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MODULAÇÃO DA VIA DAS QUINURENINAS NA LESÃO AURICULAR AGUDA INDUZIDA PELA RADIAÇÃO UVB EM CAMUNDONGOS

DISSERTAÇÃO DE MESTRADO

Manuela Bastos Piêgas

Itaqui, RS, Brasil.

2017

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Dissertação apresentada ao Programa de Pós-Graduação Bioquímica, da Universidade Federal do Pampa (UNIPAMPA, RS), como requisito parcial para a obtenção do grau de Mestre em Bioquímica.

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Área de concentração: Bioquímica Farmacêutica e Toxicológica

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Banca examinadora:

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Prof. Dr. Cristiano Ricardo Jesse (Presidente, orientador)

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Prof[a]. Dr[a]. Vanderlei Folmer (UNIPAMPA)

_____

Prof[a]. Dr[a]. Cristiani Folharini Bortolotto (UFPel)

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“Um excelente educador não é um ser humano perfeito, mas alguém que tem a serenidade para se esvaziar e sensibilidade para aprender.”

Augusto Cury

PARTE I

RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Bioquímica

Universidade Federal do Pampa, RS, Brasil

MODULAÇÃO DA VIA DAS QUINURENINAS NA LESÃO AURICULAR AGUDA INDUZIDA PELA RADIAÇÃO UVB EM CAMUNDONGOS

Autora: Manuela Bastos Piêgas

Orientador: Cristiano Ricardo Jesse

Data e Local da Defesa: Itaqui, 14 de Julho de 2017.

A radiação ultravioleta (UV) B no tecido epitelial estimula a síntese e liberação de mediadores pró-inflamatórios, induzindo à infiltração e ativação de neutrófilos e outras células fagocíticas. Assim, processos inflamatórios causados pela radiação UVB podem ser prejudiciais sobre diversas células do organismo, causando desde fotoenvelhecimento até imunossupressão. O triptofano (TRIP) é um aminoácido essencial, utilizado em inúmeros processos fisiológicos e metabolizado por duas vias principais, a serotoninérgica e a das quinureninas. Sendo esta última relacionada a enzima indoleamina 2,3 dioxigenase (IDO) que poderá ser induzida por processos inflamatórios aumentando o consumo de TRIP e formando metabólitos oxidativos, como o ácido quinolínico que está relacionado no processo de imunotolerância. Devido ao envolvimento da enzima IDO e seu possível alvo terapêutico, o objetivo desse trabalho foi avaliar a produção de metabólitos da via das quinureninas (VQs) em lesões auriculares de camundongos, provocadas pela exposição por 24 horas à radiação UVB frente ao inibidor da enzima IDO 1-metil triptofano (1-MT). Os experimentos foram realizados com 30 animais da linhagem C57B/6J. Após serem divididos em cinco grupos de seis animais cada, denominados: a) controle, sem exposição à radiação UVB; b) veículo, exposto a radiação UVB tratado com salina (10µL); c) expostos a radiação UVB e tratados com inibidor da enzima1-MT (0,5 µg/µl); d) expostos a radiação UVB e tratados com inibidor da enzima 1-MT (1,0 µg/µl) e grupo e) expostos a radiação UVB e tratados com inibidor da enzima 1-MT (2,0µg/µl). O tratamento com 1-MT e o veículo foram realizados topicamente antes da exposição à radiação UVB, e 24h depois da exposição, os camundongos foram eutanasiados e as orelhas totalmente extraídas para realização das dosagens específicas. O presente estudo demonstrou que o tratamento com 1-MT frente à radiação UVB em lesões auriculares de camundongos, foi eficaz em atenuar as seguintes alterações, a espessura e inflamação das orelhas irradiadas, o estresse oxidativo causado pela radiação UVB nas

orelhas dos camundongos. Diminuiu os níveis de fator de necrose tumoral alfa (TNF-α), interleucina 1 beta (IL-1β), interleucina 6 (IL-6), interferon gama (INF-γ), interleucina 13 (IL-13), interleucina 17 (IL-17), interleucina 10 (IL-10) e fator nuclear kappa B (NF-κB) reduzindo os níveis das citocinas inflamatórias. Aumentou os níveis de TRIP no local, diminuindo assim seus metabólitos como quinurenina (QUI), ácido quinurênico (AQUI), 3-Hidroxiquinurenina (3-HQ), ácido 3-hidroxiantranílico (3-AA), ácido antranílico (AA) e ácido quinulínico (AQ). Aumento das atividades das enzimas envolvidas na VQs, entre elas a IDO, quinurenina 3-monoxigenase (QMO), quinurenina aminotransferase (QAT) e quinureninase (QUINU). Em conclusão, esses resultados demonstram que a inibição da enzima IDO causou atenuação da inflamação, do estresse oxidativo e dos metabólitos da VQs. Além disso, sugerem que a inibição dessa enzima, seja com produtos sintéticos ou naturais podem fornecer uma nova abordagem terapêutica para o tratamento e prevenção de lesões causadas pela radiação UVB na pele.

Palavras-chave: radiação ultravioleta B; estresse oxidativo; via das quinureninas; inflamação; inibidor.

ABSTRACT

Dissertation of Master
Program of Post-Graduation in Biochemistry
Federal University of Pampa

MODULATION OF THE KYNURENINE PATHWAY IN ACUTE EAR LESION INDUCED BY UVB RADIATION IN MICE

Author: Manuela Bastos Piêgas
Advisor: Cristiano Ricardo Jesse
Site and Date of Defence: Itaqui, July 14, 2017.

Ultraviolet (UV) B radiation in the epithelial tissue stimulates the synthesis and release of pro-inflammatory mediators, inducing the infiltration and activation of neutrophils and other phagocytic cells. Thus, inflammatory processes caused by UVB radiation can be harmful to various cells in the body, from photoaging to immonussuppression. Tryptophan (TRYP) is an essential amino acid, used in numerous physiological processes and metabolized by two main pathways, serotoninergic and kynurenines. The latter is related to the indoleamine 2,3 dioxygenase (IDO) enzyme that can be induced by inflammatory processes increasing the consumption of TRYP and forming oxidative metabolites, such as the quinolinic acid that is related in the process of immunotolerance. Due to the involvement of the IDO enzyme and its possible therapeutic target, the objective of this work was to evaluate the production of metabolites of the kinurenins pathway (KPs) in mice ear lesions caused by 24-hour UVB exposure to the IDO enzyme inhibitor 1-methyl tryptophan (1-MT). The experiments were performed with 30 animals of the C57B / 6J lineage. After being divided into five groups of six animals called: a) control, without exposure to UVB radiation; B) vehicle, exposed to UVB radiation treated with saline (10μL); C) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (0,5 μg/μl); D) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (1,0 μg/μl) and group e) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (2,0 μg/μl). Treatment with 1-MT and the vehicle were performed topically prior to exposure to UVB radiation, and 24 hours after exposure, the mice were euthanized and the ears fully extracted for specific dosages. The present study demonstrated that 1-MT

treatment against UVB radiation in mouse ear lesions was effective in attenuating the following alterations, the thickness and inflammation of the irradiated ears, the oxidative stress caused by UVB radiation in the ears of the mice. It decreased levels of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interferon gamma (INF-γ), interleukin 13 (IL-13), interleukin 17 (IL-17), interleukin-10 (IL-10) and nuclear factor kappa B (NF-κB), reducing levels of inflammatory cytokines. Increased TRIP levels at the site, thereby decreasing its metabolites such as kynurenine (KYN), kynurenic acid (KYNA), 3-Hydroxyquinurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), anthranilic acid (AA) e quinolinic acid (QA). Increased activities of the enzymes involved in KPs, including IDO, kynurenine 3-monoxygenase (KMO), kynurenine aminotransferase (KAT) and kynureninase (KYNU). In conclusion, these results demonstrate that inhibition of the IDO enzyme caused attenuation of inflammation, oxidative stress and metabolites of KPs. In addition, they suggest that the inhibition of this enzyme, either with synthetic or natural products may provide a new therapeutic approach for the treatment and prevention of injuries caused by UVB radiation on the skin.

Key words: Ultraviolet B radiation; oxidative stress; Kynurenines pathway; inflammation; Inhibitor.

LISTA DE FIGURAS

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PARTE II

LISTA DE ABREVIATURAS E SIGLAS

UV = Ultravioleta

UVB = Ultravioleta B

UVA = Ultravioleta A

UVC = Ultravioleta C

EROS = Espécies Reativas de Oxigênio

DNA = Ácido Desoxirribonucleico

NF-κB = Fator Nuclear Kappa B

IL-10 = Interleucina 10

IL-6 = Interleucina 6

IL-13 = Interleucina 13

IL-1β = Interleucina 1 Beta

TNF-α = Fator de Necrose Tumoral Alfa

INF-γ = Interferon Gama

VQs = Via das Quinureninas

IDO = Indoleamina 2,3 dioxigenase

TRIP = Triptofano

QUI = Quinurenina

LPS = Lipopolissacarídeo

QMO = Quinurenina 3-monooxigenase

3-HQ = 3-hidroxiquinurenina

QUINU = Quinureninase

AA = Ácido Antranílico

3-HAA = Ácido 3-hidroxiantranílico

QAT I = Quinurenina aminotransferase I

QAT II = Quinurenina aminotransferase II

AQUI = Ácido Quinurênico

AX = Ácido Xanturênico

AQ = Ácido Quinolínico

AIDS = Síndrome da Imunodeficiência Adquirida

1-MT = 1-Metil Triptofano

NK = Natural Killers

RNA = Ácido ribonucleico

MPO = Mieloperoxidase

TBARS = Substâncias reativas ao ácido tiobarbitúrico

SUMÁRIO

APRESENTAÇÃO

Os resultados que compõem essa dissertação apresentam-se divididos em 3 partes. Na **parte I** encontram-se a **Introdução** e os **Objetivos**. A **parte II** refere-se aos resultados deste trabalho sob a forma de **Manuscrito** que abrange as seções introdução, materiais e métodos, resultados, discussão dos resultados e referências bibliográficas. O item **Conclusão** encontra-se na **parte III** desta dissertação e apresenta conclusões gerais do manuscrito contido nesse trabalho. O item **Referências** inclui somente as citações que aparecem no item introdução desta dissertação.

1. INTRODUÇÃO

1.1 Radiação Ultravioleta (UV) e danos na pele

As radiações UV do tipo A e B (UVA e UVB) são as principais responsáveis pela maioria das lesões cutâneas, devido à excessiva exposição à radiação solar da população. As manifestações da exposição aguda incluem eritema e queimadura solar (Li et al., 2016). A exposição crônica leva ao envelhecimento e é considerada um dos agentes mais importantes para o aumento de enfermidades cutâneas malignas (Li et al., 2016; Saija et al., 2000).

A exposição dos seres humanos às radiações UV geram preocupações devido aos vários relatos sobre os efeitos nocivos ocasionados pela exposição solar (Gratz & Kofler, 2015). Estudos epidemiológicos divulgam que pessoas que trabalham sob exposição solar se deparam com maior incidência de problemas de pele, desde a formação de rugas até o desenvolvimento de câncer (Harris, 2009).

As radiações UV fazem parte do espectro eletromagnético lançado pelo sol, envolvem os comprimentos de ondas na banda do UV entre 100-400 nm e é classificada em UVC (100-280 nm), UVB (280-320 nm) e UVA (320-400 nm) (Neves, 2008). As radiações UVC não atingem à superfície da Terra, pois é absorvida pela camada de ozônio. Já as UVB são parcialmente filtradas pela camada de ozônio, correspondendo cerca de 5% do total de radiação UV que chega a Terra (Neves, 2008). Quanto aos raios UVA, são considerados menos ativos na pele quando estabelecida uma relação com os raios UVB (Neves, 2008). São ainda subdivididos em UVA-II e UVA-I, sendo que UVA-II apresenta comprimento de onda que se entende entre a faixa de 320 a 340 nm e possui característica mais eritematosa e fotossensibilizante que UVA-I, que apresenta comprimento de onda entre 340- 400 nm (Wentzell, 1996). As porções de radiações UV que chegam a Terra e atingem a pele participa do processo de reflexão e dispersão, sendo parte absorvida pela camada córnea e outra porção é transmitida para as demais camadas da pele, de maneira que a energia incidente seja completamente dissipada (Yaar & Gilchrest, 2007).

