UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

AVALIAÇÃO DOS EFEITOS DA DIETILCARBAMAZINA SOBRE O PROCESSO DE INFLAMAÇÃO AGUDA PULMONAR EM CAMUNDONGOS

Aluna: Edlene Lima Ribeiro

Orientadora: Christina Alves Peixoto

Recife

2012

UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

AVALIAÇÃO DOS EFEITOS DA DIETILCARBAMAZINA SOBRE O PROCESSO DE INFLAMAÇÃO AGUDA PULMONAR EM CAMUNDONGOS

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciências Biológicas como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Pernambuco.

Aluna: Edlene Lima Ribeiro

Orientadora: Christina Alves Peixoto

Recife

2012

Catalogação na fonte
Elaine Cristina
CRB 1728

Ribeiro, Edlene Lima

Avaliação dos efeitos da dietilcarbamazina sobre o processo de inflamação aguda pulmonar em camundongos/ Edlene Lima Ribeiro. – Recife: O Autor, 2012.

70 folhas : il., fig., tab.
Orientadora: Christina Alves Peixoto
Dissertação (mestrado) – Universidade Federal de Pernambuco, Centro de Ciências Biológicas. Ciências Biológicas, 2012.

Inclui bibliografia e anexo

1. Farmacologia 2. Inflamação 3. Pulmões I. Peixoto, Christina Alves (orientadora) II. Título

615.1 CDD (22.ed.) UFPE/CCB-2012-216

BANCA EXAMINADORA

_____

Dra. Christina Alves Peixoto
Laboratório de Ultraestrutura – CPqAM/FIOCRUZ
(Orientadora)

_____

Maria Ignes Gaspar-Elsas
Laboratório de Fisiopatologia Humana, IFF, FIOCRUZ, Rio de Janeiro.

_____

Pedro Xavier-Elsas
Departamento de Imunologia, IMPPG, UFRJ, Rio de Janeiro.

À Deus

*Aos meus pais **Marizia** e **Jorge**, a minha irmã **Ediógia**, por sempre acreditarem e confiarem em mim e não mediram esforços para me verem realizada profissionalmente. Sinto muito orgulho de vocês e sou profundamente grata a tudo que fizeram por mim.*

*A **Emerson** por sempre me apoiar em todas as minhas decisões, sempre me incentivando a continuar.*

*As minhas queridas Vovós **Esmeralda** e **Antonieta** (in memoriam) amo muito vocês, maior referência em minha vida.*

AGRADECIMENTOS

À professora **Dr. Christina Alves Peixoto**, orientadora desta pesquisa. O respeito, admiração, gratidão e carinho multiplicados a cada dia. Agradeço a paciência e boa vontade que sempre demonstrou ao me atender e por todos os ensinamentos brilhantemente transmitidos ao longo destes anos. Obrigada pela oportunidade.

À Ceça, uma grande amiga a quem serei grata eternamente.

À Mari, pelo amizade e apoio.

À Fabi, pela amizade, momentos de descontração e ajuda.

À Bruna (Creide) pela amizade e ajuda.

A todos do laboratório de ultraestrutura, Sura, Karla, Ingrid, Karina pelo apoio, descontração e risadas.

À Gil, Laurimar, Eduardo e Rodrigo, do biotério experimental, pela grande ajuda e pelos momentos divertidos sempre.

À Adenilda, pela paciência.

A FACEPE pelo apoio financeiro.

RESUMO

A Dietilcarbamazina (DEC) tem sido utilizada no tratamento e controle da filariose linfática desde 1947. Apesar do seu longo período de utilização, pouco se sabe sobre o seu mecanismo de ação. Alguns estudos mostram que a DEC apresenta propriedades anti-inflamatórias como um resultado de sua interferência no metabolismo do ácido araquidônico, que inclui as enzimas lipooxigenase e ciclooxigenase (COX). O objetivo deste trabalho foi analisar a ação da DEC sobre a inflamação aguda pulmonar através das análises histológicas, contagem celular e dosagem de citocinas inflamatórias no tecido pulmonar e lavado pleural de camundongos submetidos a injeção intrapleural de carragenina. Camundongos machos da linhagem *Swiss webster* com 21 dias de idade foram utilizados. Os grupos tratados receberam DEC (50mg/kg/dia) ou Indometacina (5mg/kg/dia) (n=10) na água de beber, por três dias. Os grupos sham e carragenina receberam apenas água destilada. Após o esquema terapêutico de três dias os animais foram submetidos à injeção intrapleural de carragenina 1% ou veículo (carragenina e sham respectivamente) e após quatro horas os animais foram eutanasiados e coletado o líquido pleural para contagem celular e dosagem de citocinas. Os pulmões foram dissecados e processados para microscopia ótica, eletrônica, imunohistoquímica e Western Blot. O tratamento com DEC diminuiu significativamente a quantidade de polimorfonucleares na cavidade pleural e atenuou o grau de lesão celular, quando comparados com o grupo controle positivo. A análise ultraestrutural mostrou que a carragenina provocou dano tecidual, por outro lado, nos animais tratados com DEC, tais alterações não foram observadas. A análise da expressão de citocinas inflamatórias no tecido pulmonar e exsudato pleural demonstraram que a DEC reduziu os níveis de TNF-α, IL-1β, COX-2 e óxido nítrico quando comparados com o grupo controle positivo e padrão ouro (indometacina). Esses resultados indicam que a administração de DEC reduziu o desenvolvimento da inflamação em modelo experimental de pleurisia.

Palavras chaves: dietilcarbamazina, inflamação, pulmão.

ABSTRACT

Diethylcarbamazine (DEC) has been used in the treatment and control of lymphatic filariasis since 1947. Despite its long period of use, little is known about the mechanism of action. Some studies show that DEC has anti-inflammatory properties as a result of its interference with the arachidonic acid metabolism, including enzymes lipooxygenase and cyclooxygenase (COX). The objective of this study was to analyze the action of DEC on the acute inflammation of the lungs by histological analysis, cell count and inflammatory cytokines dosage in pleural lavage and lung tissue of mice subjected to intrapleural injection of carrageenan. Male mice of Swiss Webster strain after 21 days of age were used. The treated groups received DEC (50mg/kg/day) or indomethacin (5mg/kg/day) (n = 10) in the drinking water for three days. The positive or negative control group received only distilled water. After the treatment regimen of three days the animals underwent intrapleural injection of carrageenan 1% or vehicle (positive and negative control respectively) and after four hours the animals were euthanized and the pleural fluid collected for cell count and cytokine dosage. The lungs were dissected and processed for light microscopy, electron, immunohistochemistry and Western blot. Treatment with DEC significantly reduced the number of polymorphonuclear cells in the pleural cavity and attenuated the degree of cell damage compared with the positive control group. The ultrastructural analysis showed that carrageenan caused tissue damage, conversely, with DEC treatment these changes were not observed. Analysis of the expression of inflammatory cytokines in lung tissue and pleural exudate showed that DEC reduced the levels of TNF-α, IL-1β in addition to the levels of COX-2 and nitric oxide when compared with the positive control group and the gold standard (indomethacin). These results indicate that administration of DEC reduced the development of inflammation in an experimental model of pleurisy.

Keywords: diethylcarbamazine, inflammation, lung.

LISTA DE ABREVIATURAS E SIGLAS

AA	Ácido Araquidônico
AINE	Anti-Inflamatórios Não-Esteróides
BALF	Líquido Bronquioalveolar
CD95L	Indutor de Morte Celular
COX	Ciclooxigenase
COX-1	Ciclooxigenase-1
COX-2	Ciclooxigenase-2
DEC	Dietilcarbamazina
ERO	Espécie Reativa de Oxigênio
ICAM	Molécula de Adesão Intracelular
IL-1β	Interleucina 1β
IL-4	Interleucina-4
IL-5	Interleucina-5
INDO	Indometacina
iNOS	Sintase de óxido nítrico induzível
LTs	Leucotrienos
FAP	Fatores Ativadores das Plaquetas
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NO	Óxido Nítrico
NOS	Óxido Nítrico Sintase
NO_2	Nitrito
PGD_2	Prostaglandina D_2
PGE_2	Prostaglandina E_2
PGI_2	Prostaciclina$_2$
PMN	Polimorfonucleares
TNF-α	Fator de Necrose Tumoral
TXA_2	Tromboxano A_2
VCAM	Molécula de Adesão Vascular

SUMÁRIO

1- INTRODUÇÃO

A dietilcarbamazina (DEC) é um derivado da piperazina sintetizada como 1-dietilcarbamil-4-metilpiperazina, que tem sido utilizada no tratamento e controle da filariose linfática desde 1947 com ação nos vermes adultos e nas microfilárias (MOLYNEUX et al., 2003). É um pó branco, muito solúvel em água, estável, mesmo em condições de umidade e temperatura muito elevadas, e resiste inclusive, à autoclavagem (DREYER & NORÕES, 1997).

A DEC foi comercializada como um sal citratado por inúmeras companhias farmacêuticas, com diferentes nomes e atualmente é distribuída, sem custo, por órgãos responsáveis pelo controle de filariose linfática no Brasil. Apresenta vantagem sobre os outros fármacos utilizados no tratamento da filariose bancroftiana devido sua baixa toxicidade, intenso efeito microfilaricida e possibilidade de administração oral (DREYER et al.,1994). É rapidamente absorvida pelo trato gastrintestinal e atinge o pico da sua concentração plasmática entre uma e três horas após a ingestão (HAWKING, 1979). Vários esquemas terapêuticos de DEC têm sido utilizados tanto para cura de casos isolados, quanto para interrupção da transmissão da filariose, sendo o primeiro esquema terapêutico realizado em ciclos de 12 dias, na dose de 6mg/kg/dia (WHO, 1992, 1995).

O mecanismo de ação da DEC ainda é pouco conhecido, tem sido proposto que a DEC estimule rapidamente o sistema imune inato (MAIZELS & DENHAM 1992, SMITH, 2000) e poucos autores têm se detido sobre seus efeitos em células de vertebrados.

Florêncio e colaboradores (2005) demonstraram que após 12 dias de tratamento com DEC, os pneumócitos do tipo II apresentaram um grande número de vesículas maduras indicando uma ativação do metabolismo de surfactante pulmonar, além de ativação de macrófagos alveolares.

Observações clínicas anteriores demonstraram que a DEC é altamente eficaz no tratamento da eosinofilia pulmonar tropical, uma manifestação de hipersensibilidade causada por algumas espécies de filárias (BOGGILD et al. 2004).

Stenmark e colaboradores (1985) demonstraram que a DEC bloqueia a vasoconstrição e edema associado à produção de leucotrienos e hipóxia pulmonar. O estudo ainda demonstrou que a DEC inibe o desenvolvimento da Hipertensão Pulmonar,

hipertrofia ventricular direita e o influxo de polimorfonucleares e macrófagos alveolares.

Recentemente, estudos realizados em colaboração com o nosso laboratório demonstraram que a DEC tem importante ação no bloqueio da inflamação eosinofílica pulmonar em camundongos sensibilizados com ovalbumina. A DEC bloqueia a hiperreatividade pulmonar, a produção de citocinas Th2 e o acúmulo de eosinófilos, bem como a eosinofilopoiese *in vivo* e *in vitro* através de mecanismos iNOS/CD95L (QUETO et al., 2010).

Outros estudos em vertebrados mostram que este fármaco apresenta vários efeitos bioquímicos diretos em diferentes sistemas enzimáticos, incluindo glicólise, metabolismo do folato e aceticolinesterase (SUBRAHMANYAM, 1987).

A DEC altera o metabolismo do ácido araquidônico (AA) nas microfilárias e nas células endoteliais dos hospedeiros, visto que bloqueia a produção de leucotrienos (LTs) que são potentes vaso-bronco-constritores, como também inibe a produção de prostaglandina (PGE2), prostaciclina (PGI2) e tromboxano A2 (TXA_2), por bloquear a via da ciclooxigenase (COX) apresentando, portanto, propriedades anti-inflamatórias (revisão em MAIZELS & DENHAM, 1992).

A inflamação pode ser definida como uma resposta do sistema imune a danos celulares e teciduais causados por infecções microbianas ou estímulos nocivos de origem química ou física. A resposta inflamatória tem como principal função proteger o organismo contra infecções, bem como reparar os tecidos após eventuais lesões (HAANEN & VERMES, 1995; WEISS, 2002).

A inflamação aguda refere-se à resposta inicial promovida pela lesão tecidual, é mediada pela liberação de substâncias como histamina, serotonina, bradicinina, prostaglandinas e leucotrienos e, em geral precede o desenvolvimento da resposta imune adaptativa (PEREIRA & BOGLIOLO, 1998).

Na fase aguda da inflamação existe o envolvimento de neutrófilos, enquanto que, na fase tardia, monócitos/macrófagos migram para o sítio inflamatório (HUERRE & GOUNON, 1996).

A inflamação pulmonar, induzida por carragenina, envolve liberação de mediadores celulares, como Fator de Necrose Tumoral (TNF-α) e interleucina 1- β (IL-1β), liberação de histaminas, bradicininas, prostaglandinas, radicais livres e infiltração de neutrófilos. Atualmente os fármacos utilizados são os anti-inflamatórios não-esteróides como a indometacina, e dependendo do caso os glicocorticóides que

apresentam efeitos colaterais tais como osteoporose, intolerância à glicose, acne, catarata, ganho de peso, redução e perda de força muscular, diminuição dos níveis de testosterona, dentre outros, portanto o estudo de novos fármacos anti-inflamatórios visa contribuir com novas propostas terapêuticas. Desta forma, o presente estudo tem como objetivo caracterizar a atividade anti-inflamatória da DEC em modelo experimental de pleurisia.

1.1 OBJETIVOS

1.1.1 Objetivo Geral:

- Analisar a ação da DEC sobre a inflamação aguda pulmonar de camundongos machos *Swiss webster*.

1.1.2 Objetivos Específicos:

- Caracterizar o efeito do tratamento *in vivo* da DEC na concentração de 50mg/Kg, sobre a inflamação pulmonar de camundongos, induzida por carragenina, através da microscopia óptica e eletrônica de transmissão;
- Quantificar as concentrações de TNF-α e Óxido Nítrico (NO) no exsudato pulmonar após tratamento com DEC;
- Avaliar os efeitos anti-inflamatórios da DEC sobre a migração dos leucócitos, bem como sobre a presença de TNF-α, IL-1β, iNOS, NF-κB e COX-2 através de imunoistoquímica e Western Blot.

