiments without carefully control of the subject temperature in most cases induce HSP responses that can be achieved by heat but not by IR.

We showed that IR experiments must avoid hyperthermic or higher temperatures and that even heat increases above 38°C can trigger HSP expression and its subsequent effects. Also, IR chronic experiments can produce chronic adverse heat effects if not properly conducted.

Finally, we believe that using LEDs or at least wIRA can minimize or negate the effects of heat, and provide researchers with a better understanding of nonthermic IR effects.

9. References

Barolet, D. 2008. Light-emitting diodes (LEDs) in dermatology. Semin Cutan Med Surg 27:227-238

Beauvoit, B., Kitai, T., Chance, B., 1994. Correlation between the light scattering and the mitochondrial content of normal tissues and transplantable rodent tumors. Biophys. J. 67, 2501–2510

Benedetto AV. The environment and skin aging. Clin Dermatol 1998;16:129—39.

Boukamp P, Popp S, Bleuel K, Tomakidi E, Burkle A, Fusenig NE. Tumorigenic conversion of immortal human skin keratinocytes (HaCaT) by elevated temperature. Oncogene 1999; 18: 5638–5645.

Chen, Z., Seo, J.Y., Kim, Y.K., Lee, S.R., Kim, K.H., Cho, K.H., Eun, H.C., Chung, J.H., 2005. Heat modulation of tropoelastin, fibrillin-1, and matrix metalloproteinase-12 in human skin in vivo. J. Invest. Dermatol. 124, 70–78

Cho,S.; Lee, M.J.;Kim, M.S.; Lee, S.; Kim,Y.K.; Lee, D.H.;Lee, C.W., Cho, K.H.; Chung, J.H.; 2008. Infrared plus visible light and heat from natural sunlight participate in the expression of MMPs and type I procollagen as well as infiltration of inflammatory cell in human skin in vivo. Journal of Dermatological Science 50: 123—133

Corry PM, Robinson S, Getz S. Hyperthermic effects on DNA repair mechanisms. Radiology 1977;123:475-8.

Dams, S.D., De Liefde-van Beest, M., Nuijs, A.M., Oomens, C.W.J., Baaijens, F.P.T., 2010. Pulsed heat shocks enhance procollagen type I and procollagen type III expression in human dermal fibroblasts. Skin Res. Technol. 16, 354–364.

Danno, K.; Mori, N.; Toda, K-I.; Kobayashi, T.; Utani, A. 2001: Near-infrared irradiation stimulates cutaneous wound repair: laboratory experiments on possible mechanisms. Photodermatol. Photoimmunol. Photomed. 17: 261-265;

Davidorf, F.H., Newman, G.H., Havener, W.H., Makley, T., 1970. Conservative management of malignant melanoma. II. Transscleral diathermy as a method of treatment for malignant melanomas of the choroid. Arch. Ophthalmol. 83, 273–280.

Dewey,W.C.; Hepwood, L.E.; Sparcio,S.A. Cellular responses to combination of hyperthermia and radiation. Radiology 123:463-474, 1977

Dover JS, Phillips TJ, Arndt KA. Cutaneous effects and therapeutic uses of heat with emphasis on infrared radiation. J Am Acad Dermatol 1989;20:278—86.

Eells, J.T.; Wong-Riley, M.T.T.; Verhoeve, J..; Henryd, M.; Buchman, E.V.; Kane, M.P.; Gould,L.J.; Das,R.; Jett, M.; Hodgson, B.D.; Margolis, D.; Whelan, H.T.; 2004. Mitochondrial signal transduction in accelerated wound and retinal healing by near infrared light therapy. Mitochondrion 4:559-567

Fatemi A, Weiss MA, Weiss RA. Short-term histologic effects of nonablative resurfacing: results with a dynamically cooled millisecond domain 1320 nm Nd:YAG laser. Dermatol Surg 2002;28:172–6.

Fuchs SM, Fluhr JW, Bankova L, Tittelbach J, Hoffmann G, Elsner P. Photodynamic therapy (PDT) and waterfiltered infrared A (wIRA) in patients with recalcitrant common hand and foot warts. German Med Sci 2004; 2: Doc08.