A pele é o maior órgão do corpo, e faz a interface entre o corpo e o meio ambiente, composta basicamente por três camadas de tecido: epiderme, derme e hipoderme, atuando na manutenção do equilíbrio e harmonia do organismo e como uma barreira de proteção aos patógenos e fatores ambientais externos, além dos danos decorrentes da radiação solar. (Sander et al., 2004; Gonzalez, 2010; Maio, 2011).

As radiações UVB e UVA são responsáveis por alterações na função dos tecidos e células da epiderme e derme, ocasionando alterações agudas como o eritema e crônicas como o câncer e o envelhecimento (Ichihashi et al., 2003) Particularmente os raios UVB são competentes para alterar células epiteliais a partir do desenvolvimento de substâncias vasodilatadoras, as prostaglandinas, que principiam um processo inflamatório agudo e consequente estágio de rubor caracterizando o eritema, e os raios UVA atuam nos vasos da derme, determinam vasodilatação e eritema, todavia não ativam mediadores inflamatórios (Rijken & Bruijnzeel, 2009). Por outro lado, as radiações UV possuem ações benéficas, como contribuindo com a biossíntese de vitamina D, eliminação de patógenos na pele e na manutenção da vida na Terra (Matsumura & Aananthaswamy, 2002). Segundo Dang e colaboradores (2015), em seu estudo indica que nem toda a exposição a raios UV é ruim ou carcinogênica, que moderadamente tem um papel benéfico para aumentar a resistência para prevenção de câncer. Dentre as reações indesejáveis provenientes da radiação UV na pele são descritos o desenvolvimento do fotoenvelhecimento prematuro e da fotocarcinogênese por aumento de espécies reativas do oxigênio (EROs) (Dang et al., 2015). O envelhecimento prematuro da pele é um procedimento cumulativo, assim como o envelhecimento cronológico, porém depende especialmente do grau de exposição ao sol e tipo de pele (Fisher et al., 2002).

A radiação UV estimula também o aumento da proliferação e diferenciação celular causando aumento da espessura da epiderme, especificamente do estrato córneo, como maneira de reduzir a vulnerabilidade das células das camadas basais e supra basais aos danos induzidos pela radiação (Verschooten et al., 2006). Uma exposição aguda e crônica aos raios UV afeta diferentemente a estrutura e a função da pele (Sander et al., 2002). Danos ao ácido desoxirribonucléico (DNA), geração de inflamação e carcinogênese são características associadas a raios UVB, produzindo mutações nos dímeros de pirimidina que estão associadas ao câncer de pele não-melanoma (carcinoma de células basais e carcinoma de células escamosas) (Palm, 2007). Confrontando-se com a radiação UVA, que apresenta comprimento de onda inferior e maior quantidade de energia. Uma única exposição à radiação UV pode induzir mutação no DNA, que pode ser revertida pelo sistema de reparo celular (Palm, 2007). A exposição repetitiva à radiação UV promove envelhecimento da pele, mutações em oncogenes e genes supressores de tumor e câncer de pele (Matsumura & Ananthaswamy, 2002).

Sendo assim a radiação UVB é o agente físico mais descrito como formador de radicais livres na pele, e apesar de existir muito mais UVA do que UVB no espectro solar, a UVB é 1000 vezes mais eficaz em causar eritema do que a UVA (Armstrong & Kricker,

2001; Bickers & Athar, 2006). A radiação UVB requer de 2,0 a 5,0J/cm2 para produzir uma dose mínima eritematosa, e o eritema atinge máxima intensidade de seis a 20 horas após a exposição (Dornelles, Goldim & Cestari, 2004).

Ocorre uma cascata de eventos desencadeada após uma agressão ou estímulo, como os raios UV, resultando no aumento do calibre microvascular, aumento da permeabilidade vascular, recrutamento de leucócitos, como conseqüência de uma interação complexa entre diferentes tipos celulares residentes no tecido cutâneo (queratinócitos, fibroblastos, mastócitos, células endoteliais, macrófagos) e vários mediadores pró-inflamatórios (Nickloff & Nestlé, 2004; Sherwood & Toliver-Kinsky, 2004). Os mediadores pró-inflamatórios liberados durante o processo inflamatório, tanto pelas células residentes do tecido cutâneo quanto pelas células transientes (neutrófilos, linfócitos, monócitos), incluem os metabólitos do ácido araquidônico, histamina, citocinas, óxido nítrico e EROs (Simmons, 2006).

As alterações bioquímicas provenientes da radiação UV são propostas por dano direto ao DNA, estresse oxidativo, alteração na transdução de sinais, imunossupressão e inflamação (Vink & Roza, 2001; Dinkova-Kostova, 2008). Outros efeitos incluem: parada do ciclo celular, formação de células de queimadura solar, apoptose e hiperplasia (Ouhtit et al., 2000). Trabalhos têm apontado que o aumento na incidência do número de casos de câncer de pele está atrelado à exposição aos raios UVB, e que o papel lesivo exercido por esses raios encontra relação com a longa permanência e intensidade, ou ambos, de irradiação solar sobre a pele (Chang et al., 2010).

As EROs ou os conhecidos radicais livres são gerados em detrimento do processo de oxidação que ocorre em função da atividade endógena a partir do metabolismo celular aeróbio (redução do O_2 a H_2O) (Zhu et al., 2017). No processo exógeno, o dano celular causado pela radiação UV é caracterizado pela geração de reações em cadeia dessas espécies com outras moléculas promovendo efeitos prejudiciais para o organismo, tais como: processo inflamatório (ativação de fagócitos – neutrófilos, macrófagos, monócitos e eosinófilos) envelhecimento precoce, enfermidades crônicas como artrite e doenças terminais como o câncer (Zhu et al., 2017).

A pele dispõe desses mecanismos de defesa antioxidante. Enzimas como a glutationa peroxidase e glutationa redutase são capazes de neutralizar a ação de peróxidos de hidrogênio e a superóxido dismutase protege contra o radical superóxido e desempenha um papel importante na proteção contra os danos foto-oxidativos (Sionkowska, 2001). A partir de métodos *in vitro* é possível de mensurar uma resposta antioxidante desses agentes presentes

na pele e assim, avaliar o consumo dessas moléculas por fatores oxidantes como a radiação UV (Podda & Grundmann-Kollmann, 2001).

O estresse oxidativo causa aos componentes celulares na pele danos oxidativos, resultando em envelhecimento e carcinogênese (Kong et al., 2013). A via de sinalização do fator nuclear kappa B (NF-κB) mediada por EROs é uma importante via de sinalização celular no processo inflamatório da pele e no envelhecimento (Zhan et al., 2016). O excesso de radiação UV causa produção de EROs e promove a ativação de NF-κB, produzindo assim uma variedade de citocinas pró-inflamatórias tais como interleucina-10 (IL-10), interleucina – 6 (IL-6), interleucina-1β (IL-1β) e fator de necrose tumoral alfa (TNF-α) e promovem necrose celular e apoptose (Zhan et al., 2016). Essa liberação de mediadores pró-inflamatórios pelas células da pele podem levar a infiltração e ativação de neutrófilos e outras células fagocíticas, assim os mecanismos inflamatórios podem acentuar o efeito da radiação UV para amplificar os efeitos diretos prejudiciais sobre moléculas e células que causam fotoenvelhecimento (Bae et al., 2009).

As citocinas são mediadores protéicos cujas ações envolvem o desenvolvimento da resposta imune celular e humoral, indução da inflamação, controle da proliferação e diferenciação celular, bem como a indução da cicatrização Uchi et al., 2000). As citocinas regulam, de forma autócrina e parácrina, a resposta imune e inflamatória através da interação com receptores específicos presentes nos queratinócitos, fibroblastos, células de langerhans, células endoteliais e linfócitos T infiltrados, promovendo a mobilização de leucócitos a partir do sangue e a ativação de outras células do tecido cutâneo (Uchi et al., 2000; Williams & Kupper, 1996).

Tron e colaboradores (1998) mostraram que os efeitos da imunossupressão também são notados após a exposição à radiação UV. Demonstrou-se em camundongos e células humanas que a pele irradiada por raios UV é marcada por uma perda de epiderme e diminuição das células de Langerhans e uma penetração de leucócitos inflamatórios na derme. Foi observada infiltração de macrófagos na epiderme após 6 horas da irradiação por raios UV, acompanhada de produção e secreção de IL-10, IL-6, interleucina-13 (IL-13), interferon gama (INF-γ) e TNF-α após 72 horas, estes fatores recrutam células inflamatórias na pele e causam inflamação do tecido, e efeitos da imunossupressão em 24 horas (Tron et al., 1998, Cho et. al., 2003, Robinson & Werth, 2015). A IL-17 também esta aumentada na lesão de pele, estimula a produção das células T e aumenta o número de autoanticorpos e citocinas inflamatórias (IL-1 e IL-6) (Robinson & Werth, 2015).

A exposição à radiação UV, pode desencadear a liberação de muitas citocinas endógenas, como a expressão de TNF-α que se mostrou aumentada na exposição aguda a raios UV, e isto tem potencial para afetar a via das quinureninas (VQs) na pele, uma vez que o TNF-α é um potente indutor da enzima Indoleamina 2 3 dioxigenase (IDO) (Batycka-Baran et al, 2016). Outro estudo demonstrou que além do TNF-α, outras citocinas inflamatórias como IL-1β e IL-6 também poderiam induzir a expressão da IDO no local afetado (Ito et al., 2015). Como IDO é a primeira enzima limitante da VQs, esta ligação potencial entre a exposição à radiação UV e a VQ poderia ser a ligação que define estas duas vias (Batycka-Baran et al, 2016).

Asp e colaboradores (2011) demonstraram anteriormente, que várias doenças inflamatórias e a exposição aguda a raios UVB podem desregular a VQs induzindo a produção da enzima IDO.

1.2 Enzima Indoleamina 2,3 dioxigenase (IDO) e os metabólitos da via das quinureninas (VQs)

A IDO é uma heme proteína expressa intracelularmente de forma constitutiva ou induzida na placenta, pulmão, intestino delgado e grosso, cólon, baço, fígado, rim, estômago, e cérebro (Sugimoto et al., 2006). A IDO pode também ser induzida em células apresentadoras de antígenos, células de linhagem mielóide (células dendríticas, monócitos, macrófagos, eosinófilos), células epiteliais, fibroblastos, músculo liso vascular e células endoteliais, e certas linhagens de células tumorais (Grohmann, Fallarino & Puccetti, 2003; Yoshida et al., 1981; Mohib et al., 2008).

Dois genes homólogos intensamente ligados (IDO1, IDO2) localizados no cromossomo oito em humanos e camundongos codificam a proteína IDO, com 403 aminoácidos e peso molecular de aproximadamente 45 kDa (Ball et al., 2007; Munn et al., 1998). Em mamíferos todos os genes de IDO estudados até hoje possuem um ou mais elementos de resposta ao IFN nas suas regiões promotoras (Ball et al., 2009; Metz et al., 2007).

As oxigenases são metais contendo enzimas que catalisam a incorporação de uma molécula de oxigênio no substrato, e isso leva a um modelo no metabolismo e síntese de uma variedade de substâncias biológicas, dois tipos de oxigenases são conhecidos: monooxigenases e dioxigenases (Mellor & Munn, 2001). Estudos realizados nas décadas de 50 e 60 descrevem que dois hemes contendo dioxigenases - IDO e Triptofano 2,3-dioxigenase (TDO), catalisam o catabolismo do Triptofano (TRIP) em quinurenina (QUI). (Mellor & Munn, 2001).

A IDO está envolvida no sistema de imunoregulação, onde a supressão de células T dependente da IDO e a indução por células dendríticas sugerem que o catabolismo do TRIP tem efeitos profundos na proliferação e diferenciação das células T que implicam em manipulações imuno-terapêuticas de pacientes com câncer e doenças infecciosas crônicas (Li et al., 2004). Sugere-se que a atividade da IDO reduziria a concentração de TRIP e desta forma as células do sistema imune ativadas, não conseguiriam proliferar e entrariam em apoptose devido a este estado de carência no micro ambiente placentário (Mellor & Munn, 2001; Takikawa, 2005).