1.2 JUSTIFICATIVA

Apesar dos seus 50 anos de uso, a DEC teve o seu potencial farmacológico pouco explorado. Estudos farmacológicos mostraram que a DEC interfere no metabolismo do ácido araquidônico atuando, portanto, como um fármaco anti-inflamatório. Existem informações substanciais de que a DEC bloqueia etapas nas vias da ciclooxigenase e lipoxigenase, incluindo a inibição da quimiotaxia de leucócitos, desgranulação de granulócitos e vasodilatação periférica (MAIZELS & DEHAM, 1992).

A DEC também é eficaz no tratamento da eosinofilia tropical pulmonar, uma importante manifestação alérgica de filariose, além de alguns relatos clínicos de melhora da sintomatologia da asma, que poderia ser explicada pela inibição da produção de leucotrienos. No entanto, tais mecanismos subjacentes aos efeitos benéficos da DEC na inflamação eosinofílica pulmonar têm sido pouco investigados.

Estudos recentes demonstraram que a DEC tem importante ação no bloqueio da inflamação eosinofílica pulmonar em camundongos sensibilizados com ovalbumina, onde a DEC bloqueou hiperreatividade pulmonar, a produção de citocinas Th2 e o acúmulo de eosinófilos, bem como a eosinofilopoiese *in vivo* e *in vitro* (QUETO et al., 2010).

O atual projeto se propõe a caracterizar a ação do tratamento com DEC sobre a inflamação aguda pulmonar em camundongos *Swiss webster*.

CAPÍTULO I

1. REVISÃO BIBLIOGRÁFICA

1.1 DIETILCARBAMAZINA

A DEC é o filaricida mais amplamente utilizado no tratamento da filariose bancroftiana. Apresenta-se na forma de pó branco, muito solúvel em água, estável, mesmo em condições de umidade e temperatura muito elevadas, inclusive resistente a autoclavagem. A DEC é um derivado da piperazina sintetizada como 1-dietilcarbamil-4-metilpiperazina e preparada na forma de cloridrato, citrato ou fosfato. A partir de 1950, foi distribuída como sal citratado por inúmeras companhias farmacêuticas sob diferentes nomes, como Hetrazan, Banocide, Caricide, Carbilazine (DREYER & NORÕES, 1997). Atualmente é produzida pela Farmaguinhos e distribuída gratuitamente nos postos de saúde na Região Metropolitana do Recife.

A DEC é rapidamente absorvida pelo trato gastrointestinal e atinge o pico da sua concentração plasmática entre uma e três horas após a ingestão (HAWKING, 1979; RÉE et al., 1977; SAKUMA et al., 1967). De acordo com Ilondu e colaboradores (2000), ela está quase ausente na urina, plasma e saliva de humanos após 24 h da ingestão. Por outro lado, estudos toxicológicos e farmacológicos em camundongos indicaram que após 3 h o composto é completamente excretado pelo rim (HARNED et al., 1948). Horii e Aoki (1997) descreveram o nível plasmático de DEC em ratos após a administração de 200mg/kg, registrando valores de 30 μg/ml após 30-60 minutos da injeção, decrescendo rapidamente para 1,5 μg/ml após 4h e atingindo 0,1 μg/ml após 8 h do tratamento.

As reações adversas são o principal obstáculo ao uso terapêutico desta droga. As queixas mais comumente encontradas são náuseas, vômito, dores abdominais, diarréias, dores de cabeça, febre, sono, dores escrotais e mialgia que se estendem por um ou mais dias. Essas reações relacionam-se claramente à carga parasitária (FRANCIS et al.,1985; PARTONO et al., 1981).

A DEC possui outro papel terapêutico como uma droga anti-inflamatória (SALAZAR-MALLÉM, 1971; SRINIVAS & ANTANI, 1971; THIRUVENGADAM et al., 1974). Estudos clínicos demonstram que a DEC é altamente eficaz na eosinofilia pulmonar tropical, uma manifestação pulmonar causada por algumas espécies de filárias. Pacientes com eosinofilia pulmonar que foram tratados com DEC por 21 dias, apresentaram uma melhora acentuada de seus sintomas respiratórios. Indivíduos com eosinofilia pulmonar tropical muitas vezes recebem um diagnóstico incorreto de asma,

apesar de sua má resposta aos tratamentos convencionais para a asma. O reconhecimento precoce e tratamento da eosinofilia pulmonar tropical com dietilcarbamazina é a chave para minimizar a morbidade e mortalidade, pois a eosinofilia pulmonar tropical não-tratada pode levar à fibrose pulmonar irreversível (BOGGILD et al., 2004).

Queto e colaboradores (2010) demonstraram que a DEC diminui a expressão de citocinas como IL-5, IL-4 e eotaxina-1 na eosinofilia pulmonar em camundongos induzida por ovalbumina bem como reduz o número de células inflamatórias no lavado bronquioalveolar (BALF) e no tecido pulmonar. Além disso, estes autores demonstraram que a DEC atua através de mecanismos dependentes tanto iNOS como CD95L.

Essas observações confirmaram que a DEC promove a supressão de anormalidades funcionais e patológicas associadas à inflamação pulmonar, induzida por alérgenos, especialmente de eosinófilos presentes no sangue, BALF e tecido pulmonar.

Estudo realizado por Florêncio e colaboradores (2005) demonstrou que após 12 dias de tratamento com DEC os pneumócitos do tipo II apresentaram um grande número de vesículas maduras levando a uma ativação do metabolismo de surfactante pulmonar. Nos macrófagos alveolares foram detectadas várias características morfológicas de ativação celular, que poderiam ser explicadas por uma maior atividade endocítica dessas células, as quais são responsáveis pela fagocitose do surfactante secretado pelos pneumócitos do tipo II. O aumento da síntese e secreção de surfactante promoveria uma diminuição da tensão superficial dos alvéolos, reduzindo os esforços musculares decorrentes dos movimentos respiratórios. Tais resultados poderiam explicar o alívio dos sintomas da asma causados pelo tratamento com DEC como previamente observado por outros autores (LIU et al., 1995, 1996). Além disso, a DEC inibe a produção de leucotrienos, incluindo a cisteinil-leucotrieno (CysLT) que é considerada como o sítio da patofisiologia da asma (MATHEUS & MURPHY, 1982; ZUO et al., 2004).

Produtos da lipoxigenase são potentes agentes inflamatórios que induzem o aumento da permeabilidade vascular. Stenmark e colaboradores (1985) observaram que a DEC bloqueia a via da lipoxigenase na patogênese da hipertensão pulmonar induzida por monocrotalina em camundongos uma vez que o tratamento com DEC melhorou a pressão sistólica, peso cardíaco, número de polimorfonucleares (PMN) no BALF, diminuição de macrófagos e leucócitos ativados e diminuição nos níveis de prostaglandinas e tromboxano.

Maizels e Denham (1992) apresentaram uma hipótese para explicar o mecanismo microfilaricida *in vivo* da DEC. Este fármaco alteraria o metabolismo do ácido araquidônico nas microfilárias e nas células endoteliais do hospedeiro, visto que bloqueia a produção de LTs que são potentes vaso-bronco-constrictores. Mas, além de bloquear a via da 5- lipoxigenase, produtora de LTs, também inibe a produção pelas células endoteliais de PGE_2, PGI_2 e TXA_2 por bloquear a via da ciclooxigenase. A PGI_2, e em menor proporção a PGE_1 e PGE_2, são vasodilatadores e inibidores potentes da agregação plaquetária e da adesão endotelial. A ação da DEC sobre o metabolismo do AA, bloqueando a produção de PGE_2 e PGI_2 tanto na célula endotelial quanto nas microfilárias, levaria a uma vasoconstricção amplificando a adesão endotelial, e assim, propiciaria a imobilização do parasito circulante, aumentando a aderência e a atividade citotóxica das plaquetas e granulócitos do hospedeiro.

1.2 INFLAMAÇÃO AGUDA INDUZIDA POR CARRAGENINA

A inflamação é um conjunto complexo de interações entre fatores solúveis e células que podem surgir em qualquer tecido em resposta traumática, infecciosa, pós-isquêmica, tóxicas ou auto-imunes. Este mecanismo é composto por vários fenômenos complexos que se associam e se complementam uns aos outros formando uma reação em cascata, que envolve uma complexa interação de células inflamatórias (neutrófilos, linfócitos, monócitos/macrófagos) e das células vasculares (endoteliais e células da musculatura lisa) (TEDGUI & MALLAT, 2001).

O processo normalmente leva à recuperação da infecção e à cura. No entanto, se a destruição alvo e reparação não são devidamente efetivas, a inflamação pode levar ao dano tecidual persistente, por leucócitos, linfócitos ou deposicao de colágeno (NATHAN, 2002).

A inflamação é caracterizada, em sua forma aguda, por diversos eventos mediados por componentes solúveis, celulares e vasculares que induzem alterações morfológicas e bioquímica. Juntos esses eventos caracterizam os sinais clássicos da inflamação: dor, calor, rubor, edema e perda de função (KUMAR et al., 2005; RANG, 2001; GUALILO et al., 2000; GALLIN et al., 1992; PAUL, 1998).

A inflamação aguda é, no geral, de curta duração, podendo permanecer por alguns minutos, horas ou dias (SIQUEIRA & DANTAS, 2000). A resposta fisiológica que ocorre imediatamente após um estímulo agressivo é considerada como uma fase precoce (0-1 hora) ao contrário do que ocorre de 4-6 horas após a lesão sendo considerada a fase tardia da inflamação aguda, onde as células inflamatórias se acumulam no local lesado (ALBERTINI et al.,2004).

Vários fatores desempenham importantes papéis na modulação da resposta inflamatória de cada uma das fases da inflamação aguda. Na fase precoce, mediadores como a histamina e bradicinina modulam a resposta inflamatória aumentando o calibre e o fluxo vascular (KUMAR et al., 2005; ALBERTINI et al., 2004). Durante a fase tardia da inflamação aguda, há predominância de eventos celulares que se caracterizam pela marginação, adesão endotelial, diapedese e migração dos leucócitos para o foco da lesão, decorrentes dos estímulos quimiotáticos (KUMAR et al., 2005).

Várias células são responsáveis por iniciar a cascata de eventos do processo inflamatório, secretando ou sintetizando mediadores, sendo divididos em aminas vasoativas (histamina e serotonina); proteases plasmáticas (sistema de cinina - bradicinina, sistema complemento, sistema de coagulação-fibrinolítico); metabólitos do ácido araquidônico (via cicloxigenase e via lipoxigenase); proteases lisossômicas; radicais livres derivados do oxigênio; fatores ativadores das plaquetas (FAP); quimiocinas, citocinas e óxido nítrico (NAKAMURA et al., 2006, KUMAR et al., 2000; ALBERTINI et al., 2004). Estes mediadores têm papel determinante na gravidade, duração e recuperação da doença.

As citocinas são um tipo especial de mediadores que podem ser produzidos pelas células do tecido afetado, e atraem linfócitos e fagócitos (LUSTER et al., 2005), assim como estão envolvidos na regulação de células inflamatórias e no crescimento e diferenciação de leucócitos imaturos (LIN et al., 2000). Dentre as principais representantes podemos citar as interleucinas e fator de necrose tumoral (TNF).

O TNF-α, é uma citocina-chave que regula muitas respostas biológicas em células, incluindo inflamação, proliferação, diferenciação e morte celular (BRADHAM et al., 1998; WAJANT et al. , 2003). Promove a liberação de uma multiplicidade de citocinas, eicosanóides, glicocorticóides, e moléculas de adesão (JAWA et al., 2006). É um importante regulador da inflamação, regulando a produção de citocinas em células imunes. TNF-α induz ativação do endotélio através do aumento da produção de moléculas de adesão e mediadores químicos promovendo marginalização e migração de leucócitos para o local da inflamação (STITES et al., 1997). Os efeitos pleiotrópicos do TNF- α podem ser atribuídos à sua habilidade de simultaneamente ativar muitas vias de sinalização celular (HAN et al., 2009).

Por sua vez a IL-1β é uma das mais importantes interleucinas na resposta imune. A função predominante da IL-1β é aumentar a ativação de células T em resposta a antígenos. É secretada principalmente por macrófagos, mas também por neutrófilos, células endoteliais, célula muscular lisa, células gliais, astrócitos, células T e B, fibroblasto e queratinócitos (STITES et al., 1997).

Durante a evolução do processo de reparo, os eventos que se sucedem são a infiltração de neutrófilos, infiltração de macrófagos, fibroplasia e deposição de matriz extracelular, angiogênese, cicatrização e reepitelização. Citocinas, principalmente a IL-1β e o TNF-α, atuando sobre os receptores das células endoteliais, induzem a produção

de NO, bem como a expressão de moléculas de adesão para neutrófilos. A expressão das proteínas de adesão é, neste momento, o elemento mais importante para a migração de neutrófilos (GERSZTEN et al., 1999).

Modelos animais têm sido utilizados para avaliar o processo inflamatório através da indução de agentes químicos como, por exemplo, a carragenina (GUERINO et al., 2000; LUNARDELLI et al., 2006). A principal fonte de carragenina é a alga Chondrus Crispus, também conhecida como “Irish Nllos”, que tem origem em Carraghen (Waterford – Irlanda), onde cresce abundantemente (DI ROSA, 1972).

A carragenina é um polissacarídeo que vêm sendo utilizada em modelos experimentais como ferramenta para investigar o processo inflamatório em ratos e camundongos (LEVY, 1969).

A inflamação induzida por carragenina é um processo muito complexo envolvendo um grande numero de mediadores levando a hiperalgesia inflamatória (SAMMONS et al., 2000). Dentre esses mediadores podemos destacar produtos da cascata do ácido araquidônico (BLACKHAM et al. 1979), conteúdos de mastócitos (histamina) (BURITOVA et al., 1997), neurocininas (substancia P) (CODERRE & MELZACK, 1991), citocinas (IL-1β) (IANARO et al., 1999), oxido nítrico (SALVEMINI et al., 1996) e muitos outros.