Handley, J.M. Adverse events associated with nonablative cutaneous visible and infrared laser treatment. J Am Acad Dermatol 2006; 55:485-9

Journée-De Korver Jg, Oosterhuis Ja, Vrensen Gfjm.1995. Light and electron microscopic findings on experimental melanomas after hyperthermia at 50 °C. Melanoma Res 5: 393–402.

Juhlin L, Civier A, Shroot S, Hensby, C. 1983. Effect of infrared radiation on the recoverable levels of free arachidonic acid and prostaglandins in human forearm skin. J Invest Dermatol 81:297-300.

Karu TI, Pyatibrat LV, Kalendo GS: Photobiological modulation of cell attachment via cytochrome c oxidase. Photochem Photobiol Sci 3:211- 216, 2004

Kibbi, A.G. & Tannous, Z. Skin Diseases Caused by Heat and Cold Clinics in Dermatology. 1998;16:91-98

Kim HH, Lee MJ, Lee SR, Kim KH, Cho KH, Eun HC, et al. Augmentation of UV-induced skin wrinkling by infrared irradiation in hairless mice. Mech Ageing Dev 2005;126:1170—7

Kim MS, Kim YK, Cho KH, Chung JH. Regulation of type I procollagen and MMP-1 expression after single or repeated exposure to infrared radiation in human skin. Mech Ageing Dev 2006;127:875—82.

Kochevar, I.E.; Pathak, M.A.; Parrish, J.A. Photophysics, photochemistry, and photobiology. In: Freedberg IM, Eisen AZ, Wolff K, et al., eds. Fitzpatrick's Dermatology in General Medicine. New York: McGraw-Hill, 1999; 220–229. Apud: Schiekel, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. Photodermatol. Photoimmunol. Photomed. 19: 228-234;

Lanzafame RJ, Stadler I, Kurtz AF, et al: Reciprocity of exposure time and irradiance on energy density during photoradiation on wound healing in a murine pressure ulcer model. Lasers Surg Med 39:534- 542, 2007

Laurent-Applegate, L. & Roques, S. 2002. CHAPTER 16 Biological Actions of Infrared Radiation, in Sensing, Signaling and Cell Adaptation. Edited by K.B. Storey and J.M. Storey

Lee, H.S.; Lee, D.H.; Cho, S.; Chung, J.H. 2006 A. Minimal heating dose: a novel biological unit to measure infrared irradiation Photodermatol Photoimmunol Photomed 22: 148–152

Lee, J.H.; Roh, M.R.; Lee, K.H. 2006 B. Effects of infrared radiation on skin photo-aging and pigmentation. Yonsei Med J 47 (4):485-490

Li, W.H., Lee, Y.M., Kim, J.Y., Kang, S., Kim, S., Kim, K.H., Park, C.H., Chung, J.H., 2007. Transient receptor potential vanilloid-1 mediates heat-shock-induced matrix metalloproteinase-1 expression in human epidermal keratinocytes. J. Invest. Dermatol. 127, 2328–2335.

Maeda, Y.; Ide, M.; Kitano, H. 1999. Vibrational spectroscopic study on the structure of water in polymer systems. J. Mol. Liq. 80: 149–163;

Markandeya, N., Shenoi, S.D. Hyperpigmentation in a welder. Contact Dermatitis 2000; 43 : 361

Mayes, A.E., Holyoak, C.D., 2008. Repeat mild heat shock increases dermal fibroblast activity and collagen production. Rejuvenation Res. 11, 461–465.

Maytin, E.V.; Murphy, L.A.; Merrill, M.A. 1993. Hyperthermia Induces Resistance to Ultraviolet Light B in Primary and Immortalized Epidermal Keratinocytes Cancer Res 1993;53:4952-4959.