A IDO inicia a degradação oxidativa do aminoácido essencial TRIP ao longo da VQs (metabólito produzido como resultado da degradação do TRIP para N-formilquinurenina), através da clivagem oxidativa do anel pirrólico do TRIP, como demonstrado na **figura 1** (Ohnishi, Hirata & Hayaish, 1977; Chen & Guillemin, 2009). O TRIP é o aminoácido essencial menos abundante no organismo sendo utilizado para a síntese de proteínas e a biossíntese de diversos compostos, sendo catabolizado principalmente pela VQs (Ball et al., 2009). A QUI é o produto inicial da oxidação do TRIP pela via VQs (Cherayil, 2009). A síntese de QUI a partir de TRIP pode ocorrer pela ação de duas oxigenases, a TDO, presente no fígado, e a IDO, presente em diversos tecidos incluindo células do sistema imune (Thomas, Witting & Drummond, 2008). O TRIP pode ser catalisado por outra via metabólica, a via serotoninérgica, que leva a produção de serotonina e melatonina (**figura 2**), entre outros compostos, e essas duas vias são as responsáveis por praticamente todo metabolismo desse aminoácido (Nordlind, Azmitia & Slominski, 2008).

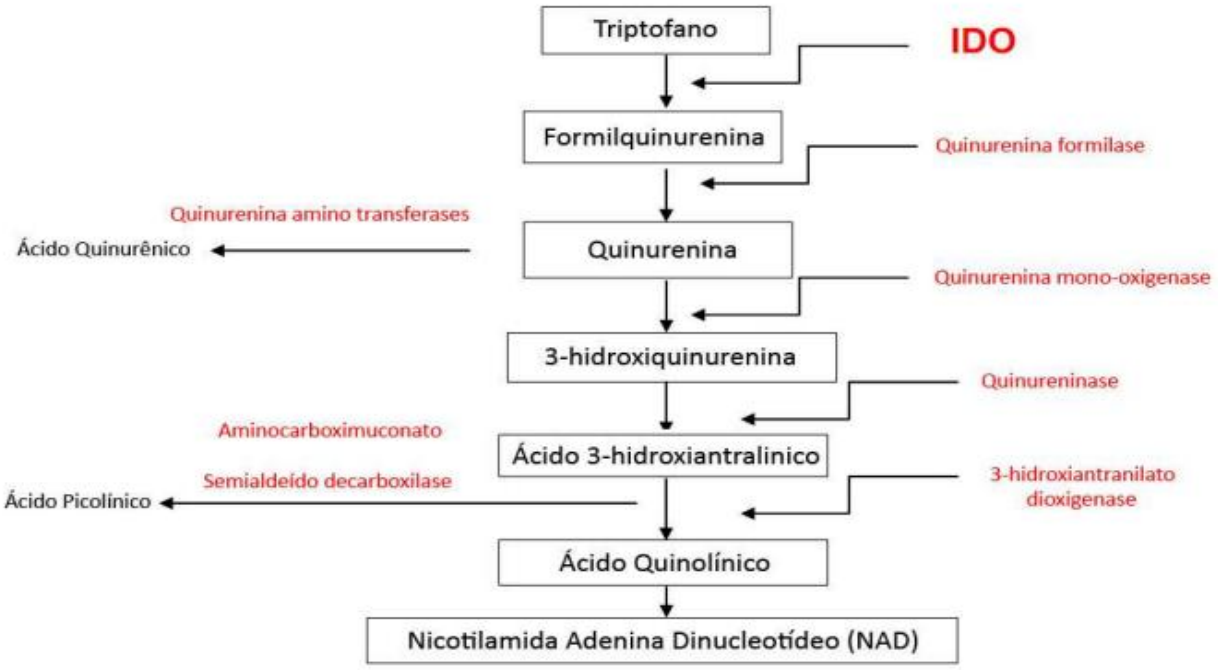

FIG. 1 - Diagrama Esquemático da Via das Quinureninas Fonte: Chen et al., 2009.

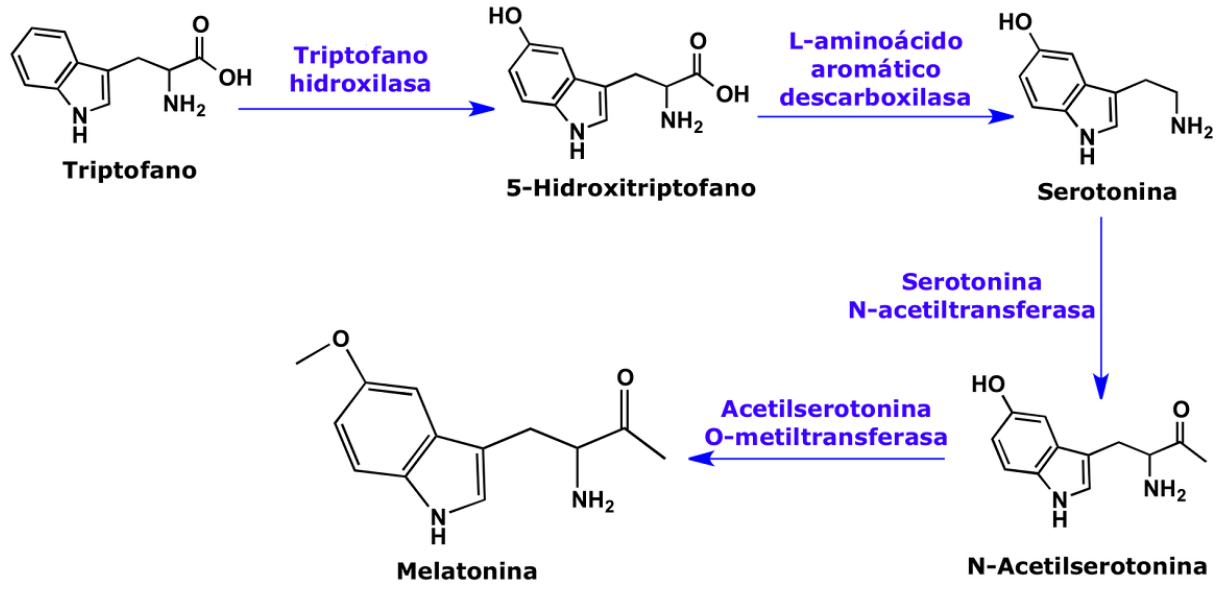

Fig. 2 – Diagrama esquemático da Via da Serotonina. Fonte: http://nutracosmeceuticos.blogspot.com.br/2012/05/la-psiconeuroinmunoendocrinologia-y-la.html

A expressão de IDO pode ser induzida pela citocina inflamatória IFN-γ, a qual regula o complexo classe II de histocompatibilidade principal, atuando sobre TRIP as células apresentadoras de antígenos e regulando a resposta inflamatória (Katz, Muller & Prendergast, 2008). Outras citocinas (IL-1, TNF-α) e o lipopolissacarídeo de membrana bacteriana (LPS) também são capazes de induzir a expressão de IDO (King & Thomas, 2007; Connick &

Stone, 1985). Infecções celulares por agentes microbianos (por exemplo, alguns vírus e outros patógenos intracelulares) também podem induzir IDO em certos tipos celulares (Thomas & Stocker, 1999). A diminuição do TRIP é considerada um dos mecanismos de defesa induzido por IFN-γ em células imunocompetentes durante a resposta imune, já que essa citocina é o principal indutor da enzima IDO, atuando como um mecanismo efetor antimicrobiano ou antitumoral e limita o crescimento de agentes patogênicos (Schrocksnadel et al., 2006). Entretanto, a degradação de TRIP também causa a supressão das células T (Katz, Muller & Prendergast, 2008).

O primeiro passo da VQs é a conversão do TRIP em N-formilquinurenina pela enzima IDO (Yeung et al., 2015). A N-formilquinurenina é inicialmente convertida em QUI e, então, pela enzima quinurenina 3-monoxigenase (QMO), na 3-hidroxiquinurenina (3-HQ) (Müller et al., 2009). Ambas, QUIN e 3-HQ podem ser oxidadas pela enzima quinureninase (QUINU) para ácido antranílico (AA) ou ácido 3-hidroxiantranílico (3-HAA), respectivamente; ou eles podem ser transaminados pelas enzimas quinurenina aminotransferase I ou II (QATI ou QATII) a ácido quinurênico (AQUI) ou ácido xanturênico (AX), respectivamente. Então, o 3-HAA, por sua vez, pode formar o ácido quinolínico (AQ) (Müller et al., 2009). Estudos com anticorpos específicos para o AQ mostram que as células do sistema imune são capazes de sintetizar e/ou estocar grandes quantidades deste metabólito (Moffett et al., 1993). A capacidade tóxica do AQ *in vivo* está associada a algumas doenças neurodegenerativas (como o Alzheimer) e com o aparecimento de demência na Síndrome da Imunodeficiência Adquirida (AIDS) (Heyes et al., 1991).

Foi proposto por Munn e colaboradores, (2005) que células dendríticas expressando IDO induzem a ativação da proteína GCN2 que seria responsável pelos efeitos causados por IDO. Os metabólitos do TRIP, como: QUI, 3-HAA e AQ são capazes de induzir apoptose e exercer efeitos citotóxicos nas células T (Parrot et al., 2016).

Entretanto, vários estudos têm sugerido um papel imunomodulador para a enzima, que pode contribuir para a indução de tolerância imunológica (Baban, Penberthy & Mozaffari, 2010). Munn e colaboradores (1998), mostraram que IDO desempenha um papel essencial na proteção do embrião contra rejeição em modelo murino de gravidez alogênica. A inibição da atividade da IDO por seu inibidor farmacológico, 1-metil triptofano (1-MT), resultou na rejeição dos fetos mediada pelas células T (Munn et al., 1998). Estes resultados não só propuseram uma função imunológica para IDO, mas também sugeriram um papel na geração de tolerância imunológica e regulação da resposta imune. Assim células expressando IDO são capazes de suprimir respostas locais da célula T e promover tolerância imune sob várias

condições fisiológicas e patofisiológicas de importância médica, inclusive doenças infecciosas, rejeição fetal, transplantes de órgãos, doenças neurológicas, desordens inflamatórias e auto-imunes e câncer (King & Thomas, 2007). Esses resultados contradiziam a função original e amplamente aceita de que a IDO agiria como um mecanismo de defesa do hospedeiro. Essa contrariedade em relação à função da IDO tem atraído grande atenção para esta enzima. Portanto, a IDO pareceu agir de uma forma dupla: 1) exercendo ação antimicrobiana, inibindo o crescimento de microorganismos e estimulando a resposta imune. 2) envolvendo-se na ativação da regulação das respostas imunes e criação de tolerância imune (Baban, Penberthy & Mozaffari, 2010).

Autores de estudos anteriores propuseram interações da IDO com o sistema imune, assumindo funções imunológicas estimulantes e reguladoras simultaneamente (Müller et al., 2009). Investiga-se que o sistema imunológico, mediado pela IDO, primeiramente reagiria ao agente infeccioso a fim de inibir seu crescimento e progressão e mais tarde numa segunda fase, o mecanismo de regulação imunológica seria ativado para proteger o hospedeiro de uma reação exacerbada induzida pelo sistema imune (Müller et al., 2009). Propõe-se tanto que a depleção de TRIP seja o principal mecanismo pelo qual a IDO exerce sua função reguladora, quanto que o real responsável seja o acúmulo de metabólitos do TRIP no microambiente (Baban, Penberthy & Mozaffari, 2010). Várias teorias têm sido sugeridas para caracterizar o papel da IDO em condições normais e em doenças, a diminuição dos níveis de TRIP e aumento nas concentrações plasmáticas de QUI já foram descritas em pacientes com doenças cardiovasculares, renais e doenças neurodegenerativas (Pawlak et al., 2009-a; Pawlak et al., 2009-b; Heyes et al., 1991). A ativação da enzima IDO inibe a proliferação de linfócitos através da depleção de TRIP e pela ação direta dos próprios metabólitos produzidos na degradação desse aminoácido, causando a imunotolerância (Mackenzie et al.,2007). Estudos tanto em animais como em células humanas demonstram que as células que expressam IDO têm uma função imunossupressora aumentando a tolerância aos linfócitos T, além disso, vários outros estudos sugerem que as células que expressam IDO esvaziam a TRIP do meio extracelular e secretam metabolitos de TRIP (incluindo QUI, 3-HQ, 3-HAA e AQ), que induzem a apoptose das células T e suprimem a imunidade respostas *in vitro* (Hoshi et al., 2012).

Uma vez que o IDO tem sido descrito como um alvo potente no tratamento de câncer, um grande número de grupos e investigadores procuram descobrir novos inibidores da IDO. A descoberta de 1-MT levou a um aumento do interesse em estudos de IDO inibição, devido ao seu papel na supressão imunológica (Jiang et al., 2015).