A pleurisia em ratos e camundongos, induzida por carragenina, permite a quantificação do volume e da concentração protéica do exsudato formado, além da avaliação da migração de células inflamatórias para a cavidade pleural (KUMAR & SHIVKAR, 2004). Este tipo de pleurisia é utilizado na investigação da fisiopatologia da inflamação aguda e avaliação da eficácia de terapias anti-inflamatórias (ARRUDA et al., 2003).

A inflamação não-infecciosa é tradicionalmente tratada com fármacos anti-inflamatórias não-esteróides (AINES) que são um grupo variado de fármacos que têm em comum a capacidade de controlar a inflamação, de analgesia e de combater a hipertermia (BRUNTON et al., 2010).

Em 1971, Vane e colaboradores demonstraram pela primeira vez que a aspirina e indometacina inibem a produção de prostaglandinas pelo bloqueio da atividade enzimática da ciclooxigenase. Desde então, verificou-se que AINEs afetam diretamente a atividade da ciclooxigenase, seja por modificação covalente da enzima, como no caso da aspirina e de inibidores seletivos da COX-2, ou por competir com o substrato para o

sítio ativo, como acontece com praticamente todos os outros AINEs (WILLIAMS et al., 1999).

Os prostanóides podem ser gerados pelas duas isoformas de enzima cicloxigenase (COX): COX-1 e COX-2, ambas encontradas nas membranas do retículo endoplasmático e na membrana nuclear. A COX-1 está presente na maioria das células e sua expressão é constitutiva, sendo responsável pela síntese fisiológica de prostanóides (ROCCA & FITZGERALD, 2002). A COX-2 é uma isoforma induzida em resposta a estímulos que incluem lipopolissacarídeos, citocinas, interferons e fator ativador de plaquetas, contribuindo para a hiperalgesia e expansão do processo inflamatório (SJODAHL, 2001). Esta distinção entre as duas isoformas da COX apresenta algumas exceções, pois a COX-1 por ser induzida sob certas condições e a COX-2 é constitutivamente expressa em células de tecido cerebral, traquéia e rins (SJODAHL, 2001).

Os AINEs são amplamente utilizados em todo mundo por sua eficácia terapêutica no combate a inflamação e dor. Entretanto, os AINEs são conhecidos por seus efeitos colaterais no tubo digestivo (gastrointestinal) (WHELTON, 2001).

Devido à relativa escassez da expressão da COX-2 no trato gastrointestinal e sua grande expressão nos tecidos inflamatórios, foram desenvolvidos e introduzidos na terapêutica, a partir de 1999, os inibidores seletivos da COX-2, com o objetivo de minimizar a toxicidade gastrointestinal dos AINEs não-seletivos (BERTOLINI, 2001).

Os inibidores de COX-2 também têm sido avaliados quanto ao seu potencial antiproliferativo em ensaios *in vitro e in vivo*. Esta enzima encontra-se presente em células neoplásicas bem como na neovasculatura de alguns tumores humanos, entre eles mama, cólon, próstata e fígado (SJODAHL, 2001). Vários trabalhos têm demonstrado que a participação da Celecoxib, um inibidor seletivo da COX-2, exerce efeitos quimiopreventivos e antitumorais em câncer de cólon, uma das mais comuns doenças malignas epiteliais sólidas em todo o mundo (BOCCA et al., 2011).

Na hipertensao pulmonar Stenmark e colaboradores (1985) utilizando DEC como inibidor da COX-2 demostraram que a inibição da COX-2 bloqueou o desenvolvimento de hipertensão pulmonar e a hipertrofia do ventrículo direito, inibindo o influxo de polimorfonucleares e macrófagos, além de melhorar a vasocontricção e edema. Estes estudos indicam que a DEC apresenta uma importante atividade anti-inflamatória que pode ser melhor esclarecida.

2. REFERENCIAS BIBLIOGRÁFICAS

ALBERTINI R, AIMBIRE FS, CORREA FI, RIBEIRO W, COGO JC, ANTUNES E, TEIXEIRA SA, DE NUCCI G, CASTRO-FARIA-NETO HC, ZÂNGARO RA, LOPES- MARTINS RA. Effects of different protocol doses of low power galliumaluminum- arsenate (Ga-Al-As) laser radiation (650 nm) on carrageenan induced rat paw ooedema. J Photochem Photobiol: Biology. 2004;74:101- 107.

ARRUDA VA, GUIMARÃES AQ, HYSLOP S, ARAÚJO MF, BOM C, ARAÚJO AL. Bothrops lanceolatus (fer de lance) venom stimulates leukocyte migration into the peritoneal cavity of mice. Toxicon. 2003; 41:99-107.

BERTOLINI A, OTTANI A, SANDRINI M. Dual acting anti-inflammatory drugs: a reappraisal. *Pharmacol Res*. 2001; 44(6):437–450.

BLACKHAM, A., HALL, D. E., MANN, J. WOODS, A. M. Effects of cyclooxygenases inhibitors in models of acute inflammation, agents-actions-suppl. 1979; 4:193-201.

BOCCA, C., BOZZO, F., CANNITO,S., PAROLA, M., MIGLIETTA, A. Celecoxib inactivates epithelial-mesenchymal transition stimulated by hypoxia and/or epidermal growth factor in colon cancer cells. Mol Carcinog. 2011; 31.

BOGGILD, A. K., KEYSTONE, J. S., KAIN, K. C. Clin Infect dis, Tropical Pulmonary Eosinophilia: A Case Series in a Setting of Nonendemicity. Clin Infect Dis 2004; 39: 1123-1128.

BRADHAM CA, PLUMPE J, MANNS MP et al. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. Am. J. Physiol. 1998; 275:G387–G392.

BRUNTON , C. GOODMAN G. Manual de Farmacologia e Terapêutica. Editora Artmed. 1º edição. 2010.

BURITOVA, J., CHAPMAN, V., HONORE P. BESSEN, J. M. The contribuition of peripheral bradykinin B2 receptors to carrageenan-evoked oedema and spinal c-Fos expression in rats. Eur. J. Pharmacol. 1997; 320:73-80.

CODERRE, T. J. MELZACK, R. Central neural mediators of secondary hiperalgesia following heart injury in rats: neuropeptides and excitatory amino acids. Neurosci. Let. 1991; 131:71-74.

DI ROSA M. Pharmacological properties of carrageenan. J Pharm Pharmac. 1972; 24: 89-102.

DREYER, G., PIRES, M. L.; DE ANDRADE, L. D.; LOPES, E.; MEDEIROS, Z.; TENORIO, J.; COUTINHO, A. NOROES, J.; FIGUEREDO-SILVA, J. Torerance of diethylcarbamazine by microfilaremic and amicrofilaremic individuals in as endemic área of bancroftian filariasis, Recife, Brazil. Transactions of the royal society of Tropical Medicine and Hygiene 1994; 88: 232-236.

DREYER, G.; NORÕES, J. Dietilcarbamazina no tratamento da filariose brancrofitiana. Revista da Sociedade Brasileira de Medicina Tropical 1997; 30(3): 229-240.
FLORÊNCIO, M. S., SARAIVA, K. L. A., PEIXOTO, C. The effects on diethylcarbamazine on the ultrastruture of lung cell in vivo. Tissue Cell 2005; 37(3): 241-246.

FRANCIS, H.; AWADZI, K.; OTTESEN, E. A. The Mazzotti reaction following treatment of onchocerciasis with diethylcarbamazine; clinical severity as a function of infection intensity. The American Journal of Tropical Medicine and Hygiene 1985; 34(3)529-536.

GALLIN, J. I.; GOLDSTEIN, I. M.; SNYDERMAN, R. Inflammation: Basic principles and Clinical Correlates. New York: Raven press, 1992.

GERSZTEN RE, GARCIA-ZEPEDA EA, LIM YC, YOSHIDA M, et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium inder flow conditions. Nature 1999; 398: 718-723.

GUALILLO O, EIRAS S, LAGO F, DIÉGUEZ C, CASANUEVA FF. Elevated serum leptin concentrations induced by experimental acute inflamation. Life Sci. 2000; 67: 2433-2441.

GUERINO MR, et al. Laser treatment of experimentally induced chonic arthritis. Appl Surf Sci. 2000; 154: 561-564.

HAANEN, C., VERMES, I. Apoptosis and inflammation. London: Rapid communications of Oxford Ltd. 1995; 5-15.

HAN D, YBANEZ MD, AHMADI S, YEH K, KAPLOWITZ N. Redox regulation of tumor necrosis factor signaling. Antioxid Redox Signal. 2009; 11(9):2245-63.

HARNED, B. K. et al. Some toxicological and pharmacological properties of 1-diethylcarbamyl- 4-methylpiperazine hydrochloride, hetrazan. Annals of New York Academy of Science 1948; 141-160.

HAWKING, F. Dietilcarbamazine and new compounds for the treatment of filariasis. Advances in Pharmacology and chemotherapy. 1979; 16:129-94.

HORII, Y.; AOKI, Y. Plasma levels of diethylcarbamazine and their effects on implanted microfilariae of *Brugia pahangi* in rats. Journal of Veterinary Medical Science 1997; 59(10) 961-963.

HUERRE, M.R., GOUNON, P. Inflammation: patterns and new concepts. Res. Immunol. 1996; 147(7):417-434.

IANARO, A.; O'DONELL, C. A.; DI-ROSA, M.; LIEW, F. Y. A nitric oixide synthase inhibitor reduces inflammation, down-regulates inflamatory cytokines and enhances interleukin-10 production in carrageenan-induced oedema in mice. Immunology 1999; 82: 370-375.

ILONDU, N. et al. Pharmacokinetics of diethylcarbamazine: production by concentration in saliva. Biological and Pharmaceutical Bulletin 2000; 23(4): 443-445.
JAWA, R.S., KULAYLAT, M.N., BAUMANN, H., DAYTON, M.T. What is new in cytokine research related to trauma/ critical care. J Intensive Care Med. 2006; 21(2):63-85.

KUMAR V, ABBAS AK, FAUSTO N. ROBBINS E COTRAN: Patologia: bases Patológicas das Doenças. 7ª ed. São Paulo: Elsevier; 2005.

KUMAR, V. L.; SHIVKAR, Y. M. Involvement of prostaglandis in inflammation induced by latex of *Calotropis procera*. Mediators inflamm. 2004; 13:151.

LEVY, L. Carrageenan paw oedema in the mouse. Life Sci. 1969; 8:601-606.

LIN E, CALVANO SE, LOWRY SF. Inflammatory cytokines and cell response in surgery. Surgery 2000; 127(2):117-26.

LIU, M. et al. Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea pigs. Clinical and Experimental Allergy 1996; 26(3)270-275.

LIU, M.; WANG, L.; ENHORNING, G. Surfactant dysfunction develops when the immunized guinea-pig is challenged with ovalbumin aerosol. Clinical and Experimental Allergy 1995; 25(11) 1053-1060.

LUNARDELLI A, LEITE CE, PIRES MGR, OLIVEIRA JR. Extract of the bristles of Dirphia sp. icrease nitric oxide in a rat pleurisy model. Inflamm Res. 2006; 55: 129-135.

LUSTER, A.D.; ALON, R.; VON ANDRIAN, U.H. Immune cell migration in inflammation, present and future therapeutic targets. Nat. Immunol. 2005; 6(12): 1182-90.

MAIZELS, R.M.; DENHAM, D.A. Dietilcarbamazine (DEC): immunopharmacological interactions of anti-filarial drug. Parasitology 1992; 105: 849-860.

MATHEWS WR, MURPHY RC. Inhibition of leukotrienes biosynthesis in mastocytoma cell by diethylcarbamazine. Biochem. Pharmacol. 1982; 31: 2129-2132

MOLYNEUX, D., BRADLEY, M., HOERAUF, A., KYELEM, D., TAYLOR, M. Mass drug treatment for lymphatic filariasis and onchocerciasis. Parasitology 2003; 19(11): 516-522.

NAKAMURA E, KITAGAWA Y, OZAWA S, SUDA K, ANDO N, UEDA M, KITAJIMA M. Role of steroid administration to reduce inflammation after thoracotomy in a rat surgical stress model. J Surg Res 2006; 135(2):364-9.

NATHAN, C. Points of control in inflammation. Nature Insight 2002; 420:846-852.
PARTONO, F. et al. The long term effects of repeated diethylcarbamazine administration with special reference to microfilaraemia and elephantiasis. Acta Tropica 1981; 38(3):217-225.

PAUL, W. E. Fundamental immunology. New York: Lippincott-Raven, 1998.
PEREIRA F. E. L, BOGLIOLO, L. Inflamações in: patologia 1998; 2 ed. Cap 7, 112-148.

QUETO, T., XAVIER, P., GARDEL, M. A., LUCA, B., BARRADAS, M., MASID, D., SILVA, P. M., PEIXOTO, C. A. VASCONCELOS, Z. M., DIAS,E. P. GASPAR, M. I. iNOS/CD95L-dependent suppression of pulmonary and bone-marrow eosinophilia by Diethylcarbamazine. Am. J. Respir. Crit. Care Med. 2010; 181: 429-437.

RANG HP, DALE MM, RITTER JM. Farmacologia. 4ª ed. Rio de Janeiro: Guanabara Kooga; 2001.

RÉE, G.H. et al. Plasma level of diethylcarbamazine in man. Transaction of Royal Society of Tropical Medicine and Hygiene 1977; 71(6): 542-543.

ROCCA, B,; FITZGERALD, G. A. cyclooxigenase and prostaglandins: shaping up the ummune response. Int. Immunopharcol. 2002; 2:603-630.

SAKUMA, S. et al. Study of the distribution of the antifilarial agent diethylcarbamazine within the body using tritium labeling and whole-body autoradiography. Japanese Journal of Parasitology 1967; (16):179-187.

SALAZAR-MALLÉN, M. Treatment of intractable asthma with diethylcarbamazine citrate. Annals of Allergy 1971; 23(11): 534-537.

SALVEMINI, D.; WANG, Z. Q.; WYATT, P. S.; BOURDON, D. M.; MARINO, M. H.; MANNING, P. T.; CURRIE, M. G. Nitric Oxide: a key mediator in the early and late phase of carrageenan-induced rat inflammation. Br. J. Pharmacol. 1996; 118:829-838.

