Universidade Estadual de Campinas

Alex Kors Vidsiunas

EFEITO DA INDOMETACINA E DA DEXAMETASONA SOBRE OS LIPÍDIOS CONTIDOS NAS CÉLULAS DECIDUAIS DE CAMUNDONGOS. ESTUDO HISTOQUÍMICO E MORFOMÉTRICO

Este exemplar corresponde à redação final da tese defendida pelo (a) candidato (a) Alex Kors Vidsiunas e aprovada pela Comissão Julgadora.

9/4/2001

Tese apresentada ao Instituto de Biologia para obtenção do Título de Mestre em Biologia Celular e Estrutural na Área de Anatomia

Orientadora: Profa. Dra Maria do Carmo Alberto-Rincon

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO INSTITUTO DE BIOLOGIA – UNICAMP

Vidsiunas, Alex Kors

V666e Efeito da indometacina e da dexametasona sobre os lipídios contidos nas células deciduais de camundongos: estudo histo-

V669e químico e morfométrico/Alex Kors Vidsiunas. - - Campinas, SP.[s.n.], 2001.

89 f: 12 ilus.

Orientadora: Maria do Carmo Alberto-Rincon

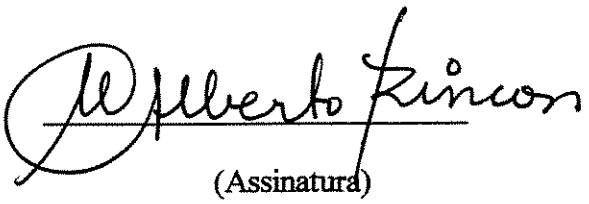
Dissertação(mestrado) – Universidade Estadual de Campinas. Instituto de Biologia.

1. Células deciduais. 2. Prostaglandinas. 3. Camundongos. I. Alberto-Rincon, Maria do Carmo. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Data da defesa: 09/04/2001

Banca examinadora

Profa. Dra. Maria do Carmo Alberto-Rincon

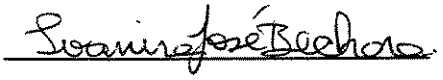


(Assinatura)

Profa. Dra. Telma Maria Tenório Zorn

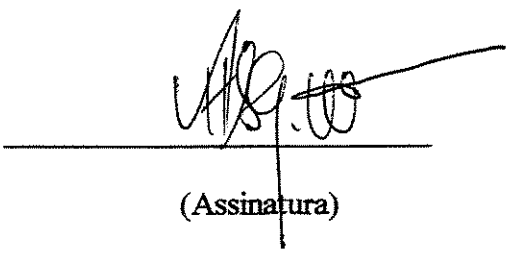
(Assinatura)

Profa. Dra. Ivanira José Bechara



(Assinatura)

Profa. Dra. Valéria Helena Alves Cagnon Quitete



(Assinatura)

Dedico este trabalho

Aos meus pais e irmão gêmeo, ***Carmen, Roberto e Eric,***

À minha família e amigos,

Pelo apoio e amor incondicionais

Que sempre me deram e contribuíram

Para que eu sempre pudesse continuar a sonhar.

Agradecimento especial

À ***Sibelle,***

Pela amizade, pela confiança, pelo incentivo,

Pelo carinho e, principalmente,

Pelo ***Amor****.*

Que sempre estiveram presentes comigo

Ao longo de todos esses dias.

Agradecimentos

À Profa. Dra. Maria do Carmo Alberto-Rincon por ter me recebido e orientado, segura e eficientemente, além, da sua amizade e confiança.

Ao Programa de Pós-Graduação de Biologia Celular e Estrutural – Área de concentração de Anatomia, do Instituto de Biologia da Universidade Estadual de Campinas, por ter me acolhido e possibilitado a execução deste trabalho.

A todos os docentes dos departamentos de Anatomia, Biologia Celular e, especialmente, Histologia e Embriologia, pelo exemplo de profissionalismo acadêmico, apoio e incentivo.

Aos membros da Pré-banca, Profa. Dra. Telma Maria Tenório Zorn, Profa. Dra. Ivanira José Bechara e Profa. Dra. Valéria Helena Alves Cagnon Quitete pela leitura da versão inicial da tese e sugestões para o aprimoramento da mesma.

Aos professores integrantes do Grupo de Biologia da Reprodução do Depto. de Histologia e Embriologia do Instituto de Biologia da Universidade Estadual de Campinas, Prof. Áureo Tatsumi Yamada, Prof. Paulo Pinto Joazeiro, Prof. Luís Antônio Violin Pereira e Profa. Dra. Maria do Carmo Alberto-Rincon, por terem tido sempre a preocupação do bom andamento do grupo como um todo, sem que houvesse prevalência de um em detrimento do todo.

Aos funcionários do Depto. de Histologia e Embriologia do Instituto de Biologia da Universidade Estadual de Campinas Marta Almeida, Marta Beatriz

Leonardo, Cleusa de Oliveira Franco e, especialmente, ao Baltazar Pereira de Paula pelo inestimável auxílio técnico.

Às secretárias do Depto. de Histologia e Embriologia do Instituto de Biologia da Universidade Estadual de Campinas Vânia, Rita, Helena e Beatriz que colaboraram para a realização deste trabalho.