1.3 Inibidor da enzima Indoleamina 2,3 dioxigenase (IDO) – 1 Metil Triptofano (1-MT)

O 1-MT foi relatado pela primeira vez como um inibidor da IDO em 1991 (Cady & Sono, 1991). Ainda recentemente, como um inibidor competitivo fraco da enzima, é ainda um dos inibidores da IDO mais conhecidos (Macchiarulo et al., 2009). Esse inibidor que tem capacidade significativa de inibir a atividade da IDO bloqueando seu efeito imunossupressor e coopera com a quimioterapia na regressão de tumores em modelos murinos (Uyttenhove et al., 2003; Muller et al., 2005; Potula et al., 2005; Muller et al., 2005). Esse agente atua reforçando as respostas das células T contra os antígenos tumorais e seus auto-antígenos (Grohmann et al.,2001). Inibe a produção de catabólitos do TRIP, como a QUIN, que diminui a proliferação de células T e de natural killers (NK) (Hwu et al., 2000; Mellor et al., 2003).

Os efeitos do 1-MT incluem resposta das células T contra os antígenos do tumor *in vitro* e *in vivo*, juntamente com a inibição da produção de catabólitos do TRIP, como a QUIN (Grohmann et al., 2001; a-Grohmann et al., 2001; Hwu et al., 2000; Mellor et al., 2003). Segundo Friberg e colaboradores (2002), um estudo demonstrou que houve atraso no crescimento de carcinoma pulmonar de Lewis em camundongos após a administração, *in vivo*, de 1-MT. Um desafio para a imunoterapia é desenvolver estratégias que possam efetivamente superar a imunossupressão induzida pelos tumores, aumentando assim, respostas antitumorais, e um fármaco que bloqueie a IDO pode ser clinicamente útil na reversão desta imunossupressão (Uyttenhove et al., 2003; Potula et al., 2005; Rutella et al., 2006). Recentemente, foi descoberto que 1-metil-D-triptofano bloqueia o RNA (ácido ribonucleico) mensageiro da IDO e com isso não deixa a enzima ser transcrita, deste modo, bloqueando a atividade da IDO (Wang et al., 2013).

Surpreendentemente, o isômero do 1-MT, o D-isômero é menos tóxico e mais efetivo como inibidor de IDO do que o L-isômero quando testado em estudos pré-clinicos com camundongos. L-isômero de 1-MT reduz o crescimento de tumor *in vivo*, e D- 1MT também é inibidor efetivo da IDO em estudos com macrófagos e células dendríticas (Duluc et al., 2007). O D-1-MT é avaliado atualmente como um potencial adjuvante em vacina para o tratamento do câncer (Kahler & Mellor, 2009). O 1-MT também foi utilizado em ensaios de pele contendo melanoma, em que a inibição da enzima IDO pode aumentar a produção de melatonina, um metabólito do TRIP, importante no restabelecimento da pele lesada (Moreno et al., 2013).

2. OBJETIVOS

2.1 Objetivo geral

No presente estudo visamos avaliar a utilização de um inibidor da enzima IDO (1-MT) em lesões auriculares agudas de camundongos, provocadas pela exposição à radiação UVB.

Os objetivos específicos serão verificar como o inibidor na enzima IDO (1-MT) age nas seguintes análises:

Espessura da orelha e na infiltração de neutrófilos, através da mieloperoxidase (MPO).

No estresse oxidativo, através dos níveis de glutationa, carbonilação de proteínas e TBARS (substâncias reativas ao ácido tiobarbitúrico).

Sobre a inflamação causada pela radiação UVB, através dos níveis fator de necrose tumoral alfa (TNF-α), interleucina 1 beta (IL-1β), interleucina 6 (IL-6), interferon gama (INF-γ), interleucina 13 (IL-13), interleucina 17 (IL-17), interleucina 10 (IL-10) e fator nuclear kappa B (NF-κB).

Sobre os metabólitos da via das quinureninas, através dos níveis de triptofano (TRIP), quinurenina (QUI), ácido quinurênico (AQUI), 3-Hidroxiquinurenina (3-HQ), ácido 3-hidroxiantranílico (3-AA), ácido antranílico (AA) e ácido quinulínico (AQ).

Sobre a atividade enzimática da via, analisando a atividade da enzimas IDO, quinurenina 3-monoxigenase (QMO), quinurenina aminotransferase (QAT) e quinureninase (QUINU).

PARTE II

Modulation of the kynurenines pathway in acute ear lesion induced by UVB radiation in mice

Manuela Bastos Piêgas, Kellen Athaide Rocha, Renata Giacomeli, Michelle Silva Antunes, Cristiano Ricardo Jesse, Márcia Rósula Poetini

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Modulation of the kynurenine pathway in auricular lesion induced by UVB radiation in mice

Manuela Bastos Piêgas, Kellen Athaide Rocha, Renata Giacomeli, Michelle Silva Antunes, Cristiano Ricardo Jesse*, Márcia Rósula Poetini

Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas – LaftamBio Pampa – Universidade Federal do Pampa, Itaqui, RS, Brazil

*Correspondence should be sent to:

Cristiano Ricardo Jesse

E-mail: cristianoricardojesse@yahoo.com.br

Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas – *LaftamBio Pampa* – Universidade Federal do Pampa, CEP 97650-000, Itaqui, RS, Brazil.

Phone and FAX number: +55-55-34331669

Highlights

- It is proposed that the 1-metyl tryptophan inhibitor reduce effects of UVB radiation on the skin
- The mechanism is based on exposing mouse ears to UVB light and checking inflammatory skin damage
- To correlate the kyrurenines pathway with damage caused by UVB vs. inhibitor

ABSTRACT

AIM:

This study investigated the effects of the inhibitor 1-methyl tryptophan (1-MT) of the enzyme indoleamine 2,3 dyoxygenase (IDO) on ear lesions of mice exposed to UVB radiation comparing the effects of this inhibition against inflammation, oxidative stress, metabolites and enzymes kynurenines pathway (KPs).

METHODS:

Mice received 10 μl of the vehicle and three doses (0,5 μg/μl, 1,0 μg/μl and 2,0 μg/μl) of the inhibitor (1-Metyl tryptophan) of the IDO enzyme topically prior to 24 hours exposure to UVB radiation.

RESULTS:

The present study demonstrated that treatment with 1-MT against UVB radiation in mouse ear lesions was effective in attenuating the following changes, decreasing the thickness and inflammation of irradiated ears, decreased the levels of carbonylation proteins and thiobarbituric reactive substances (TBARS), increased the levels of glutathione (GSH), thus reducing the oxidative stress caused by UVB radiation in the ears of mice. Decreased levels of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interferon gamma (INF-γ), interleukin 13 (IL-13), interleukin 17 (IL-17) and factor nuclear kappa B (NF-κB) reducing the levels of inflammatory cytokines. Increased TRIP levels at the site, thereby decreasing its metabolites such as kynurenine (KYN), kynurenic acid (KYNA), 3-Hydroxyquinurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), anthranilic acid (AA) e quinolinic acid (QUIN). Increased activities of the enzymes involved in KPs, including IDO, kynurenine 3-monoxygenase (KMO), kynurenine aminotransferase (KAT) and kynureninase (KYNU).

CONCLUSION:

In conclusion, these results demonstrate that the inhibition of the IDO enzyme caused attenuation of the inflammation, oxidative stress and activation of KPs. In addition, suggest that the inhibition of IDO, either with synthetic or natural products can provide a new therapeutic approach for the Treatment and prevention of injuries caused by UVB radiation on the skin.

KEY WORDS:

Kynurenines pathway; Inflammation; Inhibitor; radiation UV

1. Introduction

The skin being one of the external organs of the human body, in which the function would serve as a biological barrier, remains more exposed to several harmful sources, such as chemicals, ultraviolet radiation (UV) [1]. Low UV doses, to a lesser incidence of times, are beneficial because they are the main source for the synthesis of vitamin D3, which is involved in bone metabolism and immune system function [2]. UVB radiation is primarily responsible for cutaneous cytotoxic events, which induce various pathological changes in the skin, such as erythema, edema, sunburn, immune suppression and skin cancer [3]. When absorbed by epidermal cellular components, has developed a dermal remodeling involving mediators which diffuse from the epidermis to the dermis, where they stimulate the production of elastin fibroblasts and inflammatory cytokines [4]. UVB radiation induces a series of pathological changes, including inflammation of the skin, characterized by vasodilation with consequent edema, erythema and epidermal hyperplasia, leading to the accumulation of inflammatory cells in the skin, such as T cells and neutrophils that remain in the tissue for several days. In addition, epidermal cells release various cytokines that lead to the initiation and amplification of the immune and inflammatory responses of the skin [5]. Thus, inflammatory processes can increase the direct and harmful effects of UV radiation on the cells, causing photoaging and immobilization [6,7]. The inflammatory process induced by UVB radiation is related to the production of several events, among them vasodilation, production of reactive species and oxygen (ROS) that cause oxidative stress, induction of the production of inflammatory cytokines such as interleukins (IL) (1β, 6, 13, 17 and 10), tumor necrosis factor α (TNF-α) and migration of neutrophils and macrophages from the circulation to the skin tissue [8,9].

The essential amino acid tryptophan (TRYP), obtained through diet, is metabolized in the liver, generating biologically active compounds through two main pathways, the serotonergic route and the kynurenine pathway (KP), (**Fig. 1**) [10]. According to Sheipouri and collaborators [11], several inflammatory diseases have demonstrated significant deregulation of the KP, this includes acute exposure to UVB radiation, which generates inflammatory cytokines that may induce an increase in the indoleamine 2 3 dioxygenase (IDO) enzyme that is involved in the kynurenine pathway. In particular, studies report that proinflammatory cytokines, such as IL-1β, IL-6, IL-10, TNF-α, induce an increase in the expression of IDO in several cell types, including epithelial cells, consequently causing a decrease in TRYP and increase of kynurenine (KYN) and its metabolites (kynurenic acid, 3-Hydroxyquinurenine, 3-hydroxyanthranilic acid, anthranilic acid and quinolinic acid) in the cellular environment,

which induce apoptosis of T cells, suppresses immune responses in vitro and causes scarring [12,13,14]. Studies have shown IDO expression in several human cell types, including macrophages, microglia, dendritic cells, astrocytes, fibroblasts and epithelial cells, IDO is actually considered the primary enzyme that contributes to the production of kynurenines in inflammatory diseases [15,16].

In view of UVB radiation in the skin of the ear of mice, and the expression of the enzyme IDO and its consequences, we will test a more widely studied IDO inhibitor drug, 1-methyl tryptophan (1-MT). Previous studies with animals have shown that 1-MT significantly reduces tumor growth, possibly binding to the inhibition of IDO that is expressed by dendritic cells [17].

Thus, the objective of this study was to evaluate the production of metabolites (kynurenic acid, 3-Hydroxyquinurenine, 3-hydroxyanthranilic acid, anthranilic acid and quinolinic acid) and enzymes (IDO, kynurenine 3-monoxygenase, kynurenine aminotransferase and kynureninase) of the KPs, oxidative stress (Glutathione, protein carbonilation and thiobarbituric acid reative substances) and inflammatory processes (Inflammatory markers, ear thickness, myeloperoxidase) in auricular lesions of mice, caused by exposure to UVB radiation, in with different doses of the enzyme inhibitor IDO 1-MT (0.5, 1 and 2μg/μl).

2. Materials and methods

2.1 Animals

The experiments were performed with 30 male C57B / 6J mice weighing between 20 and 30g, aged between 60 and 120 days, obtained from the Federal University of Santa Maria (UFSM) - Santa Maria / RS. During the study, the mice will be housed in polypropylene boxes and kept at controlled temperature at 22°C ± 1°C, with free access to food and water, and respecting the light / dark cycle of 12 hours each. All the work will be carried out according to the guidelines of the Committee on the Care and Use of Experimental Animals and the Ethics Committee for the Use of Animals of the Federal University of Pampa (UNIPAMPA).

2.2 Treatment with 1-MT

The 1-MT is a pharmacological inhibitor of the enzyme IDO. The mice will be randomly divided into five groups with six animals: a) control, without exposure to UVB radiation; b) vehicle, exposed to UVB radiation treated with saline (10μL topical use); c) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (0.5μg/μl topical use); d) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (1.0μg/μl topical use) and group e) exposed to UVB radiation and treated with 1-MT enzyme inhibitor (2,0μg/μl topical use), The treatments were at the topical level and performed before exposure to radiation.