SAMMONS, J. M., RAVAL, P., DAVEY, P. T., ROGERS, D.; PARSONS, A. A. & BINGHAM, S. Carrageenan-induced thermal hiperalgesia in the mouse: role of nerve growth factor and the mitogen-activated protein kinase pathway. Brain research. 2000; 876:48-54.

SIQUEIRA JÚNIOR JF, DANTAS CJS. Mecanismos celulares e moleculares da inflamação. Rio de Janeiro: Medsi; 2000.

SJÖDAHL, R. Nonsteroidal anti-inflammatory drugs and the gastrointestinal tract. Extent, mode, and dose dependence of anticancer effects. Am J Med. 2001; 110(1A):66S-69S.

SMITH, H.L. Investigating development of infective stage larvae of filarial nematodes. Front in Biosc. 2000; 5: E95-E102.

SRINIVAS, H. V. ANTANI, J. Dietilcarbamazine in bronchial asthma. Ann Allergy. 1971; (29):418-421.

STENMARK, K. R., MORGANROTH, M. L., REMIGIO, K. L., VOELKEL, F. N., MURPH, R. C., HENSON, P. M., MATHIAS, M. M., REEVES, T. J.Alveolar

inflammation and arachidonate metabolism in monocrotaline-induced pulmonary hypertension. American journal of physiology 1985; 248(6 pt2): H859-66.

STITES, D. P., TERR, A. I., PARSLOW, T. G. Medical immunology. 9 ed. Stamford, Connecticut: APPLETON & LANGE, 1997.

SUBRAHMANYAM, D. Antifilarials and their mode of action. Ciba Found. Symp. 1987; (127):246-264.

TEDGUI A, MALLAT Z. Anti-inflammatory mechanisms in the vascular wall. Circ Res. 2001; 11: 877-887.

THIRUVENGADAM, K.V. et al. Diethylcarbamazine citrate in bronchial asthma. Journal of the Indian Medical Association 1974; 63(9): 278-281.

VANE, J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971; 231(25):232-5.

WAJANT, H., PFIZENMAIER, K., SCHEURIC, P. Tumor necrosis factor signaling. Cell death differ 2003; 10: 45-65.

WEISS, U. Inflammation. Nature Insight 2002; 420:845-891.

WHELTON, A. Renal aspects of treatment with conventional nonsteroidal anti-inflammatory drugs versus cyclooxygenase-2 specific inhibitors. Am J Med. 2001; 110:(Suppl)33S-42S..

WHO (World Health Organization). Lymphatic filariases infection & disease: control strategies. Report of a WHO/CTD/TDR Consultative Meeting held at the University sains Malaysia, Penang Malaysia 1995.

WHO (World Health Organization). Lymphatic filariases: The disease and its control. Fiftth report of the WHO Expert Committee on Filariases. Geneva. 1992.

WILLIAMS, C.S., MANN, M., DUBOIS, R.N. The role of cycloxygenases in inflammation, cancer and development. Oncogene 1999; 18:7908-7916.

ZUO L, CHRISTOFI FL, WRIGHT VP, BAO S, CLANTON TL. Lipoxygenasedependent superoxide release in skeletal muscle. J Appl Physiol 2004; 97:661–668.

CAPÍTULO II

MANUSCRIPT

Diethylcarbamazine (DEC) attenuates the development of carrageenan-induced lung injury in mice

Edlene Lima Ribeiro [a], Karla Patricia de Souza Barbosa [a] Ingrid Tavares Fragoso [a], Mariana Matos Aragão Donato[a], Fabiana Oliveira dos Santos Gomes [a], Bruna Santos e Silva [a], Amanda Karolina Soares e Silva[a], Sura Wanessa Santos Rocha [a], Valdemiro Amaro da Silva Junior[c], Christina Alves Peixoto [a,b]

[a] Laboratório de Ultraestrutura, Instituto Aggeu Magalhães, FIOCRUZ, Recife, Brazil.

[b] Laboratório de Microscopia e Microanálise, Centro de Tecnologias Estratégicas do Nordeste, Recife, Brazil.

[c] Departamento de morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Recife, Brazil.

Correspondence to:

Ms. E. L. Ribeiro, Instituto Aggeu Magalhães, FIOCRUZ, Av. Av. Professor Moraes Rego, s/n - Campus da UFPE - Cidade Universitária CEP: 50.670-420 Recife, Brazil;

ed_crbio@hotmail.com

ABSTRACT

The diethylcarbamazine (DEC), an antifilarial drug has a potent anti-inflammatory properties as a result of its interference with the arachidonic acid metabolism. In this study we evaluated the anti-inflammatory activities of DEC in mice model of acute inflammation, carrageenan-induced pleurisy. We evaluated here that DEC (administrated 50mg/kg, oral route, 3 day prior to carrageenan) exerts potent anti-inflammatory effects in this model. Injection of carrageenan into the pleural cavity induced accumulation of fluid containing a large number of PMNs in the pleural cavity, infiltration of PMNs in lung tissues, and increased production of nitrite, tumor necrosis factor-α, (TNF- α) increased expression of interleukin-1β (IL-1β), cyclooxygenases (COX-2) and inducible nitric oxide synthase (iNOS). Furthermore, carrageenan induced the expression of nuclear factor-κB (NFκB). Administration of DEC 3 day before the challenge with carrageenan significantly reduced of all the parameters of inflammation measured. The study shows that DEC can be a potential drug for the treatment of acute lung inflammation.

Keywords: Diethylcarbamazine, pleurisy, cytokines, cyclooxygenase, lung injury

1. INTRODUCION

Since 1947, diethylcarbamazine citrate (DEC) has been used in the treatment and control of lymphatic filariasis caused by the nematodes *Wuchereria bancrofti, Brugia malayi* and *B. timori.* DEC continues to play an important role as one of the drugs used in the Global Programme for the Elimination of Lymphatic Filariasis [1]. However, despite its long period of use, little is known regarding its mechanism of action.

Pharmacological studies have demonstrated that DEC affects the metabolism of arachidonic acid, thereby acting as an anti-inflammatory drug. Substantial evidence has demonstrated that DEC blocks a number of steps in both the cyclooxygenase (COX) and lipoxigenase pathways. This drug is a potent blocker of leukotriene production (LTs), bronchial vaso-constrictor substances, but also inhibits the production of prostaglandin (PGE2), prostacyclin (PGI2) and thromboxane A2 (TXA2) [2].

According to Mathews and Murphy (1982) [3], DEC inhibits the formation of LTB_4 and the sulfidopeptide leukotrienes, which are potent vaso/bronchoconstrictors, in mastocytoma cell while actually stimulating the formation of 5-hydroxyeicosatetraenoic acid (5-HETE), suggesting that the site of action of DEC in inhibiting leukotrienes formation may be the leukotrienes A_4 synthetase reaction. Also Bach and Brashler (1986) [4] showed that DEC inhibited the formation of sulfidopeptide leukotrienes in rat basophil leukemia cell.

Clinical studies have described that DEC is quite effective in the symptomatic treatment of bronchial asthma [5] [6]. Recent studies carried out in collaboration with our laboratory demonstrated that DEC has important role in blocking the pulmonary eosinophilic inflammation in mice sensitized with ovalbumin, effectively preventing the effects of subsequent airway resistance, Th1/Th2 cytokine production, pulmonary

eosinophil accumulation and eosinophilopoiesis *in vivo* and *ex vivo*. Besides, DEC directly suppressed IL-5–dependent eosinophilopoiesis in naive bone marrow [7].

Carrageenan-induced inflammation is a model of local acute inflammation commonly used to evaluate activity of anti-inflammatory drugs [8] and useful to assess the contribution of cells and mediators to the inflammatory process [9]. The inflammatory process is invariably characterized by the production of prostaglandin, leukotrienes, histamine, bradykinin, platelet-activating factor, interleukins and migrating cells [10].

The recruitment of polymorphonuclear cell (PMNs) out of the circulation into the inflamed tissue have a key function in the breakdown and remodeling of injured tissue [11] [12]. Moreover, macrophages participate in the progression of experimental pleurisy producing proinflammatory cytokines such as tumor necrosis factor-α (TNFα) and IL-1β [13].

The initial phase of carrageenan-induced acute inflammation (0-1h), which is not inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, has been attributed to the release of histamine, 5-hydroxytryptamine and bradykinin, followed by a late phase (1-6h) mainly sustained by prostaglandin release attributed to the induction of cyclooxygenase (COX-2) [13] [14].

Since DEC has anti-inflammatory properties as a result of its effect on the metabolism of arachidonic acid, the purpose of the present study was to investigate the anti-inflammatory action of DEC in a model of carrageenan-induced pleurisy (4h), we have determined the following end points of the inflammatory response: 1) PMN infiltration. 2) lung injury (histology and ultrastructure). 3) expression of TNF- α (ELISA and immunohistochemistry). 4) expression of IL-1β protein, COX-2 protein

(immunohistochemistry and western blot), iNOS protein (immunohistochemistry) and NFkB protein (western blot). 5) nitric oxide (NO) synthesis (nitrite concentration).

2. MATERIAL AND METHODS

2.1. Animals

Male Swiss mice (weight 20–25 g; CPqAM/PE, Brazil) were used following protocols institutionally approved (CEUA#LW-47/10). The animals were housed in a controlled environment and provided with standard rodent chow and water.

2.2. Experimental groups

Mice were randomly allocated into the following groups:

(I) Sham + water group: Sham group in which identical surgical procedures to the CAR group was performed, except that the saline was administered instead of carrageenan (N= 10);

(II) CAR + water group: Mice were subjected to carrageenan-induced pleurisy (N= 10);

(III) CAR + DEC group. Mice were subjected to carrageenan-induced pleurisy and diethylcarbamazine (50mg/kg oral route) 3 days prior to carrageenan (N= 10);

(IV) CAR + INDO group. Mice were subjected to carrageenan-induced pleurisy and Indomethacin (5mg/kg oral route) 3 days prior to carrageenan (N= 10);

The lymphatic filariasis therapeutic dose regimens recommended by World Health Organization (WHO) is 6 mg/Kg for 12 days [15]. Considering that the total metabolism rate of a mouse is approximately seven times that of the human the present study used 50 mg/Kg of DEC adjusted according to the mice body weight [16]. Indomethacin mg/kg was chosen in agreement with previous study [17].

2.3. Carrageenan-induced pleurisy

Mice were anaesthetized with the combination of 10% ketamine hydrochloride (115mg/kg) and xylazine 2% (10mg/kg) intramuscularly. After confirmation of analgesia of the animals a shaving the right chest was performed and in the sixth intercostal space was administered sterile saline or sterile saline containing 1% λ-carrageenan (0,1 ml) into the pleural cavity. At 4 h after the injection of carrageenan, the animals were killed by inhalation of CO2. The chest was carefully opened and the pleural cavity rinsed with 1ml of saline solution containing heparin (5 U/ml) [18]. The exudate and washing solution were removed by aspiration. Any exudate, which was contaminated with blood, was discarded. Samples of the fluid pleural cavity were collected to determine the total leukocyte contents.

Total leukocytes were determined in a Neubauer chamber diluting the exudates in Turk solution (1:20) [17]. Considering that the inflammatory response induced by carrageenan in the pleural space of the mice has a biphasic profile, peaking at 4 and 48 h after pleurisy induction, in this study, we measured the expression of inflammatory mediators 4 h after injection of carrageenan on the basis of previous studies [8].

2.4. Histological examination

Lung base biopsies were taken at 4 h after injection of carrageenan. The lung fragments were washed twice in PBS pH 7.2 and fixed in Bouin's solution for 8 hours (1% saturated picric acid, formaldehyde and 40% glacial acetic acid), dehydrated in increasing ethanol series, cleared in xylene, embedded and included purified paraffin (VETEC, São Paulo, SP, Brazil). Tissue sections of 5µm were cut using a microtome (Leica RM 2125RT) deparaffinized with xylene, stained with haematoxylin/eosin, and studied using light microscopy [19].

2.5. Electron transmission microscopy

The fragments of lung were fixed overnight in a solution containing 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer. After fixation, the samples were washed twice in the same buffer and then they were post-fixed in a solution containing 1% osmium tetroxide, 2 mM calcium chloride and 0.8% potassium ferricyanide in 0.1 M cacodylate buffer, pH 7.2, dehydrated in acetone, and embedded in Embed 812. Polymerization was performed at 60 °C for 3 days [20]. Ultrathin sections were collected on 300-mesh nickel grids, counterstained with 5% uranyl acetate and lead citrate and examined using a FEI Morgani 268D transmission electron microscope.

2.6. Immunohistochemical localization of TNF-α, IL-1β, COX-2 and iNOS

Five sections (5 µm in thickness) of each group were cut and adhered to slides treated with 3-amino-propyl-trietoxi-silane (APES [Sigma, USA]). Briefly, sections were deparaffinized with xylene and rehydrated in graded ethanol (100 to 70%). To minimize endogenous peroxidase activity, the slides were treated with 10% (v/v) H_2O_2 in water for fifteen minutes. The sections were washed with 0.01M PBS (pH 7.2) and then blocked with 1% BSA, 0.2% Tween 20 in PBS for 1h at room temperature. The sections were incubated overnight at 4ºC with anti-TNF-α antibody (ABCAM, CA, USA, 1:250), anti-IL-1β antibody (GenWay, San Diego, CA, 1:250), anti-COX-2 antibody (ABCAM, CA, USA, 1:400) and anti-iNOS (ABCAM, CA, USA, 1:50). The antigen-antibody reaction was visualized with avidin-biotin peroxidase (Dako Universal LSAB ® + Kit, Peroxidase) using 3.3-diaminobenzidine as the chromogen. The slides were counterstained in hematoxylin. Positive staining resulted in a brown reaction product. Five pictures at the same magnification were quantitatively analyzed using Gimp 2.6 software (GNU Image Manipulation Program, UNIX platforms).

2.7. Measurement of TNF-α levels

TNF-α levels were evaluated in the exudates at 4 h after the induction of pleurisy by carrageenan injection. The assay was carried out by using a colorimetric, commercial ELISA kit (ABCAM, CA, USA# cat. ab100747). Lower detection limit of the assay was of 60 pg/ml.