McDaniel, D.H.; Weiss, R.A.; Geronemus, R. Ginn, L.; Newman, J. 2002. Light-tissue interactions I: photothermolysis vs photomudlation laboratory findings. Lasers Surg Med 14:25

Morita, S.; Narita, M.; Kitatani, N,; Terauchi, T. 2006. Effect of light-emitting diode irradiation on skin. J. Dermatol. Science, 42: 133-198;

Moritz, A.R., Henriques, F.C. Jr. Studies in thermal injury. II. The relative importance of time and surface temperature in the causation of cutaneous burns. American Journal of Pathology 1947; 23: 695–720

Nakazawa, K.; Sahuc, F.; Damour, O.; Collombel, C.; Nakazawa, H. Regulatory Effects of Heat on Normal Human Melanocyte Growth and Melanogenesis: Comparative Study with UVB. J Invest Dermatol 110:972–977, 1998

Park, HJ, Youn,JL, Lee, YS, The influence of infrared radiation on ultraviolet induced skin injury.Korean J Dermatol 22:176-182, 1984

Park CH, Lee MJ, Ahn J, Kim S, Kim HH, Kim KH, et al. Heat shock-induced matrix metalloproteinase (MMP)-1 and MMP-3 are mediated through ERK and JNK activation and via an autocrine interleukin-6 loop. J Invest Dermatol 2004;123: 1012—9.

Parrish, J.A., Anderson, R.R., Harrist, T. *et al.* Selective thermal effects with pulsed irradiation from lasers: from organ to organelle. *Journal of Investigative Dermatology* 1983; **80**: 75s–80s.

Piazena H, Meffert H. Dosis-Wirkungs-Beziehungen bei Infrarot- Bestrahlung der Haut und die Extrapolation der Daten von in vitro-Messungen auf die Bedingungen in vivo - Erfordernisse und Fehler [Dosis related effects during infrared irradiation of the skin and the extrapolation of the data from in vitro measurements to in vivo conditions - requirements and errors]. Lecture presented at the symposium entitled "Water-filtered infrared-A (wIRA) in dermatology" of the Dr. med. h.c. Erwin

Braun Foundation in Liestal/Basel, Switzerland, November 20, 2004. Apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Piazena H, Meffert H. Therapie mit Infrarotstrahlen – Physikalische Grundlagen und Anwendung in der Sauna und in Infrarotgeräten. In: Bühring M, Kemper FH, Hrsg. Naturheilverfahren [Loseblattsammlung]. Berlin, Heidelberg, New York: Springer; 2002. Bd. 1, 03.09. S.1-22 apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Pogue BW, Lilge L, Patterson MS, et al: Absorbed photodynamic dose from pulsed versus continuous wave light examined with tissue-simulating dosimeters. Appl Opt 36:7257-7269, 1997

Pujol JA, Lecha M. Photoprotection in the infrared radiation range. Photodermatol Photoimmunol Photomed 1992; 9: 275–278.

Rendell MS, Milliken BK, Finnegan MF, Finney DA, Healy JC. The skin blood flow response in wound healing. Microvasc Res 1997; 53: 222–234.

Rittie L, Fisher GJ, Voorhees JJ (2006) Retenoid therapy for photoaging. In: Skin Aging (Gilchrest BA, Krutmann J, eds), New York: Springer, 143–56

Schieke S, Stege H, Kurten V, Grether-Beck S, Sies H, Krutmann J. Infrared-A radiation-induced matrix metalloproteinase 1 expression is mediated through extracellular signalregulated kinase 1/2 activation in human dermal fibroblasts. J Invest Dermatol 2002;119:1323—9

Schieke, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. Photodermatol. Photoimmunol. Photomed. 19: 228-234

Schindl, A.; Schindl, M.; Pernerstorfer-Schön, H.; Schindl, L. 2000. Low intensity laser therapy: A review. *J. Invest. Med.* **48,** 312–326.

Schroeder P, Pohl C, Calles C, Marks C, Wild S, Krutmann J (2007) Cellular response to infrared radiation involves retrograde mitochondrial signaling. Free Radic Biol Med 43:128–35

Schroeder,P.; Lademann, J.; Darvin,M.E.; Stege,H.; Marks, C.; Bruhnke, S.; Krutmann,J. 2008. Infrared radiation-induced matrix metalloproteinase in human skin: implications for protection. Journal of Investigative Dermatology 128: 2491-2497

Seo, J.Y. & Ching, J.H. 2006.Thermal aging: A new concept of skin aging. Journal of Dermatological Science Supplement. 2: S13—S22.