2.3 UVB radiation model

The UVB irradiation source consisted of a Philips TL40W/12 RS lamp (Medical-Eindhoven, The Netherlands) mounted 20 cm above where the mice were placed and which emitted a continuous light spectrum between 270 and 400nm with peak emission at 313nm, according to Casagrande and collaborators [18].

Soon after the end of exposure to UVB radiation, the animals were euthanized by cervical dislocation, since the administration of anesthetic drugs may influence the biochemical analyzes to be performed [19]. The whole ear was withdrawn, homogenized in acetate buffer solution (80mM, pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide, homogenized, centrifuged at 3000rpm for 10 minutes, supernatant removed and The samples immediately frozen at -20ºC to carry out the proposed analyzes.

2.4 Measurement of ear edema

Photodynamics of the skin was induced by irradiation with UVB and the inflammatory process was evaluated through the formation of edema in the ear. Edema was measured by increasing ear thickness after inflammatory stimuli. The thickness of the ear was evaluated before and 24h after UVB irradiation using a digital micrometer (Digimess) in animals anesthetized with isoflurane [20]. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. The thickness was expressed in mm. To minimize variation, a single investigator performed measurements throughout each experiment.

2.5 Myeloperoxidase (MPO) analysis

Leukocyte migration induced by UVB irradiation on the skin of the ear will be evaluated using the MPO enzyme activity assay. For the evaluation of MPO enzyme activity, the sample will be incubated with acetate buffer and 3,3', 5,5'-tetramethylbenzidine solution

(18.4mM) at 37°C. The reaction will be stopped on ice by the addition of acetic acid. The color formed will be analyzed by a spectrophotometer at 630nm [21].

2.6 Glutathione (GSH) assay

GSH skin levels were determined using a fluorescence assay as previously described [22]. The total skin of ear mice (1:3, w/w dilution) was homogenized in 100Mm NaH2PO4 (pH 8.0) containing 5mM EGTA using a Turrax TE-102 (Turratec). Whole homogenates were treated with 30% trichloroacetic acid, centrifuged at 1900g for 6 min at 4°C and the fluorescence of the resulting supernatant measured in a Hitachi F-4500 fluorescence spectrophotometer. Briefly, 100µL of sample supernatant was mixed with 1mL of 100mM NaH_2PO_4 (pH 8.0) containing 5mM EGTA and 100µL of OPT (1mg/mL in methanol). The fluorescence was determined after 15 min (λexc = 350nm; λem = 420nm) and the values referred to a standard curve prepared with 0–40 µM GSH. The results are presented as µm of GSH per mg of skin.

2.7 Protein Carbonyl Levels

The carbonyl content was assayed by a method based on the reaction of protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH) forming dinitrophenylhydrazone, a yellow compound, measured spectrophotometrically at 370 nm [23]. The homogenate (without centrifugation) was diluted with Tris–HCl buffer, pH 7.4, at a ratio of 1:8 (w/v). Three tubes containing aliquots of 1 ml of the diluted homogenate were incubated at 37 C for 2 h. A volume of 200 µl of 10 mM DNPH dissolved in 2.0 M HCl was added to two tubes (duplicate). In the third tube, 200 µl of 2.0 M HCl solution was added (blank). After incubation, at room temperature, for 1 h in a dark ambient, 500 µl of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3 % sodium dodecyl sulfate), 1.5 ml of heptane (99.5 %), and 1.5 ml of ethanol (99.8 %) were sequentially added. The tubes were shaken with a vortex mixer for 40 s and centrifuged for 15 min. Next, the protein isolated from the interface was washed two times with 1 ml of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 ml of denaturing buffer. Total carbonylation was calculated using a molar extinction coefficient of 22,000 M-1 cm^{-1}. Results were reported as carbonyl content (µM/mg protein).

2.8 Lipid peroxidation

A 200-µl aliquot of S1 from tissue of mice belonging to the experimental groups was added to the reaction mixture containing 500 µl of 0.8% thiobarbituric acid, 200 µl of 8.1% sodium dodecyl sulfate, and 500 µl of acetic acid (pH 3.4), and was incubated at 95°C for 2 h. Thiobarbituric-acid-reactive species (TBARS) were determined as described by Ohkawa, Ohishi & Yagi [24]. Malondialdehyde (MDA), formed as an endproduct of lipid peroxidation, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. Lipid peroxidation was expressed as nanomoles of MDA equivalents per mg of tissue

2.9 Cytokine levels and inflammatory markers

The levels of TNF-α, IL-1β, IL-6, interferon gama (INF-γ), IL-13, IL-17, IL-10 and nuclear factor kappa B (NF-κB) in the ear tissue were measured using 100 µl sample aliquots and ELISA kits Rat Cytokine DuoSet from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions (protein range 31.25-2000pg). The cytokine level was estimated by interpolation from a standard curve by colorimetric measurements at 450nm (correction wavelength 540nm) on an ELISA plate reader (Berthold Technologies-Apollo 8-LB 912, KG, Germany). Results are reported as pg/mg of skin tissue.

2.10 Analysis of the TRYP, KYN, KYNA, 3-HK, 3-HAA, anthranilic acid (AA) and QUIN by High Pressure Liquid Chromatography (HPLC)

TRYP, KYN, KYNA, 3-HK, 3-HAA, AA and QUIN levels were measured in hippocampus samples using HPLC, according to Ferraz and collaborators [25]. The mobile phase contained 50 nM glacial acetic acid, 100 mM zinc acetate and 3% acetonitrile dissolved in double- distilled NANOpure water HPLC grade H_2O. The pH was adjusted to 4.9, using 5M NaOH. Hippocampal tissue was sonicated in 1 ml of mobile phase containing 7% perchloric acid spiked with 50 ng/20 µl of N-methyl 5-HT as internal standard. Hippocampal homogenates were centrifuged at 20,000 rpm for 20 min and the supernatants were placed into new eppendorf tubes, using a syringe fitted with a 0.45 µm filter (phenomenex). 20 µl of the filtered supernatant was injected using a Waters autosampler and a Reverse Phase analytical column (Kinetex™ Core Shell Technology column with specific area of 4.6 mm and particle size of 2.6 µl Phenomenex) was used for separation of metabolites. A PDA-UV detector (Shimadzu SPD-M10A VP), calibrated to integrate at 230 and 250 nm, as well as a fluorescent detector (Shimadzu RF- 20A XS prominence fluorescence detector), set to excitation wavelength 254 nm; emission wavelength 404 nm, were used to detect the

metabolites. Chromatographs were generated by CLASS-VP software (Shimadzu). Results are expressed as ng/mg protein.

2.11 Determination of IDO, KMO, KAT and KYNU activities

IDO, KMO and KAT activities were determined as described previously, with minor modifications [26]. To the IDO activity, ear homogenate of mice (50 µl) were incubated with 200 µl of assay buffer (400 mM L-tryptophan, 20 mMascorbate, 10 µM methylene blue, 100 µg catalase, in 50 mM potassium phosphate buffer pH 6.5) at 37 ºC for 1 h. The reaction was stopped by adding 50 µl of 10% sulfosalicylic acid solution (SSA) and then incubated for an additional 30 min at 50 ºC to hydrolyze N-formylkynurenine to L-kynurenine. The reaction mixture was then centrifuged at 13,000g for 10 min at 4 ºC and filtered through 0.2 mM filter tubes at 13,000g for 5 min. KYN was analyzed by HPLC, as previously described by Zwilling and collaborators [26]. The results are expressed as nmolKYN/h/mg protein.

To measurement the KMO activity was made using the method previously described by Winkler and collaborators [27]. The ear tissue was homogenized 1:5 (wt/vol) in ultrapure water and further diluted 1:5 (vol/vol) in 100 mMTris–HCl buffer (pH 8.1) containing 10 mMKCl and 1 mM EDTA. Eighty µl of the tissue preparation were incubated for 40 min at 37°C in a solution containing 1 mM NADPH, 3 mM glucose-6-phosphate, 1 U/ml glucose-6 phosphate dehydrogenase, 100 µM kynurenine, 10 mMKCl and 1 mM EDTA in a total volume of 200 µl. The reaction was stopped by the addition of 50 µl of 6% perchloric acid. Blanks were obtained by adding the specific enzyme inhibitor Ro 61-8048 (100 µM) in the incubation solution. After centrifugation (16,000 x g, 15 min), 20 µl of the supernatant were applied to HPLC to measure 3-HK.

The KAT activity was made using the method previously described by Guidetti, Okuno & Schwarcz [28]. Briefly, the ears was harvested and homogenized in distilled water. After centrifugation (12 000 g, 10 min), KAT activity was measured in a total volume of 200 µl, containing 80 µl of supernatant fluid, 150 mm Tris-acetate buffer, pH 7.4, 2 lm kynurenine, 1 mm pyruvate and 80 lm pyridoxal-5-phosphate. Samples were incubated for 24 h at 37 ºC, and the reaction was terminated by adding 50% (w ⁄ v) trichloroacetic acid. After successive washes with 0.1 m HCl and distilled water, KYNA was eluted from the column with 2 X 1 mL of distilled water and quantified by HPLC.

Kynureninase activity was measured according to a previously published method by Ubbink, Vermaak & Bissbort [29] by high-performance liquid chromatography (Agilent 1100; Agilent Technologies). The intraassay precision was estimated from the 5 replicates of

pooled samples at 3 different concentrations (low, medium, and high) based on the standard curve, ranging from 0.2% to 3.0% (coefficient of variance). The interassay precision is similarly evaluated over a 5-day period, ranging from 0.7% to 3.2%. The variability between duplicates was much lower than the 12% limit recommended by the manufacturer.

2.12 Statistical analysis

The data will be tabulated in the Excel software and statistical procedures will be performed in the SPSS program for Windows, version 21.0, and Graphad Prisma 6.0. One ANOVA followed by post hoc Newman–Keuls multiple comparison test. The level will be considered statistically significant when the value of $p < 0.05$.

3. Results

3.1. Evaluation of ear edema and leukocyte infiltration - induced by UVB radiation

The UVB-induced ear edema model was used to evaluate the influence of the 1-MT inhibitor on inflammatory parameters induced by UVB radiation. UVB irradiation in the ear in mice induced a marked increase in ear thickness when evaluated 24 hours after UVB radiation. By topical treatment with 1-MT, ear edema induced by UVB irradiation and infiltration of inflammatory cells were effectively reduced as the dose in the inhibitor increased. The highest dose of 2 μg/μl of 1-MT was the one that most blocked the increase in the thickness of the mouse ear, compared with naive and becoming significantly different from the vehicle (**Fig. 2A**). MPO plays an important role in the innate immune system being considered a biochemical marker of infiltration of inflammatory cells in the injured tissue. In order to evaluate the effect of the inhibitor of the IDO enzyme on infiltration of inflammatory cells, MPO activity was evaluated 24 h after UVB radiation. Irradiation with a source of UVB promoted an increase of MPO activity in vehicle treated radiated groups as compared with the group not exposed to UVB radiation. All tested doses of the inhibitor 1-MT were able to decrease MPO activity induced by UVB radiation with a maximum inhibition at the highest dose of 2 μg/μL, with $p < 0.05$ being different from the vehicle almost equal to Naive (**Fig. 2B**).

3.2 Evaluation of oxidative stress

It has been reported that UV radiation generates ROS production, inflammation and skin damage. To investigate the possible antioxidant effect of the inhibition of the IDO

enzyme with 1-MT, we evaluated the ability of the treatments to reduce oxidative stress parameters through levels of TBARS and carbonylation proteins. The UVB radiation promoted an increase of all oxidative stress parameters evaluated. The IDO inhibitor readily decreased lipid peroxidation through the TBARS test and carbonylation proteins (**Fig. 3B and 3C**). Inhibition of the IDO enzyme by 1-MT prevents GSH depletion of the skin induced by UVB. There was a depletion of GSH levels in the skin exposed to UVB radiation. Topical treatment of the skin of 1-MT mice prior to UVB exposure resulted in inhibition of GSH depletion, maintaining a similar level to the unirradiated and untreated naive group (**Fig. 3A**). This result suggests that the inhibition of the IDO enzyme against UVB radiation increases antioxidant protection in the skin. Animals that received UVB radiation showed an increase in oxidative stress after 24 hours of exposure compared with naive (4-fold increase in carbonylation protein levels, 2-fold increase in TBARS and 2-fold reduction in GSH levels).