2.8 Measurement of NO

For the measurement of nitric oxide was used the Griess colorimetric reaction, which is the detection of nitrite ($NO2^-$), oxidation of NO in the pleural fluid. 50µl of the pleural fluid in duplicate ware added to an ELISA plate 96 wells, , followed by the same volume of Griess reagent, which is composed of 1% sulfanilamide diluted in 2.5% H_3PO_4 (solution A) and N-1-naphtyl-ethtylenodiamina also diluted in 2.5% H_3PO_4 (solution B). To prepare a standard curve, a solution of sodium nitrite in the initial concentration of 100µM was serially diluted in PBS. After incubation for 10 minutes in the dark, reading in the spectrophotometer at 490nm was performed. The absorbance of different samples was compared with the standard curve, and the results expressed as mean ± standard error of the duplicate, using GraphPad Prism software (v. 5.0) [21].

2.9 Western blot analysis for COX-2, IL-1β and NFkB

Lungs were quickly dissected, and then homogenized in a Wheaton Overhead Stirrer (nº 903475) in an extraction cocktail (10 mM Ethylenediamine tetraacetic acid (EDTA), 2 mM phenylmethylsulfonyl fluoride (PMSF), 100 mM sodium fluoride (NaF), 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate ($NaVO_4$), 10 mg of aprotinin and 100 mM Tris(hydroxymethyl)aminomethane, pH 7.4). Homogenates were centrifuged at 3000 xg for 10 min and the supernatant was collected and stored at -70º C until use for immunoblotting. Protein levels were determined using the Bradford method using bovine serum albumin as the standard [22]. The proteins (40 µg/µl) were

separated on 10% (COX-2 and NFkB) or 14% (IL-1β) sodium dodecyl sulfate polyacrylamide by gel electrophoresis under reduced conditions and were electrophoretically transferred onto nitrocellulose membrane (Bio Rad, CA, USA, Ref. 162-0115). After blocking overnight at 4ºC with 5% non-fat milk in TBS-T (Tris-buffered saline 0.1% plus 0.05% Tween 20, pH 7.4), the membranes were incubated at room temperature, for 3 h, with rabbit polyclonal antibody against COX-2 (1:1,000 dilution; ABCAM, CA, USA), IL-1 β (1:2,000 dilution, Genway, San Diego, CA), NFkB (1:200 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) diluted in buffer solution TBS-T containing 3% non-fat milk. After washing (six times, 10 min each) in TBS-T, the membranes were further reacted with horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (1:80,000 (Ref. A6154), and 1:80,000 (Ref. A5420) respectively, Sigma, USA), diluted in TBS-T with 1% nonfat milk, for 1h30min, at room temperature. An enhanced chemiluminescence reagent (Super Signal, Pierce, Ref. 34080) was used to make the labeled protein bands visible and the blots were developed on X-ray film (Fuji Medical, Kodak, Ref. Z358487-50EA). For quantification, the density of pixels of each band was determined by the ImageJ 1.38 program (available at http://rsbweb.nih.gov/ij/download.html; developed by Wayne Rasband, NIH, Bethesda, MD). For each protein investigated the results were confirmed in three sets of experiments. Immunoblot for β-actin was done as a control for the above protein blots.

After protein blots visualization with enhanced chemiluminescence, the protein antibodies were stripped from the membranes, which were reprobed with monoclonal anti-β-actin antibody (1:2,000 dilution, Sigma, USA) and subsequently protein densitometry was done.

2.11. Data analysis

All values are expressed as mean standard error (± S.E.M.) of the mean of n observations. For the in vivo studies n represents the number of animals studied. The results were analysed by one-way ANOVA followed by a Tukey post-test using GraphPad Prism (V. 5.0). A p-value less than 0.05 was considered significant.

3. RESULTS

3.1 Effect of DEC in leukocyte migration

The injection of carrageenan into the pleural cavity of mice induced an acute inflammatory response characterized by accumulation of fluid containing a large amount of PMNs. The number of PMNs was significantly reduced with prior treatment for 3 days with DEC and INDO 0.22 ± 0.09 and 3.72 ± 5.05, respectively, compared to the group that received carrageenan 21.63 ± 2.27 (Fig. 1).

3.2 Effect of Diethylcarbamazine on tissue damage induced by Carrageenan

Histological analysis showed that animals with carrageenan-induced pleurisy presented discrete alveolar thickening due to increased cellularity, mild hemorrhage and congestion, apoptotic cells, inflammatory cells (mononuclear and polymorphonuclear cells), pulmonary edema and emphysema (Fig. 2 A).Treatment for three days with DEC attenuated the degree of injury and the infiltration of PMNs (Fig. 2B). In the sham group lungs showed morphological characteristic preserved.

Pulmonary ultrastructural analyses of sham group animals showed preserved morphological pattern as respiratory spaces, including the alveolar epithelium composed by pneumocytes (not shown). The lung tissue of animals with carrageenan-induced pleurisy revealed pneumocytes type II with lamellar bodies containing electrodense granules, vacuoles and myelin bodies, characterizing cell suffering. Also numerous collagen fibers were observed in the interstitial space increasing its thickness (Fig. 3A and B). Animals treated with DEC present a preserved alveolar epithelium similar to the sham group (Fig. 3C).

3.3 Effect of DEC on of TNF-α, IL-1β, COX-2 and iNOS expression

In this study we have evaluated the TNF-α, IL-1β, COX-2 and iNOS expression in the lung tissue by immunohistochemical detection. Tissue sections obtained of from mice of the CAR group demonstrate positive staining for TNF-α in alveolar cell, macrophages, vascular wall (Fig. 4A, densitometry analysis 4C). In contrast, no staining for TNF-α was found in the lungs of mice treated with DEC (Fig 4B, densitometry analysis 4C). No positive staining for TNF-α was found in lung tissue from sham-treated mice.

Lung tissue sections from mice of the CAR group showed a positive reaction for IL-1β in alveolar macrophages (Fig. 5A, densitometry analysis 5C). Treatment with DEC for three days significantly reduced the degree of expression of IL-1β (Fig. 5B, densitometry analysis 5C). There was no labeling for IL-1β in lung tissues obtained from mice of the sham group.

Analysis by immunohistochemical of cyclooxygenase-2 in lung tissue sections obtained from mice treated with carrageenan revealed a higher expression for COX-2 (Fig. 6A, densitometry analysis C). The degree of COX-2 was significantly reduced in lung sections obtained from mice treated with DEC (Fig. 6B, densitometry analysis C). Sections of lung of the sham group mice expressed COX-2 levels at baseline.

Immunohistochemical analyses of lung sections obtained from mice treated carrageenan revealed positive staining for iNOS in alveolar macrophages (Fig. 7A, densitometry analysis C). DEC treatment significantly attenuated this iNOS expression (Fig. 7B, densitometry analysis C). Little staining for iNOS was observed in the lung tissue obtained from the sham group.

3.4 Effect of DEC on TNF-α e NO concentration in pleural exudate

The concentration of TNF-α in pleural exudate was analyzed by enzyme-linked immunosorbent assays (ELISA). The induction of pleurisy by carrageenan administration induced high levels of TNF-α in pleural exudate compared with the sham group. On the other hand treatment with DEC for 3 days before induction of pleurisy attenuated the production of TNF-α significantly. Treatment with indomethacin, in turn, produced no reduction of this proinflammatory cytokine levels (Fig. 8A).

Through the Greiss reaction were analyzed NO levels in pleural exudate. NO levels increased significantly in the exudate of CAR group compared with the exudate of sham group. Treatment with DEC and INDO significantly reduced NO levels when compared to the CAR group (Fig. 8B).

3.5 Western Blot analysis for COX-2, IL-1 β and NFkB

The presence of COX-2 in lung homogenate was investigated by Western blot 4 hours after induction of pleurisy. The basal levels of the COX-2 were detected in the sham group animals, and significantly increased in lung tissue of the CAR group. However treatment with DEC significantly reduced the expression of COX-2. Unexpectedly, treatment with indomethacin did not reduce the levels of COX-2 when compared with the CAR group (Fig. 9).

The expression of IL-1β in the lung homogenate baselines levels were detected in the sham group. The CAR group present increased levels of IL-1β when compared to the sham group. Conversely, DEC and INDO decreased significantly the IL-1β levels compared with the CAR group (Fig. 10).

NFkB p65 levels in the lung were also significantly increased at 4 h after carrageenan infection compared to the sham group. DEC and INDO decreased significantly the NFkB levels compared with the CAR group (Fig. 11).

4. DISCUSSION

The acute pulmonary inflammation is associated with an enhanced formation of the proinflammatory cytokines TNF-α and IL-1β, inducible COX-2, production de ROS, such as hydrogen peroxide, superoxide and hydroxyl radicals [9] [23]. We demonstrate that the injection of carrageenan in the pleural cavity induced infiltration de PMNs, lung injury, production proinflammatory cytokines as well as COX-2 and NOS.

The present work demonstrated by histological and ultrastructural analyses that DEC efficiently blocked the lung carrageenan injury characterized by cellular infiltration, edema, alveolar thickness, myelin bodies and large vacuoles.

The oxidative stress has been shown to play a critical role in the acute and chronic inflammatory response such as lung injury. Leukocyte activation prior to the cell responses involved in the acute inflammatory process such as neutrophils, promotes the release of several types of ROS [17]. The NO is another free radical, present during inflammation, synthesized by iNOS and capable of interacting with ROS to increase free radical actions [24]. These radicals are released by various cell types in response to stimulation with TNF-α, IL-1β, all of which activate a cytoplasmic form of the transcription factor NF-kappa B by releasing an inhibitory protein subunit [25].

Experimental evidence have clearly suggested that NF-κB plays a central role in the regulation of many genes responsible for the generation of mediators or proteins in acute lung inflammation associated with carrageenan administration such us TNF-α, IL-1β, nitric oxide synthase inducible (iNOS) and COX-2 [8]. We confirmed here that the inflammatory process caused by injection of carrageenan into the pleural cavity leads to

a substantial increase in the levels of TNF-α and IL-1β in the exudates as well as in lung tissue. Therefore, the inhibition of the liberation of TNF-α and IL-1β by DEC described in the present study could be attributed to the inhibitory effects of the activation of NF-κB. However further studies are necessary clarify the signal transduction patways involved.

In human lungs, neutrophil, eosinophil, macrophage, platelet, and airway epithelial cell have been described as the main cellular sources of lipoxygenase-derived arachidonic acid products and DEC has been utilized as a potent lipoxygenase inhibitor of alveolar macrophages by blocking the release of chemotactic activity [26]. Also, Stenmark et al. [27] found that DEC blocks the lipoxygenase pathway in the pathogenesis of pulmonary hypertension induced by monocrotaline in mice. Lipoxygenase products are potent inflammatory agents inducing vascular permeability and bronchoconstriction. According to these authors, the treatment with DEC improved systolic blood pressure and heart weight, reduced the number of PMN in bronchoalveolar lavage fluid, and decreased levels of prostaglandins and thromboxane.

Carrageenan-induced pleurisy is a well-characterized experimental model of inflammation utilized to evaluate cellular migration and other inflammatory parameters. Nonsteroidal anti-inflammatory drugs are effective in inhibiting both cell migration and exudation [28]. According to our results, the injection of carrageenan in the pleural cavity induced infiltration de PMNs however treatment with DEC significantly reduced the number of leucocytes in the exudate.

Tomlinson et al. [9] utilizing a carrageenan-induced model of acute inflammation demonstrated that after induction of pleurisy the influx of PMNs increases the production of COX-2 and iNOS. As the inflammation progressed and the cell population changed from PMN to mononuclear profile, there was a decrease in COX an

NOS activity. These authors suggested that in acute inflammation the use of selective inhibitors of COX-2 and iNOS may be more beneficial than existing therapies. In general iNOS-derived NO and COX-2-derived PGs are involved in acute inflammation as well as in chronic inflammation [29].

There is good evidence in carrageenan and other models of inflammation that enhanced formation of prostanoids following induction of COX-2 contributes to the pathophysiology of local inflammation [30] [31] and that selective inhibitors of COX-2 exert potent anti-inflammatory effects. Our results showed that after treatment with DEC significantly decreased the levels of COX-2 in pulmonary tissues as observed in other non-steroidal anti-inflammatory drugs. DEC inhibits platelet aggregation, possibly due to its effects on the COX pathway [2], which has similarities with the NO pathway since both have constitutive and inducible isoforms of their enzymes and are key regulators of inflammatory responses [32] [33].

In a study with knockout mice for the iNOS gene (iNOS -/-), McGarry et al. [34] demonstrated that nitric oxide synthase (iNOS) pathways likely exert an effect on DEC activity through the interaction with the cyclooxygenase. The authors found that DEC had no microfilaricide activity in iNOS-deficient mice infected with *B. malayi* and there was a remarkable reduction in COX-1 protein in the peritoneal exudate. Therefore, the iNOS/COX pathway appears to be an essential event in the rapid sequestration of microfilariae following treatment with DEC.

Queto et al. [7] demonstrated that DEC has an important action in blocking the eosinophilic lung inflammation in mice sensitized with ovalbumin reduced the amount of eosinophils in bronchoalveolar fluid in the tissue infiltrates in the generation of cytokines involved in the production, activation and migration of eosinophils, providing the first evidence of a therapeutic mechanism in a model of DEC pulmonary

eosinophilic inflammation. Interestingly DEC blocks the pulmonary hyperreactivity, Th2 cytokine production and accumulation of eosinophils, as well as eosinofilopoiese in vivo and in vitro through mechanisms iNOS/CD95L.

Inhibitors of NOS activity reduce the development of carrageenan-induced inflammation and support a role for NO in the pathophysiology associated with this model of inflammation [35] [36]. The present results showed the inhibiton of NOS activity after treatment with DEC since the formation of nitrite were evidently reduced in pleural exudates confirming.

In conclusion, our results demonstrate for the first time that administration of DEC in a model of acute inflammation induced by carrageenan decreased lung injury, cell migration of PMNs, formation of NO production and release of proinflammatory cytokines as well as COX-2 confirming previous observation that DEC effectively acts through NOS/COX mechanism.