Shin, M. H., Seo, J.E.; Kim, y.K.; Kim, K.H.; Chung, J.H. 2012. Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mechanisms of Ageing and Development 133: 92–98

Shin, M.H., Moon, Y.J., Seo, J.E., Lee, Y., Kim, K.H., Chung, J.H., 2008. Reactive oxygen species produced by NADPH oxidase, xanthine oxidase, and mitochondrial electron transport system mediate heat shock-induced MMP-1 and MMP-9 expression. Free Radic. Biol. Med. 44, 635–645.

Song, C.W., Rhee, J.G., Levitt, S.H., 1982. Effect of hyperthermia on hypoxic cell fraction in tumor. Int. J. Radiat. Oncol. Biol. Phys. 8, 851–856

Suzuki, T., Hirayama, T., Aihara, K., Hirohata, Y. Experimental studies of moderate temperature burns. Burns. 1991; 17: 443–51.

Toyokawa, H.; Matsui, Y.; Uhara, J.; Tsuchiya,H.; Teshima, S.; Nakanishi, H.; Kwon, A.; Azuma, Y.; Nagaoka, T.; Ogawa, T.; Kamiyama, Y. 2003. Promotive effects of far-infrared ray on full-thickness skin wound healing in rats. Exp Biol Med. 228: 724–729;

Vaupel, P., Stohrer, M., Groebe, K. and Rzeznik, J. 1991. Localized hyperthermia in superficial tumors using water- filtered infrared-A-radiation: evaluation of temperature distribution and tissue oxygenation in subcutaneous rat tumors. Strahlenther Onkol. 167, 353-354.

Walther, M.; Plochocka, P.; Fischer, B.; Helm, H.; Jepsen, P. U. 2002. Collective vibrational modes in biological molecules investigated by terahertz time-domain spectroscopy. Biopolymers 67, 310–313;

Warren R, Gartstein V, KligmanAM,MontagnaW, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. J Am Acad Dermatol 1991;25:751—60.

Weenink, A.C., van Best, J.A., Oosterhuis, J.A., Keunen, J.E.E., 1998. Lens transmission by fluorophotometry after brachytherapy and thermotherapy of choroidal melanoma. Ophthalmic Res. 30, 402–406.

Weiss RA, McDaniel DH, Geronemus RG. 2003 A. Review of nonablative photorejuvenation: reversal of the aging effects of the sun and environmental damage using laser and light sources. Semin Cutan Med Surg 2003;22:93–106

Weiss RA, Weiss MA, McDaniel DH, et al. 2003 B. Comparison of nonablative fibroblast photoactivation with and without application of topical cosmeceutical agents. Lasers Surg Med 2003;15:23.

Welch, W. How cells respond to stress. Scientific American May:34-41, 1993.

Westermarck, J., Kahari, V.M., 1999. Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J. 13, 781–792.

4. Considerações Finais e Recomendações

Os resultados desta Tese contribuem para o melhor entendimento dos efeitos da exposição crônica com a radiação infravermelha e a radiação ultravioleta, assim como da interação entre as duas. Estes resultados estabelecem que a exposição crônica à radiação infravermelha pode resultar em alterações epiteliais a longo prazo e em algumas situações até ampliar as lesões ocasionadas pela radiação ultravioleta B.

A importância deste fato, se amplia pelo Brasil ser um país tropical de vasta extensão territorial abrangendo regiões onde a camada de ozônio por vezes fica rarefeita, regiões de altos índices de UVB, assim como uma alta taxa de radiação infravermelha anual. Ressaltando-se também a importância de evitar exposição à radiação UVB entre os horários das 10 horas da manhã e as 16 horas da tarde. Como a exposição inicial à radiação infravermelha reduziu algumas das lesões ocasionadas pela radiação ultravioleta, deve-se destacar a prevenção ocasionada pelos primeiros raios de Sol em relação ao UVB.

Dependendo do estado das células do ser vivo exposto, à exposição ao infravermelho antes da exposição ao ultravioleta B pode auxiliar na sobrevivência das células epiteliais reduzindo o número de células apoptóticas e necróticas. Contudo, como não houve indução do gene p53, estes resultados podem indicar uma permanência de células lesionados pelo UVB que não foram reparadas pelo organismo. Mais estudos são necessários para esclarecer se a radiação infravermelha apresenta papel na carcinogênese epitelial.