3.3 Inhibition of the IDO enzyme through 1-MT prevents the increase of the proinflammatory mediators induced by UVB

In this study, the results showed an increase in the amounts of IL-1b and TNF-α, respectively, when compared with naive and vehicle groups. Inhibition of the IDO enzyme by 1-MT in the higher dose was made to be different from the vehicle (**Fig. 4A and 4B**). Other interleukins were observed in this study, which were significantly elevated in exposure to UVB radiation, the use of the inhibitor at the highest dose suppressed the levels of all four cytokines, being different from vehicle values, IL-6 (**Fig. 4C**), IL-13 (**Fig. 4E**), IL-17 (**Fig. 4F**) e IL-10 (**Fig. 4G**). INF-γ another marker that increases in inflammatory opposite UVB radiation responses, induces the production of the enzyme IDO and IL-13 production, in our study 1-MT inhibitory at the highest dose, reduced the expression (± 10pg / mg) are showing different from the vehicle almost equal to naive (**Fig. 4D**). Since the NF-κB pathway plays a key role in the induction of inflammatory cytokine expression by UV radiation, we examined whether 1-MT inhibits the activation of UV radiation of NF-κB. The UVB radiation in the mouse ear increased almost 3-fold the expression of NF-κB, compared with the 1-MT inhibitor levels reduced almost if associated to the naive and different from the vehicle (**Fig 4H**).

3.4 The role of the metabolites of the KPs against the topical use of the 1-MT inhibitor in the ear of the mice

As IDO is an enzyme that catalyzes the degradation of TRYP to KYN, increased IDO activity leads to depletion of TRYP and an increased concentration of KYN in the local microenvironment. Therefore, we evaluated the effect of TRYP and KYN on UVB radiation and on the 1-MT inhibitor, the results show that by inhibiting IDO at the highest dose, it increased TRYP and reduced levels of KYN almost matching the naive (**Fig. 5A and 5B**). By exposing the skin to UVB radiation we know that there is an increase in the expression of the IDO, so the rest of the metabolites of the pathway would be at their high levels as well. Our study also measured in the auricular tissue irradiated with UVB levels of KYNA (**Fig. 5C**), 3-HK (**Fig. 5D**), 3-HAA (**Fig. 5E**), AA (**Fig. 5F**) and QUIN (**Fig. 5G**), which opposite the inhibitor 1-MT were reduced all different vehicle.

3.5 Role of the enzymes participating in the KPs against topical use of the 1-MT inhibitor in the ear of the mice

IDO (**Fig. 6A**) is the first enzyme participating in the kynurenines pathway, followed by KAT (**Fig. 6B**), KMO (**Fig. 6C**) and KYNU (**Fig. 6D**). Our study shows that the exposure to UVB radiation was twice as large as those enzymes. The use of the 1-MT inhibitor at the highest dose (2μg / μl) reduced their levels, where they were different from the vehicle, almost matching the naive.

4. Discussion

This paper describes the involvement of the KPs modulated the inflammatory and oxidative markers in the damage caused by UVB radation in ear mice. UVB radiation mainly affects epidermal cells, being more genotoxic and approximately 1000 times more capable of causing burn than UVA radiation [30]. Their adverse biological effects are complex because UVB rays act through direct and indirect mechanisms and the direct effects induced by UVB include damage to the lipids of DNA, protein and stratum corneum, while also being able to generate ROS by indirect mechanisms that induce imbalance in the oxidative state in the skin [31]. Acute skin exposure to UVB irradiation results in inflammation associated with edema formation and leukocyte infiltration [32,33,34]. According to Silva and collaborators [35], the animals exposed to UVB radiation in the legs developed edema, as an inflammatory response, when compared with the control, reinforcing our findings regarding the ear edema of the mice exposed to UVB radiation. These results suggest that MPO is responsible for the development of UVB-induced skin inflammation, possibly by regulating the cytokines expressed in the

skin [36]. According to Polyzos and collaborators [37], the inhibition of IDO increased the innate immune responses in the vascular wall, decreasing inflammation, relating to our findings, noting that the IDO inhibitor, 1-MT, is possibly able to block the KP thereby reducing inflammation.

UVB-induced damage is generally associated with overproduction of ROS, free radicals and the damage of antioxidant defense mechanisms [38,39]. To investigate whether the inhibition of the IDO enzyme through the 1-MT inhibitor would have an antioxidant capacity, we measured oxidative stress using levels of GSH, carbonylation proteins and TBARS. Animals that received UVB radiation showed an increase in oxidative stress after 24 hours of exposure compared with control. In the presence of the 1-MT inhibitor at higher doses (2μg/μl), all markers of oxidative stress, in this study analyzed, were different from the vehicle. Inflammation induced by UVB irradiation may be mediated in the affected tissue by oxidant / antioxidant balance impairment, which results in an increase in cellular ROS levels leading to damage of cellular proteins and lipids and to DNA oxidation [40,38]. In addition, a set of data on IDO activity has suggested that even though it exists in some human cell lines constitutively, in most cells proinflammatory stimuli considerably increase their activity [41]. Thus, it may contribute to the immunosuppressive microenvironment due to tryptophan depletion and the accumulation of the metabolites of the KP, which would be reversed using the IDO inhibitor, 1-MT [42].

Expression of the IDO enzyme, which is distributed in mammalian tissues and cells, and converts TRYP to KYN, is induced by proinflammatory cytokines in the course of an inflammatory response, induced by UVB, in cell types including macrophages, fibroblasts, and epithelial cells [13]. A previous study demonstrated that several cytokines, including TNF-α, IL-6 and IL-1β, were increased at some wound sites, these cytokines may be involved in the induction of IDO expression at wound sites [43]. In addition, ROS production is believed to activate the production of NF-κβ, which exerts detrimental functions, inducing the expression of pro-inflammatory mediators that orchestrate and sustain the inflammatory response and cause tissue damage [44,45]. Thus the NF-κβ pathway is involved in skin UVB mediated injury, including skin carcinogenesis, and its regulation by antioxidants has emerged as a new area with promising therapeutic implications [46,47]. Another proinflammatory cytokine IFN-γ is released by activated T cells during an immune response [48]. Thus, our study evaluated the main inflammatory cytokines, IL-6, IL-1β, IL-10, IL-13, IL-17, TNF-α and IFN-γ, the cell marker NF-κB, which are increased by exposure to UVB radiation when

compared with control and vehicle groups, demonstrated that the induction of inflammation occurs in the ears of mice, consequently increasing the expression of the enzyme IDO. This is confirmed by the study where it says that exposure to UVB may also lead to systemic immunosuppression possibly through the release of soluble mediators such as IL-1β, IL-10, IL-13, IL-17 and TNF-α from the UVB-irradiated epidermis for circulation [49]. The use of inhibitors such as 1-MT in addition to decreasing the expression of the IDO enzyme may provide partial protection against UVB-induced inflammation. These results are in agreement with Moreno and collaborators [50], they associate that the inhibition of the IDO and the KP by 1-MT can increase the production of melatonin in the skin cells, due to its antioxidant power and to reduce ROS in the local.

It is well established that the activation of the IDO enzyme results in a decreased concentration of TRYP and an increased concentration of TRYP metabolites, including KYN, in the local microenvironment [13]. In the present study we demonstrated that the expression of IDO is increased against acute exposure to UVB radiation and the inhibition of IDO by the topical administration of 1-MT showed a significant decrease, almost matching the naive. The decrease in the concentration of TRYP and increase in the concentration of KYN and consequently its metabolites, impair the proliferation and activation of immune cells [51,43]. QUIN is attributed to an increase in intracellular calcium concentration. Influx through the activation of glutamate receptors, inducing the activation of a cascade of intracellular enzymes such as nitric oxide synthase and xanthine oxidase, which then produce ROS, leading to oxidative stress and macromolecular damage and consequently cell death [48]. Our study demonstrates an increase of QUIN against UVB radiation, which relates to the production of oxidative stress, indicating that the inhibition of IDO by 1-MT could reduce the damage caused by this acid. KYNA is characterized as a non-selective antagonist at glutamate receptors, with high affinity for the co-agonist site of glycine and with the ability to antagonize the toxic effects of QUIN [48]. Glutamatergic receptors have been found to be present in non-neuronal tissue and have been shown to play functional roles in keratinocytes, suggesting that calcium influx through glutamatergic receptors in keratinocytes inhibits the re-epithelial process, affecting cell proliferation, differentiation, and migration in the cell cycle [52]. The other additional metabolites produced in the KP also show significant ability to cause toxic effects, such as 3-HK, 3-HAA and AA, produce highly reactive free radicals that play an important role in some pathogenic disorders, especially UV skin that are of great interest [48].

In addition to the IDO enzyme, we can show in our study that UVB radiation can also alter the expression of several other enzymatic transcripts of the KP, such as KMO, KYNU and KAT. As noted in previous studies, IDO is likely to be the main determinant of this response [53]. The enzyme KAT is responsible for the production of the metabolite, KYNA [54]. In our studies they are high in the auricular lesion caused by the UVB radiation when purchased from the naive. These results are in agreement with studies by Sheipouri and collaborators [11], which demonstrated that human skin fibroblasts and keratinocytes can release KYNA in response to UVB treatment, indicating that transcriptional changes in response to inflammatory cytokines and UVB are present in these cells. In our studies, the enzyme KYNU that produces AA is more expressed in the skin against UVB radiation. If our results are confirmed when compared with Guillemin and collaborators [55], which reports an increase in the transcript encoding KYNU after UVB radiation, it suggests that UVB radiation and/or release of inflammatory cytokines is likely to increase metabolite levels and induce toxicity. However, the KMO enzyme will eventually produce QUIN [11]. In our findings, they are increased against UVB radiation, indicating that the inhibition of IDO by 1-MT would block the whole cascade of events related to the KP, avoiding the production of toxic metabolites that would further impair the cells on UVB radiation.

Studies of the KP in the skin are very limited. Thus this pathway and the expression of the enzyme IDO against skin diseases should be a new path of studies. We thus characterized the quantification of the KP and their enzymes, using a novel approach, inducing inflammation through UVB radiation in the ears of mice. It is important to highlight that they demonstrate the relationship that these changes in the enzymatic activity of KP have on the level of their metabolites. Our findings report an increase in inflammation and oxidative stress, an increase in the production of the enzyme IDO against UVB radiation. This leads to an increase in other pathway enzymes and all of their metabolites, ultimately reducing the tryptophan in the medium, making it difficult for the lesion caused by UVB radiation in the mouse ear. When exposed with the inhibitor of the enzyme IDO 1-MT at the highest dose (2μg/μl) these results were reversed almost comparing naive. This provides important insights into the role of KP activation in inflammatory skin diseases. It also highlights potential therapeutic targets for IDO inhibitors, some of which are already under investigation. In a future work we can evaluate some natural inhibitor of this enzyme, which protects against inflammations such as those caused by UVB radiation on the immunomodulatory effects of the activation of the KP.

5. Conclusion

In conclusion, the data reported here demonstrated that attenuation of inflammation, oxidative stress, and reduction of kynurenines pathway metabolite levels are involved in the protective effect of the pharmacological inhibitor (1-MT) of the IDO enzyme on topical use. In addition, they can provide a novel therapeutic approach for the treatment and prevention of injuries caused by UVB radiation in the skin.

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Legends

Fig.1. Kynurenine pathway of tryptophan degradation in mammals. Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) catalyze the first step of the kynurenine pathway. 5-HT: serotonin; KATs: kynurenine aminotransferases; KYNU: kynureninase; KMO: kynurenine 3-monooxygenase; KYNA: kynurenic acid; 3-HK: 3-hydroxykynurenine; 3-HAO: 3-hydroxyanthranilic acid dioxygenase; QUIN: quinolinic acid.

Fig.2. Effect of 1-MT (0.5, 1 and 2 µg/µl) treatment on UVB irradiation-induced skin injury in mice. Ear edema **(A)** and myeloperoxidase activity **(B)** in ear mice submitted to UVB irradiation. Values are mean ± S.E.M. (n=6 per group). *: $p<0.05$, **: $p<0.01$ and***: $p<0.001$ when compared with naïve group; #: $p<0.05$ when compared with Vehicle/UVB (One ANOVA followed by post hoc Newman–Keuls multiple comparison test).

Fig.3. Effect of 1-MT (0.5, 1 and 2 µg/µl) treatment on stress oxidative parameters induced by UVB irradiation in mice. GSH **(A)**, protein carbonilation **(B)** and lipid peroxidation **(C)** levels in ear mice submitted to UVB irradiation. Values are mean ± S.E.M. (n=6 per group). *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ when compared with naïve group; #: $p<0.05$ when compared with Vehicle/UVB (One ANOVA followed by post hoc Newman–Keuls multiple comparison test).