5. ACKNOWLEDGEMENTS

This study was supported by the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), Aggeu Magalhães Research Center of the Oswaldo Cruz Foundation in Recife, Brazil (CPqAM/FIOCRUZ), Center for Strategic Technology in the Northeast (CETENE) and National Institute of Structural Biology and Bioimagem (INBEB).

6. REFERENCES

[1] Hewitt RIS, Kushner HW, Stewart E, White WS, Wallace Y. Experimental chemotherapy of filariasis III. Effect of 1-diethylcarbamazine-4-methylpiperazine hydrochloride against naturally acbquired filarial infections in cotton rats and dogs. J Lab Clin Med 1947; 32:1341-1329.

[2] Maizels RM, Denham DA. Dietilcarbamazine (DEC): immunopharmacological interactions of anti-filarial drug. Parasitology 1992; 105: 849-860.

[3] Mathews WR, Murphy RC. Inhibition of leukotrienes biosynthesis in mastocytoma cell by diethylcarbamazine. Biochem Pharmacol 1982; 31: 2129-2132.

[4] Bach MK, Brashler JR. Inhibition of the leucotriene synthetase of rat basophil leukemia cell by diethylcarbamazine, and synergism between diethylcarbamazine and piriprost, a 5- lipoxygenase inhibitor. Biochem Pharmacol 1986; 35: 425-433.

[5] Srinivas HV, Antani J. Dietilcarbamazine in bronchial asthma. Ann Allergy 1971; 29: 418-421.

[6] Thiruvengadam KV, Subramaniam N, Devarajan TV, Zachariah MGM. Diethylcarbamazine citrate in bronchial asthma. J Indian Med Assoc 1974; 63: 278-281.

[7] Queto T, Xavier P, Gardel MA, Luca B, Barradas M, Masid D, Silva PM, Peixoto CA, Vasconcelos, ZM, Dias EP, Gaspar MI. iNOS/CD95L-dependent suppression of pulmonary and bone-marrow eosinophilia by Diethylcarbamazine**.** Am J Respir Crit Care Med 2010; 181: 429-437.

[8] Impellizzeri D, Esposito E, Mazzon E, Paterniti I, Di Paola R, Bramanti P, Cuzzocrea S. Effect of apocynin, a NADPH oxidase inhibitor, on acute lung inflammation. Biochemical Pharmacology 2011; 81: 636–648.

[9] Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoughby D. Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. Br J Pharmacol 1994; 113(3):693-698.

[10] Vane J, Botting R. Inflammation V and the mechanism of action of antiinflammatory drugs. FASEB J 1987; 1(2):89-96.

[11] Salvemini D, Manning PZ, Zweifel BS, Seibert K, Connor J, Currie MG, Needleman P, Masferrer JL. Dual inhibition of nitric oxide and prostaglandin production contributes to the anti-inflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 1995; 96(1):301-308.

[12] Wagner JG, Roth RA. Neutrophil migration mechanisms, with an emphasis on the pulmonary vasculature. Pharmacol Rev 2000; 52(3):349-374.

[13] Crisafulli C, Mazzon E, Paterniti I, Galuppo M, Bramanti P, Cuzzocrea S. Effects of Liver x receptor agonist treatment on signal transduction pathways in acute lung inflammation. Respir Res 2010; 22: 11-19.

[14] Nantel F, Denis D, Gordon R, Northey A, Cirino M, Metters KM, Chan CC. Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation. Br J Pharmacol 1999; 128(4):853-859.

[15] World Health Organization. Global Programme to Eliminate Lymphatic Filariasis. Annual report on lymphatic filariases. Geneva 2002; 113.

[16] Terpstra AHMDifferences between humans and mice in efficacy of the body fat lowering effect of conjugated linoleic acid: role of metabolic rate. J Nutr . 2001; 131:2067-2068.

[17] Dalmarco EM, Budni P, Parisotto EB, Filho DW, Frode T S. Antioxidante effects of mycophenolate mofetil in a murine pleurisy model. Transpl Immunol 2009; 22:12-7.

[18] Menegazzi M, Di Paola R, Mazzon E, Genovese T, Crisafulli C, Dal Bosco M, Zou Z, Suzuki H, Cuzzocrea SG. lycyrrhizin attenuates the development of carrageenan-induced lung injury in mice. Pharmacological Research 2008; 58: 22–31.

[19] Saraiva KLA, Silva Junior VA, Dias ESF, Peixoto CA. Morphological changes in the testis induced by diethylcarbamazine. Reprod Toxicol 2006; 22:754–759.

[20] Saraiva KLA, Silva AKS, Wanderley MI, Araújo AA, Souza JRB, Peixoto CA. Chronic treatment with sildenafil stimulates Leydig cell and testosterone secretion. Int J Exp Pathol 2009; 90(4):454–462.

[21] Guevara I, Iwanejko J, Dembin´Ska-Kiec´ A, Pankiewicz J, Wanat A, Anna P, Gołabek I. Bartus S, Malczewska-Malec, M, Szczudlik A. Determination of nitrite / nitrate in human biological material by the simple Griess reaction. Clinica Chimica Acta 1998; 274: 177–188.

[22] Bradford MA. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1970; 72:240–54.

[23] Cuzzocrea S, Sautebin L, De Sarro G, Costantino G, Rombolà L, Mazzon E, Ialenti A, De Sarro A, Ciliberto G, Di Rosa M, Caputi AP, Thiemermann C. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. J Immunol 1999; 163(9):5094-104.

[24] Bakhle YS, Botting RM. Cyclooxygenase-2 and its regulation in inflammation Mediators of Inflammation 1996; 5:305-323.

[25] Shreck R, Bauerle PA. A role for oxygen radicals second messengers. Trends in Cell Biology 1991; 1: 39-42.

[26] Nomura H, Sato E, Konama S, Haniuda M, Kuba K, Nagai S, Izumi T. Histamine stimulates alveolar macrophages to release neutrophil and monocyte chemotactic activity. Lab Clin Med 2001; 138:226-235.

[27] Stenmark KR, Morganroth ML, Remigio KL, Voelkel FN, Murph RC, Henson PM, Mathias MM, Reeves TJ. Alveolar inflammation and arachidonate metabolism in monocrotaline-induced pulmonary hypertension. Am J Physiol 1985; 248(6 pt2) : H859-66.

[28] Saleh TSF, Calixto, JB, Medeiros YS. Effects of anti-inflamatory drugs upon nitrate and mieloperoxidase levels in the mouse pleurisy induced by carrageenan. Peptides 1999; 20: 949-956.

[29] Sakaguchi Y, Shirahase H, Kunishiro K, Ichikawa A, Kanda M, Uehara Y. Effects of combination of nitric oxide synthase and cyclooxygenase inhibitors on carrageenan-induced pleurisy in rats. Life Sciences 2006; 79: 442-447.

[30] Salvemini D, Manning PZ, Zweifel BS, Seibert K, Connor J, Currie MG, Needleman P, Masferrer JL. Dual inhibition of nitric oxide and prostaglandin production contributes to the antiinflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 1995; 96(1):301-308.

[31] Sautebin L, Ialenti A, Di Rosa M. Relationship between nitric oxide and prostaglandins in carrageenan pleurisy. Biochem Pharmacol 1998; 55:1113-7.

[32] Sano H, Hla T, Maier J, Crofford L, Case J, Maciag T, Wilder RL. In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. J Clin Invest 1992; 89: 97-108.

[33] Clancy RM, Abramson SB. Nitric oxide: a novel mediator of inflammation. Proc Soc Exp Biol Med 1995; 210: 93-10.

[34] McGarry HL, Plant LD, Taylor MJ Diethylcarbamazine activity against *Brugia malayi* microfilariae is dependent on inducible nitric-oxide synthase and the cyclooxygenase pathway. Filaria J 2005; 4:4.

[35] Van de Loo FA, Arntz OJ, Van den Berg WB Effect of interleukin 1 and leukaemia inhibitory factor on chondrocyte metabolism in articular cartilage from normal and interleukin-6-deficient mice: role of nitric oxide and IL-6 in the suppression of proteoglycan synthesis. Cytokine 1997; 9(7):453-62.

[36] Cuzzocrea, S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabo´ C Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radical Biol Med 1998a; 24(3):450-9.

7. LEGENDS TO FIGURES

Figure 1: Effect of Diethylcarbamazine (DEC: 50mg/kg, 3 days before) on cell migration in the initial phase (4 h) of the inflammatory reaction induced by carrageenan in mice. Data are the mean ± SEM of 10 mice for each group. *P <0.05 vs. carrageenan.

Figure 2: Effect of DEC treatment on histological alterations of lung after carrageenan-induced injury. (A-A1) Lung sections taken from mice with carrageenan-induced pleurisy demonstrated tissue injury as evidenced in: edema, cellularity enhancement and polimorphonuclear infiltration. (B-B1) Treatment with DEC 3 days prior the pleurisy demonstrated reduced lung injury and infiltration of PMNs. The figure is representative of at least 3 experiments performed on different experimental days. n= 10 mice for each group. Scale bar = 100 µm and 20 µm

Figure 3: Ultrastructural analysis of lung after carrageenan-induced injury and DEC treatment. (A and B) Lung sections from mice with carrageenan-induced pleurisy showing enhanced thickness of the interstitial space filled with collagen fibers (thin arrows), myelin bodies (arrowheads), vacuoles (asterisks) lamellar bodies containing electrodense granules (short arrows). (C) Lung treated with diethylcarbamazine presenting preserved pneumocytes cells. Bar = 2,000nm.

Figure 4: Effect of DEC on immunocytochemical localization TNF-α in the lung after carrageenan-induced pleurisy. (A)In tissue sections obtained from CAR group mice positive staining for TNF-α were mainly localized in inflammatory cells. (B) After treatment with DEC was reduced the degree of positive staining for TNF-α in the lung

tissues. Densitometry analysis of immunocytochemistry photographs for TNF-α from lung tissues was assessed (C). The figure is representative of at least 3 experiments performed on different experimental days. Data are expressed as mean ± S.E.M. from n = 5 mice for each group. ND: Not detected. * P<0,05 vs carrageenan. Scale bar = 20 µm

Figure 5: Effects of DEC on immunocytochemical localization of IL-1β. (A) At 4 h after carrageenan injection, the staining intensity for IL-1β substantially increased in alveolar macrophages. (B) No positive staining for IL-1 when DEC was administred 3 days before carrageenan injection. Densitometry analysis of immunocytochemistry photographs for IL-1-β from lung tissues (C). The figure is representative of at least 3 experiments performed on different experimental days. Data are expressed as mean ± S.E.M. from n = 5 mice for each group. ND: Not detected. * P<0,05 vs carrageenan. Scale bar = 20 µm

Figure 6: Effect of DEC on immunocytochemical localization of COX-2 in lung tissue after pleurisy induced by carrageenan. (A) In tissue sections of the CAR group positive labeling was detected on type II pneumocytes. (B)Treatment with DEC significantly reduced the COX-2 staining when compared to the carrageenan group, achieving levels similar to the sham group. Densitometry analysis of immunocytochemistry photographs for COX-2 from lung tissues (C). The figure is representative of at least 3 experiments performed on different experimental days. Data are expressed as mean ± S.E.M. from n = 5 mice for each group. ND: Not detected. *P<0,05 vs carrageenan. Scale bar = 20 µm

Figure 7: Effect of DEC on immunocytochemical localization of iNOS in lung tissue after pleurisy induced by carrageenan. (A) In tissue sections of the CAR group positive labeling was detected on alveolar macrophages. (B)Treatment with DEC significantly

reduced the iNOS staining when compared to the carrageenan group, achieving levels similar to the sham group. Densitometry analysis of immunocytochemistry photographs for iNOS from lung tissues (C). The figure is representative of at least 3 experiments performed on different experimental days. Data are expressed as mean ± S.E.M. from n = 5 mice for each group. *P<0,05 vs carrageenan. Scale bar = 20 µm

Figure 8: Effect of DEC on carrageenan-induced TNF-α and NO production in the lung. (A)TNF-α level were significantly elevated at 4h after carrageenan administration in CAR group when compared with sham mice. DEC significantly reduced TNF-α level, however, INDO did not reduce TNF-α level when compared with CAR group. Nitrite and nitrate level, stable NO metabolites, were significantly increased in the pleural exudates at 4h after carrageenan administration when compared with sham mice. DEC and INDO significantly reduced the nitrite and nitrate level exudates (B). Data are expressed as mean ± S.E.M. from n = 8 mice for each group. * P<0,05 vs carrageenan.

Figure 9: Effects of DEC on carrageenan-induced COX-2 expression in the lung. Basal expression of COX-2 was detected in lung samples of sham group, whereas COX-2 levels were substantially elevated in lung tissue obtained from animals at 4 h after carrageenan injection. DEC treatment reduced the expression of COX-2, however treatment with indomethacin did not decrease the levels of COX-2 (A and B). A - Representative blot of lysates obtained from pool 4 animals per group. B - Data are expressed as mean ± S.E.M. of 4 replications for each group. *P<0,05 vs carrageenan °P<0,05 vs DEC.

Figure 10: Effects of DEC on carrageenan-induced IL-1β expression in the lung. Basal expression of IL-1β was detected in samples of lung obtained from sham group, whereas levels of IL-1β was significantly increased in the lung tissue of animals 4 hours after injection of carrageenan. Treatment with DEC reduced expression of IL-1β as well as group INDO compared with CAR group. A- Representative blot of lysates obtained from pool 4 animals per group. B - Data are expressed as mean ± S.E.M. of 3 replications for each group. *P< 0,05 vs carrageenan.

Figure 11: Effects of DEC on carrageenan-induced NFkB expression in the lung. Basal expression of NFkB was detected in samples of lung obtained from sham group, whereas levels of NFkB was significantly increased in the lung tissue of animals 4 hours after injection of carrageenan. Treatment with DEC reduced expression of NFkB as well as group INDO compared with CAR group. A- Representative blot of lysates obtained from pool 4 animals per group. B - Data are expressed as mean ± S.E.M. of 3 replications for each group. *P<0,05 vs carrageenan.