Por fim, a Tese ressalta a importância de que os experimentos com radiação infravermelha sejam realizados sem a presença de calor, indicando a utilização de LEDs de infravermelho para uma melhor obtenção de resultados confiáveis.

5. Literatura Citada

Aufiero, B.M.; Talwar, H.; Young, C.; Krishnan, M.; Hatfiedl, J.S.; Lee, H.K.; Wong, H.K.; Hamzavi, I.; Murakawa, G.J. Journal of Photochemistry and Photobiology B: Biology 82 (2006) 132–139.

Bachelor, M. A., Bowden, G. T. 2004. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. Seminars in Cancer Biology. 14: 131-138,

Barolet, D. 2008. Light-emitting diodes (LEDs) in dermatology. Semin Cutan Med Surg 27:227-238

Beauvoit, B.; Kitai, T.; Chance, B. 1994. Contribution of the mitochondrial compartment to the optical properties of the rat liver: a theoretical and practical approach. Biophys. J. 67(6): 2501-2510;

Berneburg, M., Plettenberg,H.; Krutmann, J. Photodermatol Photoimmunol Photomed 2000; 16: 239–244.

Brenneisen P, Sies H, Scharffetter-Kochanek K (2002) Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. Ann N Y Acad Sci 973:31–43

Chapman, R.S.; Cooper, K.D.; Fabo, E.C.; Frederick, J.E.; Gelatt, K.N.; Hammond, S.P.; Hersey, P.; Koren, H.S.; Ley, R.D.; Noonan, F.; Rusch, G.M.; Sarasin, A.; Selgrade, M.J.; Sobolev, I.; Ward, B.J. e Werkema, T.E. 1995. Solar Ultraviolet Radiation and the risk of infectious disease: sumary of a workshop. Photochem. Photobiol. 61(3): 223-247;

Continenza, M.A.; Ricciardi, G.; Franchitto, A. 1993. Effects of low power 904 nm radiation on rat fibroblasts explanted and in vitro cultured. J. Photochem. Photobiol. B: Biol. 19(3): 231-234;

Danno, K.; Mori, N.; Toda, K-I.; Kobayashi, T.; Utani, A. 2001: Near-infrared irradiation stimulates cutaneous wound repair: laboratory experiments on possible mechanisms. Photodermatol. Photoimmunol. Photomed. 17: 261-265;

Eells, J.T.; Wong-Riley, M.T.T.; Verhoeve, J..; Henryd, M.; Buchman, E.V.; Kane, M.P.; Gould,L.J.; Das,R.; Jett, M.; Hodgson, B.D.; Margolis, D.; Whelan, H.T.; 2004. Mitochondrial signal transduction in accelerated wound and retinal healing by near infrared light therapy. Mitochondrion 4:559-567

Frank, S.; Menezes, S.; Lebreton-De Coster, C.; Oster, M.; Dubertret, L.; Coulomb, B. 2006. Infrared radiation induces the p53 signaling pathway role in infrared prevention of ultraviolet B toxicity. *Experimental Dermatology* 15(2): 130-137;

Fuchs SM, Fluhr JW, Bankova L, Tittelbach J, Hoffmann G, Elsner P. Photodynamic therapy (PDT) and waterfiltered infrared A (wIRA) in patients with recalcitrant common hand and foot warts. German Med Sci 2004; 2: Doc08.

Gouveia, G.R; Lopes, T.M.; Neves, C.A.; Nery, L.E.M.; Trindade, G.S. 2004. Ultraviolet radiation induces dose-dependent pigment dispersion in crustacean chromatophores. Pigment Cell Res 17: 545-548;

Gouveia, G.R.; Marques, D.S.; Cruz, B.P.; Geracitano, L.A.; Nery, L.E.M.; Trindade, G.S. 2005. Antioxidant defenses and DNA damage induced by UV-A and UV-B radiation in the crab chasmagnathus granulata (Decapoda, Brachyura). Photochem. Photobiol. 81: 398-403;

Jantschitsch C, Majewski S, Maeda A., Schwarz,T., Schwarz, A. 2009. Infrared radiation confers resistance to UV-induced apoptosis via reduction of DNA damage and upregulation of antiapoptotic proteins. J Invest Dermatol 2009:129:1271–1279.