Fig.4. Effect of 1-MT treatment on inflammatory markers induced by UVB irradiation in mice. TNFα **(A)**, IL-1β **(B)**, IL-6 **(C)**, INFγ **(D)**, IL-13 **(E),** IL-17 **(F)**, IL-10 **(G)** and NF-κB **(H)** levels in ear mice submitted to UVB irradiation. Values are mean ± S.E.M. (n=6 per group). *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$when compared with naïve group; #: $p<0.05$ when compared with Vehicle/UVB (One ANOVA followed by post hoc Newman–Keuls multiple comparison test).

Fig.5. Effect of 1-MT treatment on metabolites of KP induced by UVB irradiation in mice. Tryptophan **(A)**, kynurenine **(B)**, kynurenic acid **(C)**, 3-hydroxykynurenine **(D)** 3-hydroxyanthranilic acid **(E)**, anthranilic acid **(F)** and quinolinic acid **(G)** levels in ear mice submitted to UVB irradiation. Values are mean ± S.E.M. (n=6 per group). *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ when compared with naïve group; #: $p<0.05$ when compared with Vehicle/UVB (One ANOVA followed by post hoc Newman–Keuls multiple comparison test).

Fig.6. Effect of 1-MT treatment on enzymes of KP induced by UVB irradiation in mice. IDO **(A)**, KAT **(B)**, KMO **(C)** and Kynu **(D)** activities in ear mice submitted to UVB irradiation. Values are mean ± S.E.M. (n=6 per group). *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ when compared with naïve group; #: $p<0.05$ when compared with Vehicle/UVB (One ANOVA followed by post hoc Newman–Keuls multiple comparison test).

Figure 1

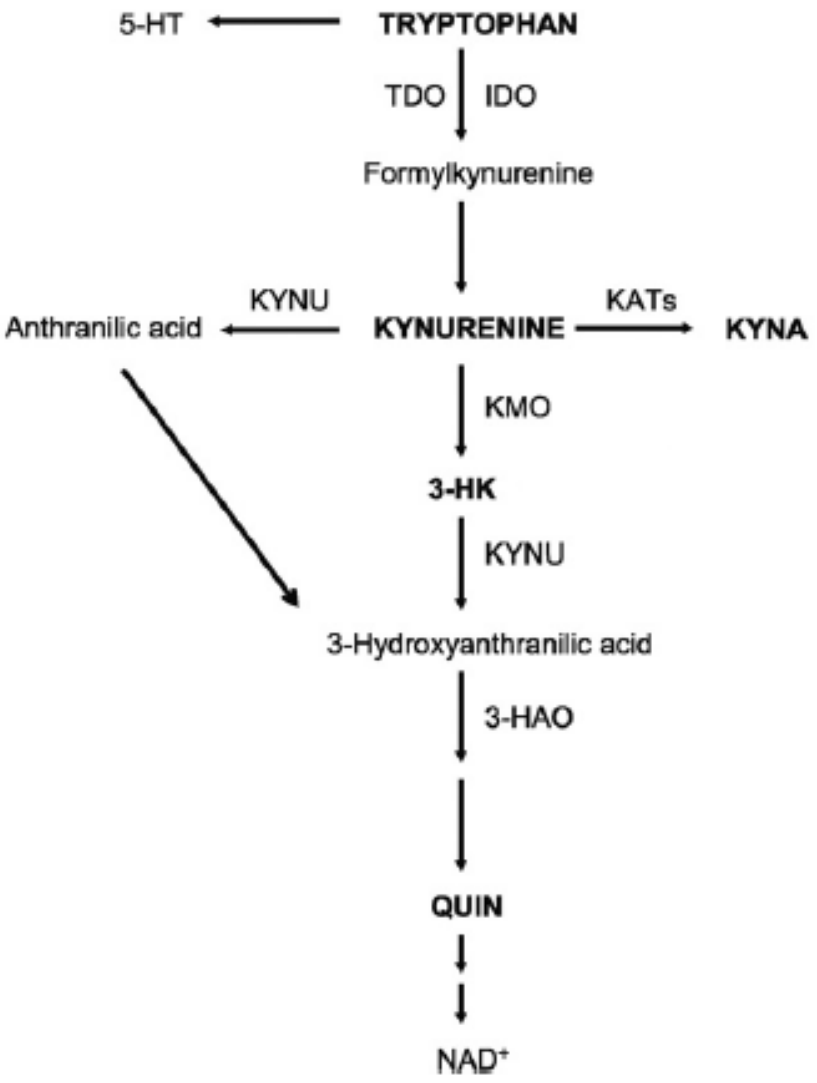

5-HT
TRYPTOPHAN
TDO
IDO
Formylkynurenine
KYNU
Anthranilic acid
KYNURENINE
KATs
KYNA
KMO
3-HK
KYNU
3-Hydroxyanthranilic acid
3-HAO
QUIN
NAD+

Figure 2

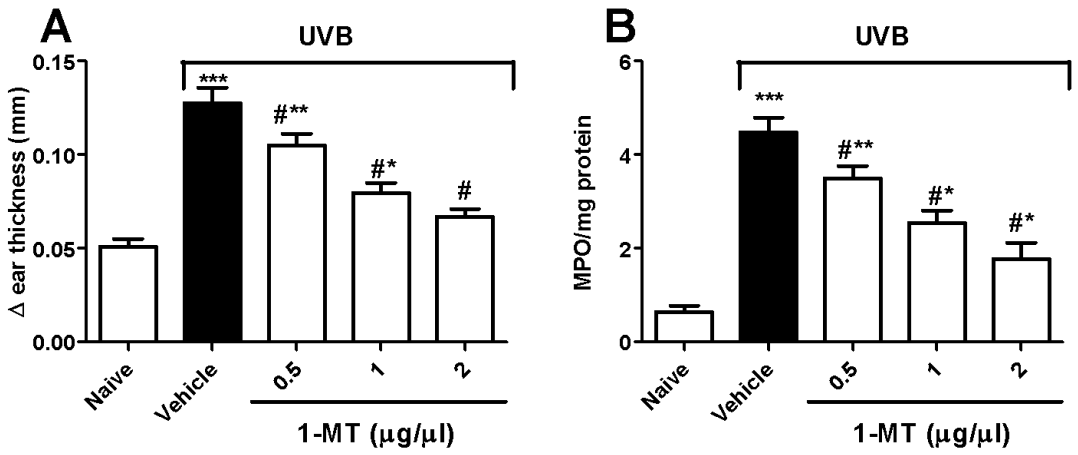

A
UVB
Δ ear thickness (mm)
0.15
0.10
0.05
0.00
***
#**
#*
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
B
UVB
MPO/mg protein
6
4
2
0
***
#**
#*
#*
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)

Figure 3

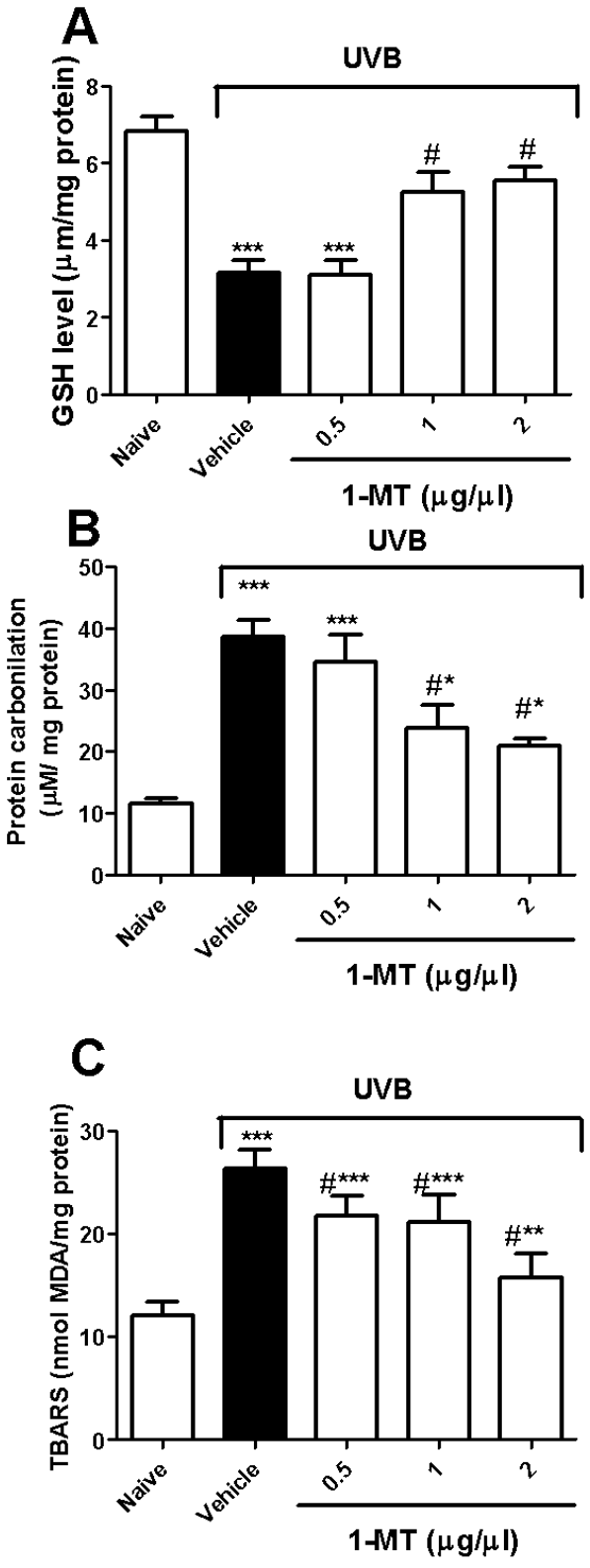

A
UVB
GSH level (μm/mg protein)
8
6
4
2
0
***
***
#
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
B
UVB
Protein carbonilation (μM/ mg protein)
50
40
30
20
10
0
***
***
#*
#*
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
C
UVB
TBARS (nmol MDA/mg protein)
30
20
10
0
***
#***
#***
#**
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)