FIGURE 1

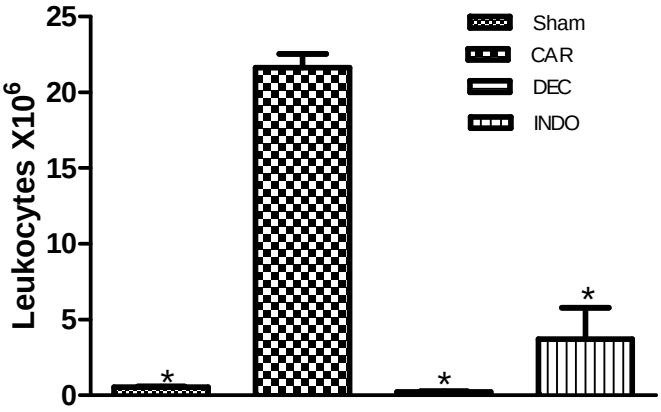


FIGURE 2

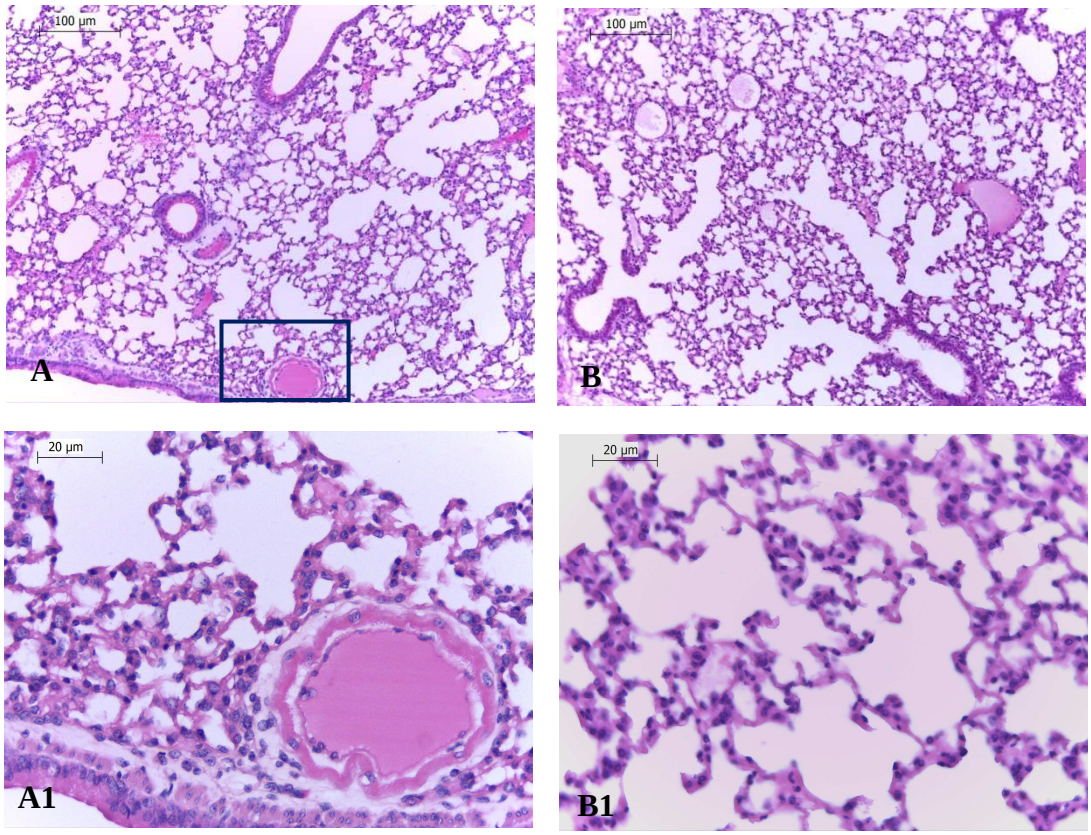


FIGURE 3

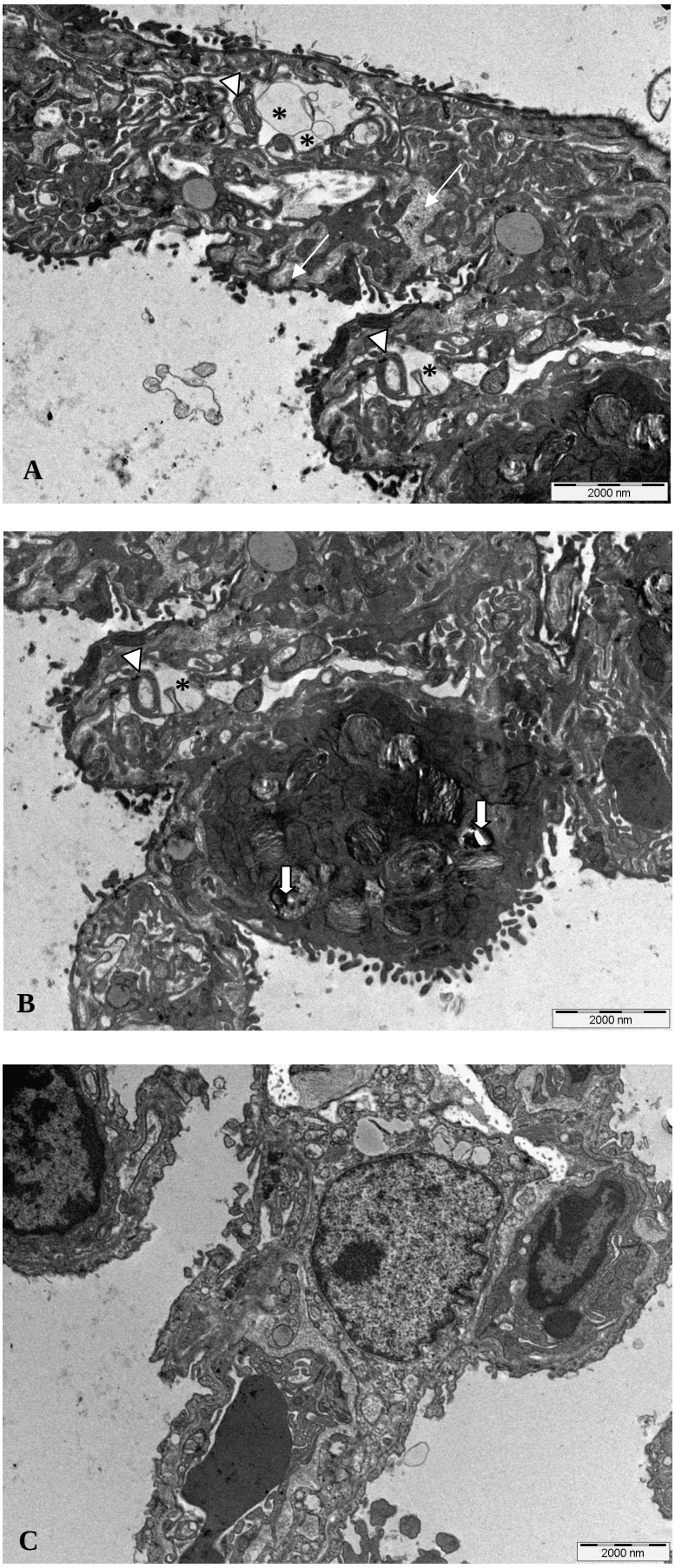


FIGURE 4

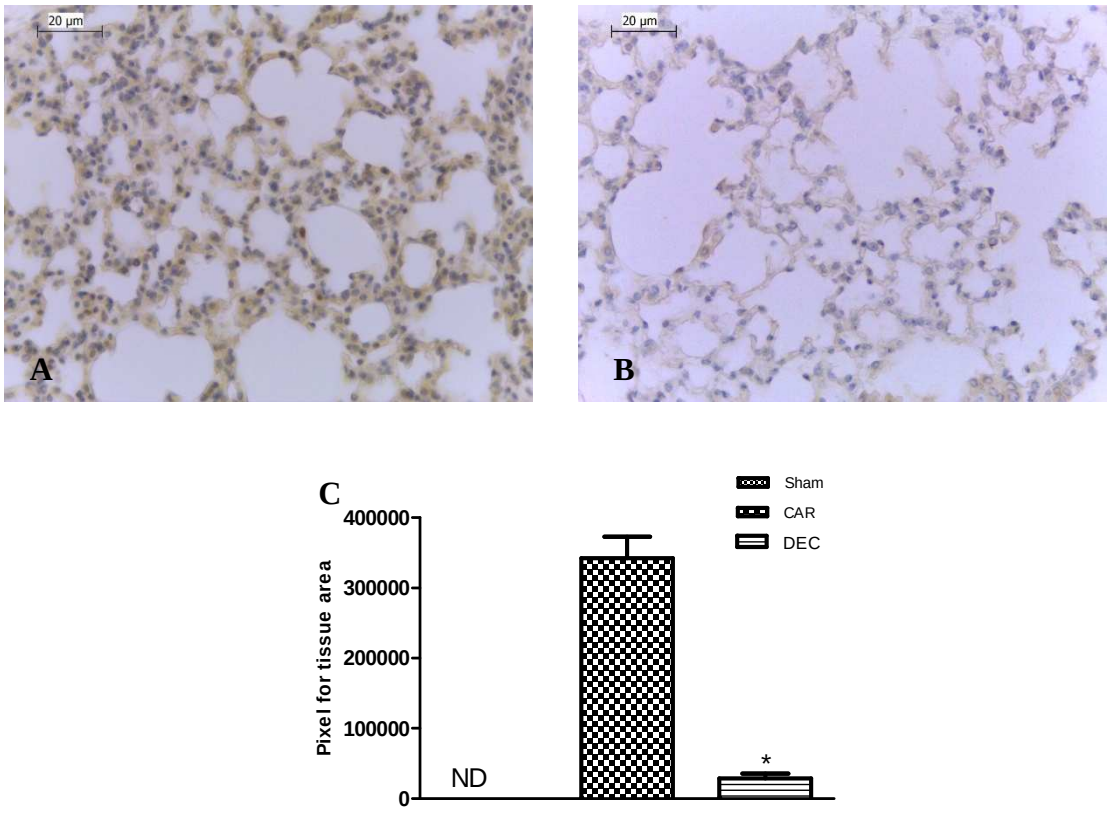

20 µm
A
20 µm
B
C
Sham
CAR
DEC
Pixel for tissue area
400000
300000
200000
100000
0
ND
*

FIGURE 5

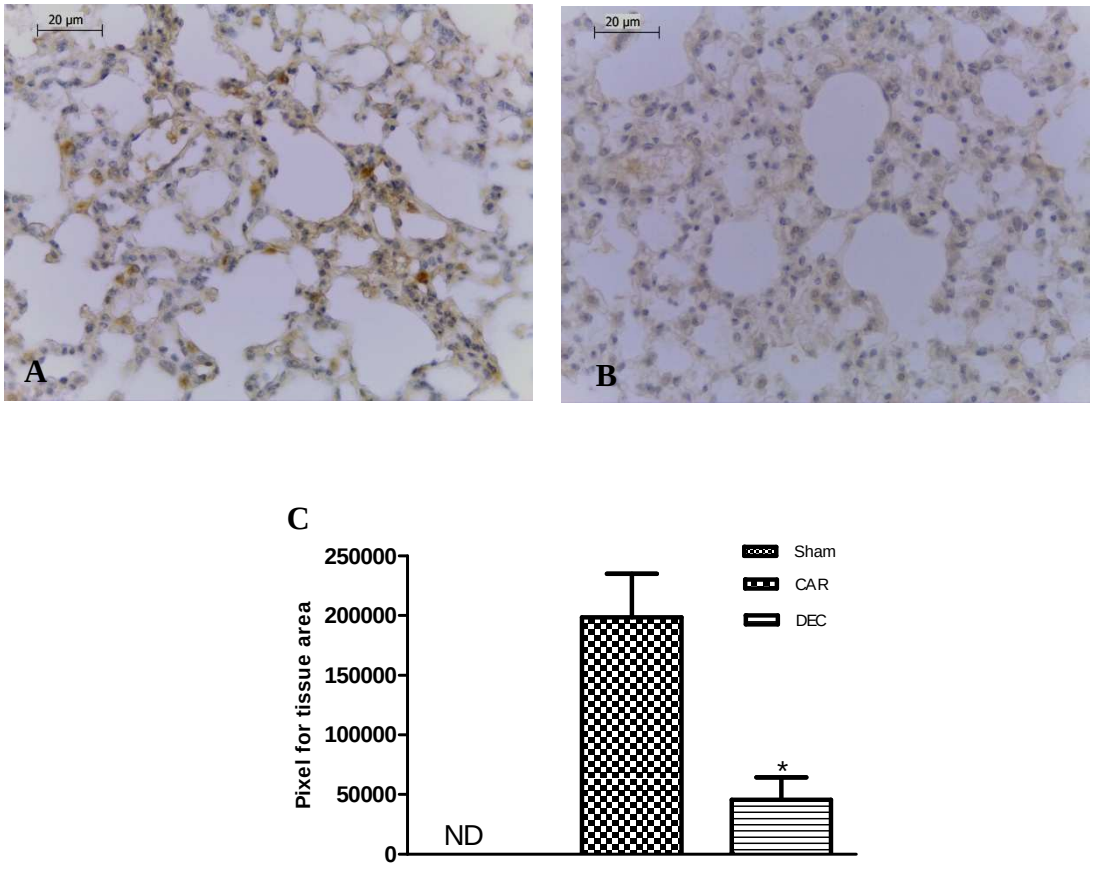

20 µm
A
20 µm
B
C
Pixel for tissue area
250000
200000
150000
100000
50000
0
ND
Sham
CAR
DEC
*