Jantschitsch,C.; Weichenthal, M.; Maeda, A.; Proksch, E.; Schwarz, T.; Schwarz, A. 2011. Infrared radiation does not enhance the frequency of ultraviolet radiation-induced skin tumors, but their growth behavior in mice. Experimental Dermatology 20, 346-350

Karu, T. 1999. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B. Biol. 49(1): 1-17;

Karu, T.I.; Pyatibrat, L.V.; Afanasyeva, N.I. 2004. A novel mitochondrial signaling pathway activated by visible-to-near infrared radiation. Photochemistry and photobiology 80:366-372,

Kerr, J.B. e McElroy, C.T. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science 262: 1032-1034;

Kibbi, A.G. & Tannous, Z. Skin Diseases Caused by Heat and Cold Clinics in Dermatology. 1998;16:91-98

Kim HH, Lee MJ, Lee SR, Kim KH, Cho KH, Eun HC, et al. Augmentation of UV-induced skin wrinkling by infrared irradiation in hairless mice. Mech Ageing Dev 2005;126:1170—7

Kim MS, Kim YK, Cho KH, Chung JH. Regulation of type I procollagen and MMP-1 expression after single or repeated exposure to infrared radiation in human skin. Mech Ageing Dev 2006;127:875—82.

Kullavanijaya, P.; Lim, H. W. 2005. Photoprotection. J. Am. Acad. Dermat. 52: 937-958;

Laurent-Applegate, L. & Roques, S. 2002. CHAPTER 16 Biological Actions of Infrared Radiation, in Sensing, Signaling and Cell Adaptation. Edited by K.B. Storey and J.M. Storey

Lee, H.S.; Lee, D.H.; Cho, S.; Chung, J.H. 2006. Minimal heating dose: a novel biological unit to measure infrared irradiation Photodermatol Photoimmunol Photomed 22: 148–152

Maeda, Y.; Ide, M.; Kitano, H. 1999. Vibrational spectroscopic study on the structure of water in polymer systems. *J. Mol. Liq.* 80: 149–163;

Manney, G.L.; Froidevaux, L.; Waters, J.W.; Zurek, R.W.; Read, W.G.; Elson, L.S.; Kumer, J.B.; Mergenthaler, J.L.; Roche, A.E.; O'Niell, A.; Harwood, R.S.; MacKenzie, I. e Swinbank, R. 1994. Chemical depletion of ozone in the Arctic lower stratosphere during winter 1992-93. Nature 370: 429-434;

Maytin, E.V.; Murphy, L.A.; Merrill, M.A. 1993. Hyperthermia Induces Resistance to Ultraviolet Light B in Primary and Immortalized Epidermal Keratinocytes Cancer Res 1993;53:4952-4959.

Menezes, S.; Coulomb, B.; Lebreton, C. and Dubertret, L. Non-coherent near infra-red radiation protects normal human dermal fibroblasts from the solar ultraviolet toxicity. J. Invest Dermatol 111:629-633, 1998

Morita, S.; Narita, M.; Kitatani, N,; Terauchi, T. 2006. Effect of light-emitting diode irradiation on skin. J. Dermatol. *Science*, 42: 133-198;

Möckel, F.; Hoffman,G.; Obermüller, R.; Drobnik, W.; Schmitz,G.2006. Influence of water-filtered infrared-A (wIRA) on reduction of local fat and body weight by physical exercise. German Medical Science 4,Doc05