Figure 4

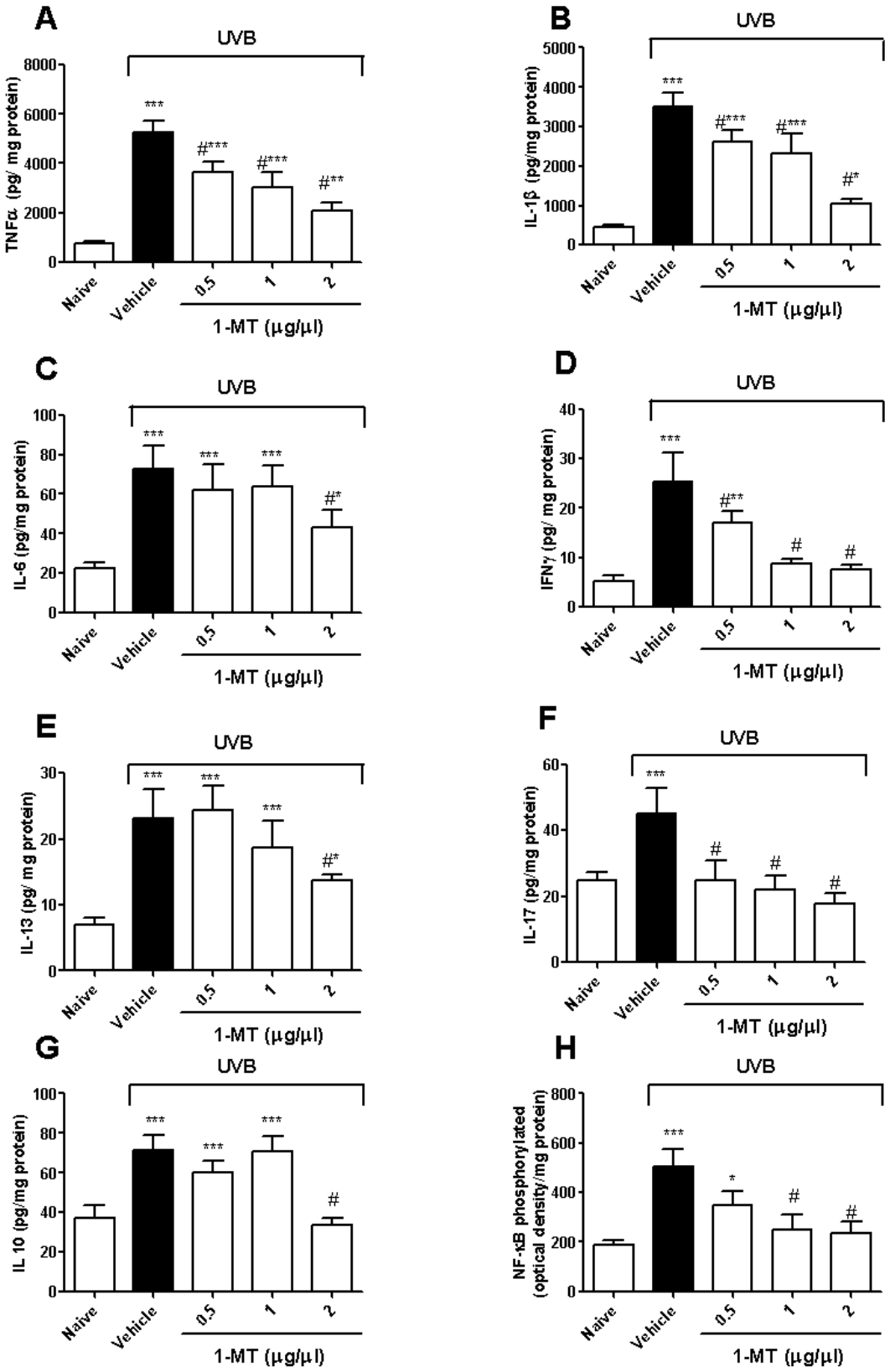

A
UVB
TNFα (pg/ mg protein)
8000
6000
4000
2000
0
***
#***
#***
#**
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
B
UVB
IL-1β (pg/mg protein)
5000
4000
3000
2000
1000
0
***
#***
#***
#*
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
C
UVB
IL-6 (pg/mg protein)
100
80
60
40
20
0
***
***
***
#*
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
D
UVB
IFNγ (pg/ mg protein)
40
30
20
10
0
***
#**
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
E
UVB
IL-13 (pg/ mg protein)
30
20
10
0
***
***
***
#*
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
F
UVB
IL-17 (pg/mg protein)
60
40
20
0
***
#
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
G
UVB
IL 10 (pg/mg protein)
100
80
60
40
20
0
***
***
***
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
H
UVB
NF-κB phosphorylated (optical density/mg protein)
800
600
400
200
0
***
*
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)

Figure 5

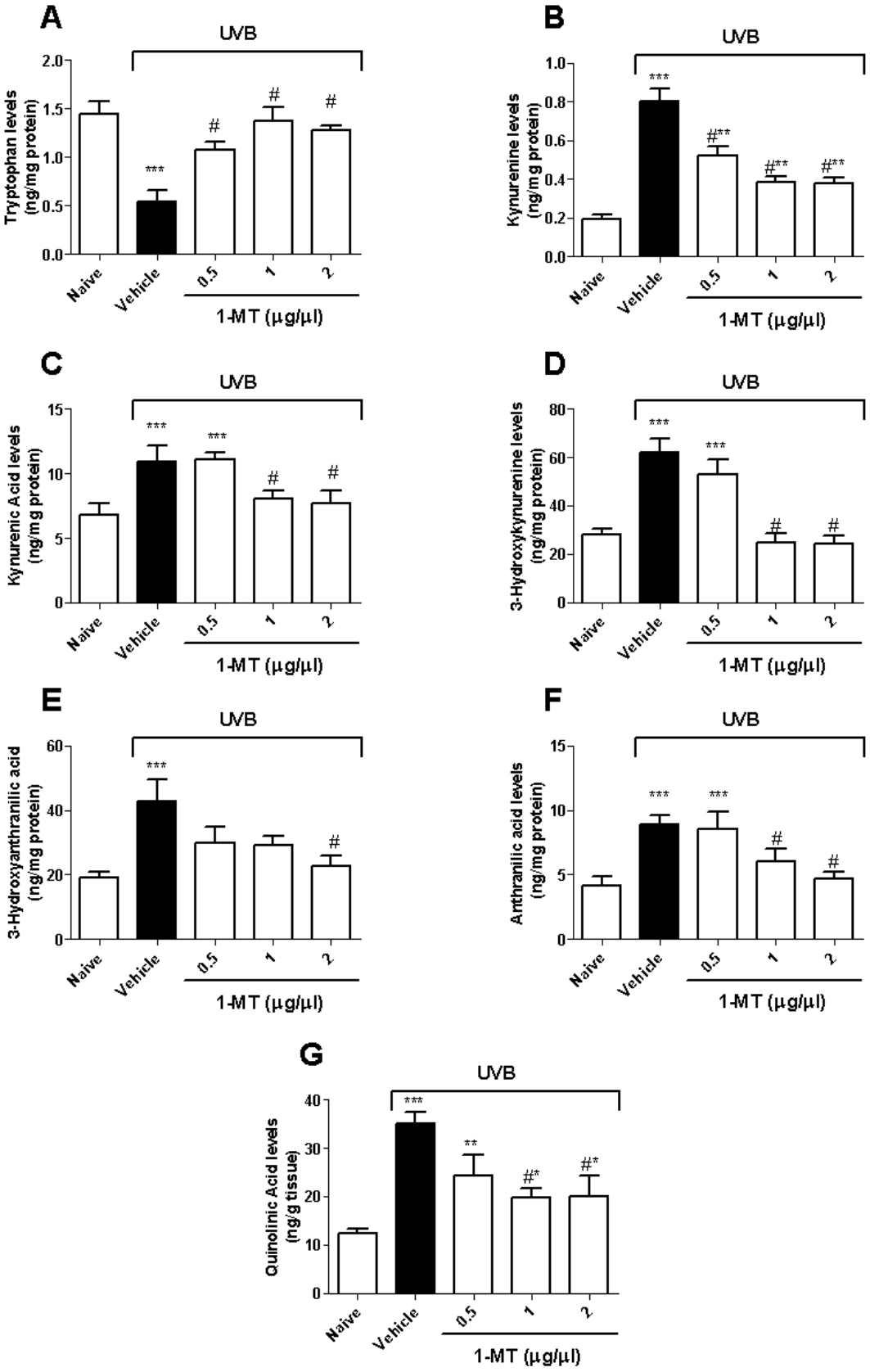
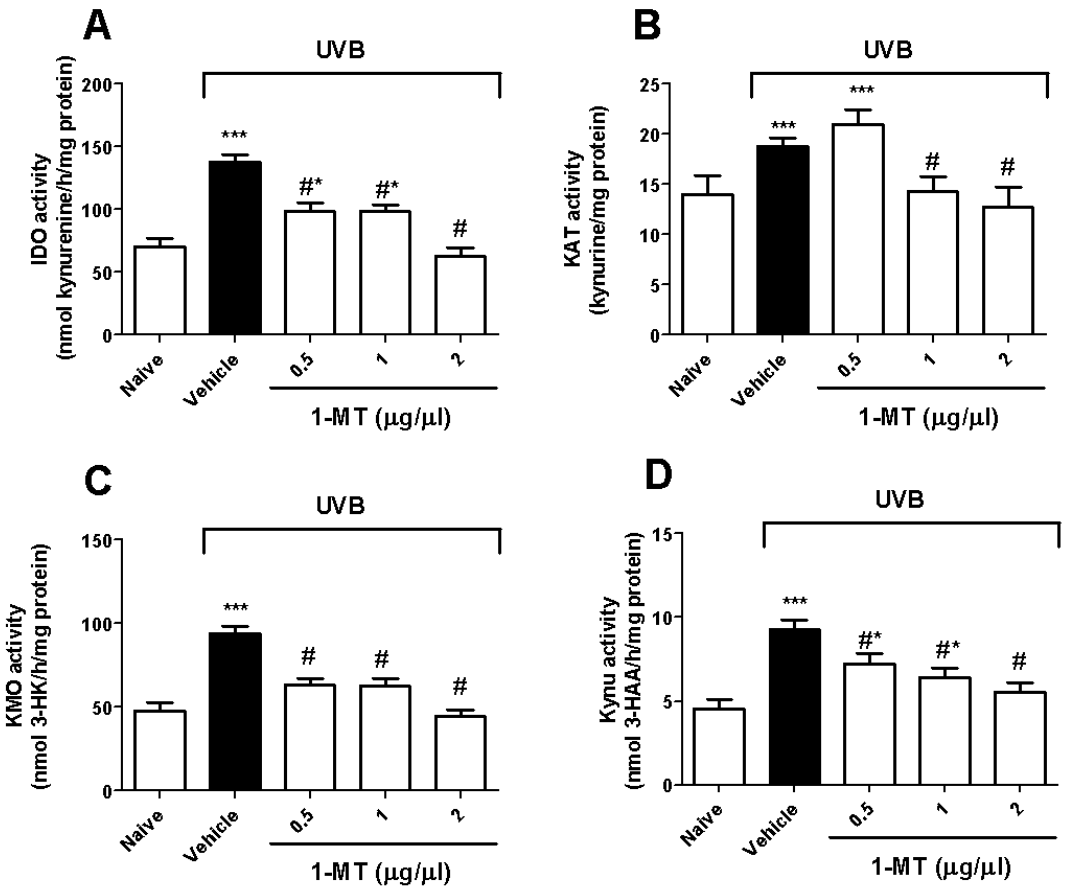

A
UVB
Tryptophan levels (ng/mg protein)
2.0
1.5
1.0
0.5
0.0
***
#
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
B
UVB
Kynurenine levels (ng/mg protein)
1.0
0.8
0.6
0.4
0.2
0.0
***
#**
#**
#**
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
C
UVB
Kynurenic Acid levels (ng/mg protein)
15
10
5
0
***
***
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
D
UVB
3-Hydroxykynurenine levels (ng/mg protein)
80
60
40
20
0
***
***
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
E
UVB
3-Hydroxyanthranilic acid (ng/mg protein)
60
40
20
0
***
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
F
UVB
Anthranilic acid levels (ng/mg protein)
15
10
5
0
***
***
#
#
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)
G
UVB
Quinolinic Acid levels (ng/g tissue)
40
30
20
10
0
***
**
#*
#*
Naive
Vehicle
0.5
1
2
1-MT (µg/µl)

Figure 6


A
UVB
IDO activity
(nmol kynurenine/h/mg protein)
200
150
100
50
0
***
#*
#*
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
B
UVB
KAT activity
(kynurine/mg protein)
25
20
15
10
5
0
***
***
#
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
C
UVB
KMO activity
(nmol 3-HK/h/mg protein)
150
100
50
0
***
#
#
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)
D
UVB
Kynu activity
(nmol 3-HAA/h/mg protein)
15
10
5
0
***
#*
#*
#
Naive
Vehicle
0.5
1
2
1-MT (μg/μl)

PARTE III

CONCLUSÃO

Em conclusão, os dados demonstram que a atenuação da inflamação, do estresse oxidativo e dos níveis dos metabólitos da via das quinureninas, estão envolvidos no efeito protetor do inibidor farmacológico (1-MT) da enzima IDO em uso tópico. Além disso, podem fornecer uma nova abordagem terapêutica para o tratamento e prevenção de lesões causadas pela radiação UVB na pele.

Como perspectivas futuras podem ser utilizadas diferentes dosagens do inibidor 1-MT, a exposição a radiação UVB pode ser mais prolongada e ainda podem ser utilizados inibidores naturais da IDO, como a cúrcuma, que já vem sendo estudada em vários artigos científicos.

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ANEXO

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÂO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55) 3413 4321, E-mail: ceua@unipampa.edu.br

Data: 22 de Março de 2013

PROTOCOLO N° 002/2013

Pesquisador: CRISTIANO RICARDO JESSE

Campus: Itaqui

Telefone: (55) 3433-1669

Título: AVALIAÇÃO DOS MECANISMOS ENVOLVIDOS NOS EFEITOS TIPO ANTIDEPRESSIVO E ANTI-HIPERALGÉSICO CAUSADO PELA HESPERIDINA EM CAMUNDONGOS

E-mail: cristianoricardojesse@yahoo.com.br

Após a análise detalhada do projeto de pesquisa a relatoria do CEUA-Unipampa emite parecer **FAVORÁVEL** para o cadastro do protocolo e execução do referido projeto.

Luiz E. Henkes
Professor Adjunto
Coordenador do CEUA/Unipampa