FIGURE 6

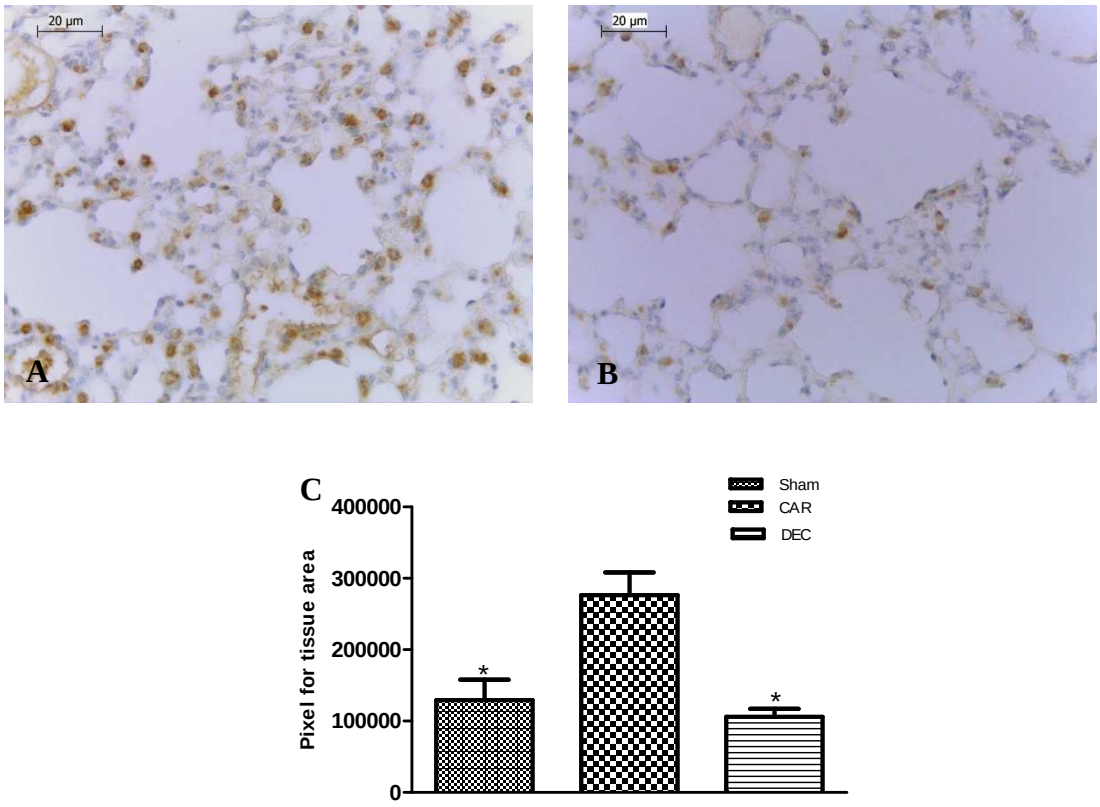

20 µm
A
20 µm
B
C
Sham
CAR
DEC
Pixel for tissue area
400000
300000
200000
100000
0
*
*

FIGURE 7

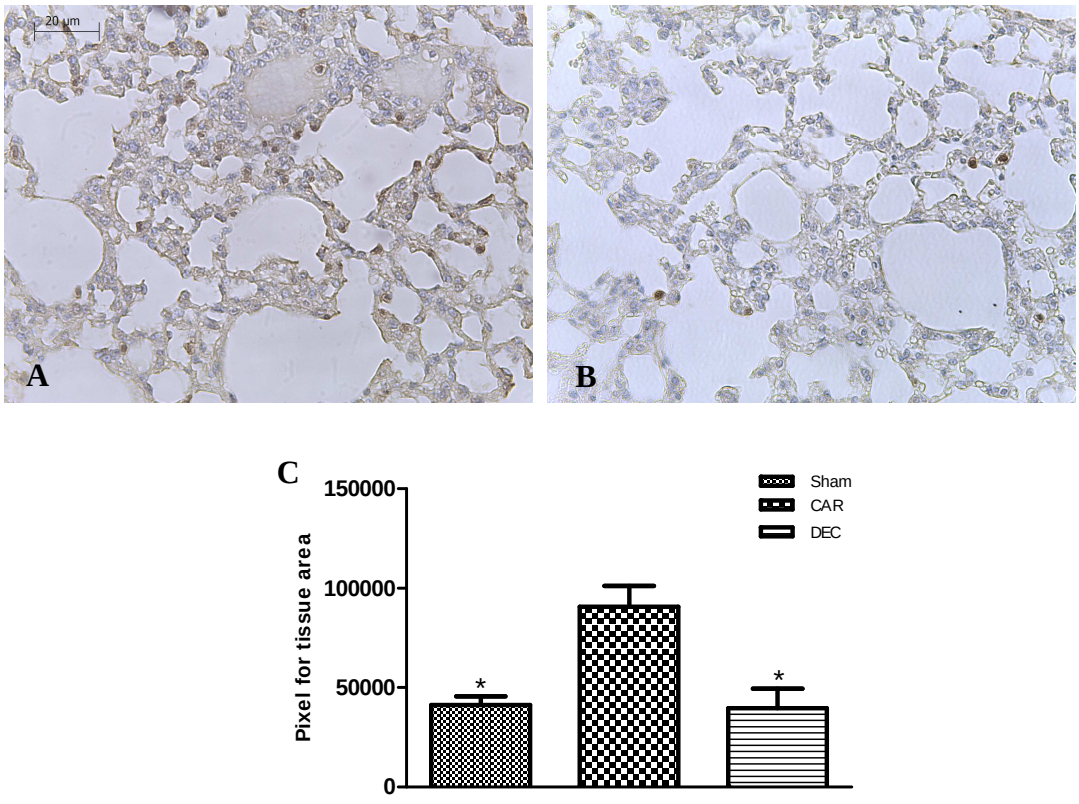

20 µm
A
B
C
Pixel for tissue area
150000
100000
50000
0
Sham
CAR
DEC
*
*

FIGURE 8

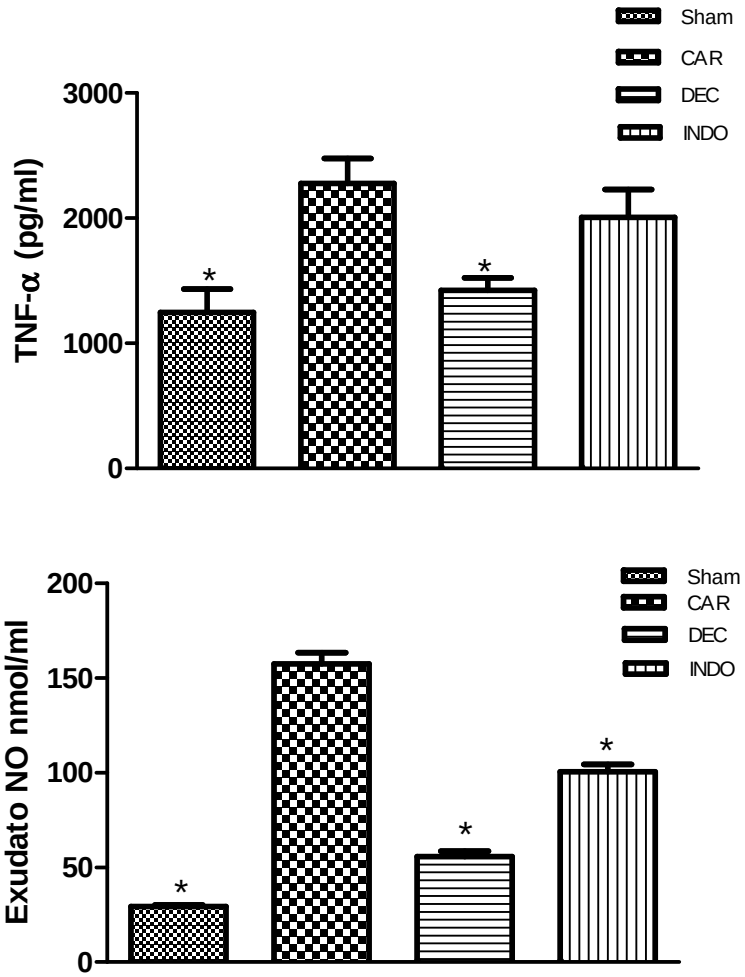
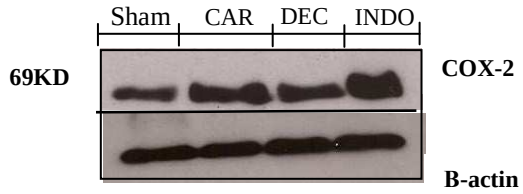

Sham
CAR
DEC
INDO
TNF-α (pg/ml)
3000
2000
1000
0
*
*
Sham
CAR
DEC
INDO
Exudato NO nmol/ml
200
150
100
50
0
*
*
*

FIGURE 9


Sham
CAR
DEC
INDO
69KD
COX-2
B-actin

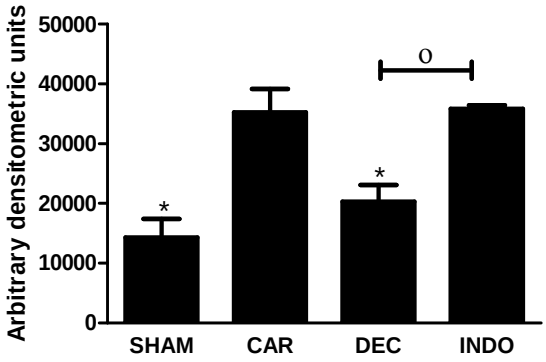

Arbitrary densitometric units
50000
40000
30000
20000
10000
0
o
*
*
SHAM
CAR
DEC
INDO

FIGURE 10

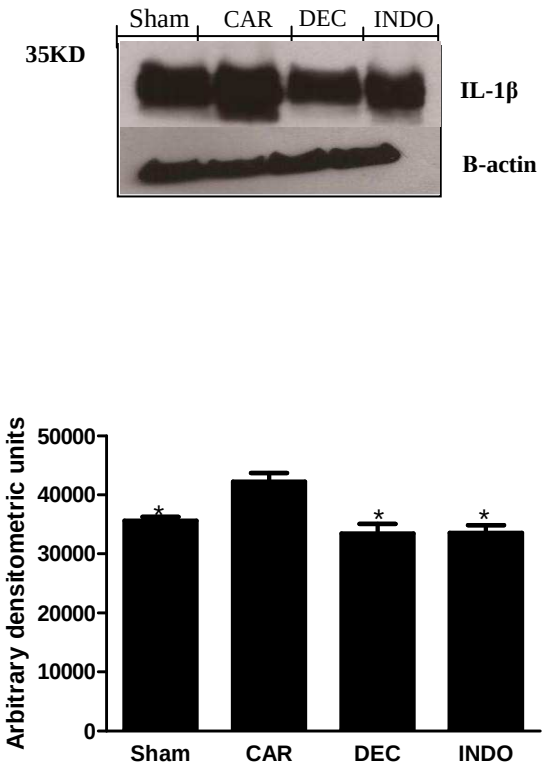
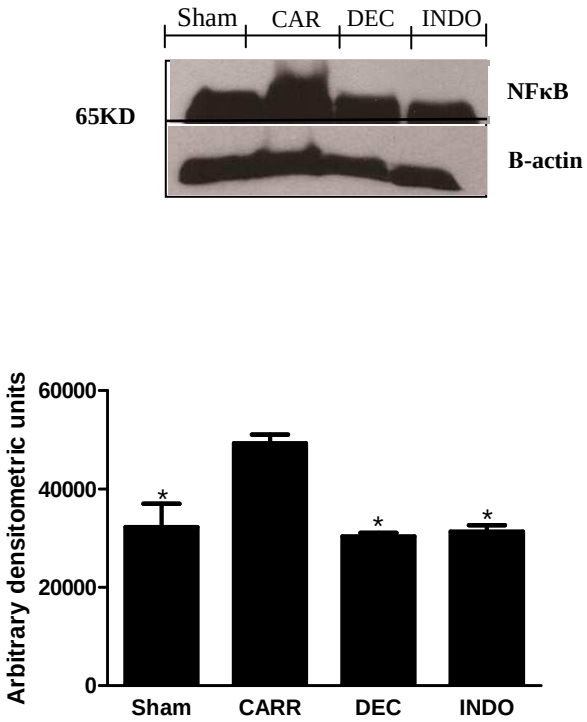

Sham
CAR
DEC
INDO
35KD
IL-1β
B-actin
Arbitrary densitometric units
50000
40000
30000
20000
10000
0
*
*
*
Sham
CAR
DEC
INDO

FIGURE 11


Sham
CAR
DEC
INDO
65KD
NFκB
B-actin
Arbitrary densitometric units
60000
40000
20000
0
*
*
*
Sham
CARR
DEC
INDO

CONCLUSÕES

- A injeção de carragenina na cavidade pleural induziu infiltração de PMNs, injuria pulmonar, produção de citocinas pró-inflamatórias como TNF-α e IL-1β, bem como COX-2 e NO, portanto, o modelo proposto efetivamente induziu um processo inflamatório agudo.

- A administração de DEC inibiu significativamente a migração de PMNs, injúria pulmonar, produção de TNF-α, IL-1β, COX-2 e NO tanto no tecido pulmonar quando no exsudato pleural, indicando que a DEC atuou como potente fármaco anti-inflamatório em modelo de pleurisia.

- A análise comparativa entre os efeitos anti-inflamatórios promovidos pela DEC e Indometacina confirma que ambos os fármacos preveniram o desenvolvimento do processo inflamatório pulmonar, sendo a DEC inclusive mais eficaz em inibir a migração de PMNs e expressão de COX-2.

ANEXO

Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz

Vice-presidência de Pesquisa e
Laboratórios de Referência

Comissão de Ética
no Uso de Animais

LICENÇA

LW-47/10

Certificamos que o protocolo (P-61/10-3), intitulado "Avaliação dos Efeitos da Dietilcarbamazina sobre o Processo de Inflamação Aguda Pulmonar em Camundongos", sob a responsabilidade de EDLENE LIMA RIBEIRO, atende ao disposto na Lei 11794/08, que dispõe sobre o uso científico no uso de animais, inclusive aos princípios da Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL). A referida licença não exime a observância das Leis e demais exigências legais na vasta legislação nacional.

Esta licença tem validade até 06/12/2012 e inclui o uso total de :

Mus musculus

- 180 Machos de Swiss Webster, Idade: 21 Dia(s), Peso: 22,0000 Grama(s).

Rio de Janeiro, 6 de dezembro de 2010

Octavio Augusto França Presgrave
Coordenador da CEUA

Comissão de Ética no Uso de Animais
Vice-presidência de Pesquisa e Laboratórios de Referência - Fundação Oswaldo Cruz
Av. Brasil, 4036 - Prédio da Expansão - sala 200 - Manguinhos - Rio de Janeiro / RJ
Telefone: (21) 3882.9121 e-mail: ceua@fiocruz.br