Piazena H, Meffert H. Dosis-Wirkungs-Beziehungen bei Infrarot- Bestrahlung der Haut und die Extrapolation der Daten von in vitro-Messungen auf die Bedingungen in vivo - Erfordernisse und Fehler [Dosis related effects during infrared irradiation of the skin and the extrapolation of the data from in vitro measurements to in vivo conditions - requirements and errors]. Lecture presented at the symposium entitled "Water-filtered infrared-A (wIRA) in dermatology" of the Dr. med. h.c. Erwin Braun Foundation in Liestal/Basel, Switzerland, November 20, 2004. Apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Piazena H, Meffert H. Therapie mit Infrarotstrahlen – Physikalische Grundlagen und Anwendung in der Sauna und in Infrarotgeräten. In: Bühring M, Kemper FH, Hrsg. Naturheilverfahren [Loseblattsammlung]. Berlin, Heidelberg, New York: Springer; 2002. Bd. 1, 03.09. S.1-22 apud Gebbers,N.; Hirt-Burri, N.; Scaletta,C.; Hoffman,G.; Applegate, L.A. Water-filtered Infrared-A radiation (wIRA) is not implicated in cellular degeneration of human skin. 2007. German Medical Science (GMS) 5, Doc8

Pujol JA, Lecha M. Photoprotection in the infrared radiation range. Photodermatol Photoimmunol Photomed 1992; 9: 275–278.

Schieke, S.M.; Schroeder, P.; Krutmann, J. 2003. Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. *Photodermatol. Photoimmunol. Photomed.* 19: 228-234;

Schramm, J.M; Warner, D.; Hardesty, R.A.; Oberg, K.C. 2003. A unique combination of infrared and microwave radiation accelerates wound healing. *Plast. Reconstr. Surg*. 111(1): 258-266;

Schroeder P, Pohl C, Calles C, Marks C, Wild S, Krutmann J (2007) Cellular response to infrared radiation involves retrograde mitochondrial signaling. Free Radic Biol Med 43:128–35

Schroeder,P.; Lademann, J.; Darvin,M.E.; Stege,H.; Marks, C.; Bruhnke, S.; Krutmann,J. 2008. Infrared radiation-induced matrix metalloproteinase in human skin: implications for protection. Journal of Investigative Dermatology 128: 2491-2497

Seo, J.Y. & Ching, J.H. 2006.Thermal aging: A new concept of skin aging. Journal of Dermatological Science Supplement. 2: S13—S22.

Shin, M. H., Seo, J.E.; Kim, y.K.; Kim, K.H.; Chung, J.H. 2012. Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mechanisms of Ageing and Development 133: 92–98

Song, C.W., Rhee, J.G., Levitt, S.H., 1982. Effect of hyperthermia on hypoxic cell fraction in tumor. Int. J. Radiat. Oncol. Biol. Phys. 8, 851–856

Toyokawa, H.; Matsui, Y.; Uhara, J.; Tsuchiya,H.; Teshima, S.; Nakanishi, H.; Kwon, A.; Azuma, Y.; Nagaoka, T.; Ogawa, T.; Kamiyama, Y. 2003. Promotive effects of far-infrared ray on full-thickness skin wound healing in rats. *Exp Biol Med.* 228: 724–729;

Walder, E.Y.J. e Hargis, A.M. 2002. Chronic moderate heat dermatitis (erythema ab igne) in five dogs, three cats and one silvered langur. *Vet. Dermatol*. 13; 283-292;

Walther, M.; Plochocka, P.; Fischer, B.; Helm, H.; Jepsen, P. U. 2002. Collective vibrational modes in biological molecules investigated by terahertz time-domain spectroscopy. *Biopolymers* 67, 310–313;

Weiss RA, McDaniel DH, Geronemus RG. 2003. Review of nonablative photorejuvenation: reversal of the aging effects of the sun and environmental damage using laser and light sources. Semin Cutan Med Surg 2003;22:93–106

Wong-Riley, M.T.T.; Liang, H.L.; Eells, J.T.; Chancel, B.; Henry, M.M.; Buchmann, E.; Kane, M.; Whelan, H.T. 2005. Photobiomodulation Directly Benefits Primary Neurons Functionally Inactivated by Toxins – Role of Cytochrome c Oxidase. *J. Biol. Chem*. 280 (6): 4761-4771;

Yu, S.; Chiu, J.; Yang, S.; Hsu, Y.; Lui, W.; Wu, C. 2006. Biological effect of far-infrared therapy on increasing skin microcirculation in rats. *Photodermatol. Photoimmunol. Photomed.* 22: 78–86;