Ao Laboratório de Microscopia Eletrônica do Instituto de Biologia da Universidade Estadual de Campinas, pelo apoio técnico e uso de seus equipamentos.

Ao PIBIC/CNPq e CAPES pelas concessões de bolsas de iniciação científica e de mestrado, respectivamente.

A todos os amigos e amigas do Programa de Pós-Graduação de Biologia Celular e Estrutural, pelos ótimos momentos compartilhados.

Às minhas amigas, amigos e a todos aqueles que, direta ou indiretamente, apoiaram-me e incentivaram-me para que eu concluísse este trabalho.

Índice

Resumo

A reação decidual envolve acentuadas transformações morfológicas das células do estroma endometrial tais como hipertrofia, poliploidia, formação de junções comunicantes (gap junctions) e *adherens*, remodelação de matriz extracelular, desenvolvimento de organelas responsáveis pela síntese e secreção de macromoléculas, acúmulo de filamentos intermediários, gotículas lipídicas e glicogênio. Estas modificações culminam na transformação de um tecido conjuntivo frouxo em outro com características epitelióides – o tecido decidual.

Para que a decidualização ocorra é necessário que o útero esteja preparado hormonalmente e que um estímulo, normalmente produzido pelo blastocisto, seja aplicado à luz uterina durante o processo de implantação embrionária. A decidualização também pode ser induzida artificialmente através da administração intra-uterina de várias espécies de substâncias incluindo óleo e polissacarídeos.

Porém, a natureza exata dos estímulos que desencadeiam a decidualização é ainda desconhecida e motivo de controvérsias. Acredita-se que as prostaglandinas, uma classe de lipídios bioativos, possam estar envolvidas na decidualização.

Considerando-se essa premissa, objetivou-se estudar os efeitos da indometacina (I), droga de ação anti-inflamatória não-esteróide e da dexametasona (D), um potente glicocorticóide sintético (inibidores das enzimas: prostaglandina sintase e fosfolipase A_2, respectivamente) sobre os lipídios contidos no interior das

células deciduais, os quais provavelmente, moléculas precursoras da síntese de prostaglandinas.

Camundongos fêmeas, no 5° dia de gestação (ddg), foram divididos em dois grupos de tratamento. No primeiro grupo, os animais receberam ou veículo (óleo de gergelim) ou uma única dose de 0,25 mg de I no 5° ddg. 48 horas após de o veículo ou a droga terem sido administrados, os animais foram sacrificados no 7° ddg.

No outro grupo, os animais receberam, diariamente, no 5°, 6° e 7° ddg, doses de 0,20, ou 0,24 ou 0,36mg de D. Os animais controles receberam somente o veículo (solução salina) nos dias correspondentes. Todos os animais foram sacrificados no 7°ddg duas horas após de a última dose ter sido administrada.

Os sítios de implantação embrionária foram dissecados, fixados em solução de formalina contendo 3% de $CaCl_2$ para reação de Sudan Black (SB) para a detecção de lipídios totais; ou fixados em formalina contendo 3% de $HgCl_2$ para reação de detecção de fosfolipídios contendo colina (FCC).

Para as análises ultra-estruturais e morfométricas, as amostras foram fixadas em paraformaldeído 2% e glutaraldeído 2% em PBS 0.1M, pós-fixadas com OsO_4 1% e embebidas em epóxi.

A intensidade da reação de SB observada nos sítios de implantação embrionária de animais que receberam I foi maior do que aquela observada por animais que receberam óleo de gergelim. O mesmo foi constatado para a reação de FCC.

Com relação ao tratamento com o glicocorticóide sintético, a D, a reação de SB mostrou nenhuma diferença entre o grupo que recebeu dose fisiológica, isto é, 0,20 mg D, quando comparada com o grupo controle (recebeu solução salina).

Contudo, doses mais elevadas de D intensificaram as reações para SB e FCC de forma dose-dependente, ou seja, maior intensidade para doses mais elevadas. Dentre as doses antiinflamatória e imunosupressora, esta última dose é a que apresentou maior intensidade tanto para SB como FCC.

Comparando-se os dois diferentes tratamentos, isto é, o grupo tratado com I e o grupo que recebeu diferentes doses de D, as intensidades das reações de SB e FCC foram maiores para aquele tratado com I. Nenhum dos sub-grupos do tratamento por D apresentou intensidade igual àquela observada pelo grupo que recebeu I.

Os resultados ultra-estruturais e morfométricos confirmaram os dados obtidos pelas reações histoquímicas de SB e de FCC, isto é, maior acúmulo de lipídios observados no interior do citoplasma das células deciduais de camundongos no 7° ddg.

De acordo com as frações volumétricas calculadas (Ppi) para as diferentes doses de D, foi observado que não houve diferença significativa entre o grupo controle e o que recebeu dose fisiológica da droga (0,20 mg D).

Porém, quando as outras duas dosagens foram comparadas ao controle, houve diferença significativa entre os referidos tratamentos. Sendo que a dose imunosupressora apresentou maior índice volumétrico ocupado pelas gotículas

lipídicas contidas no interior das células deciduais de camundongos no sétimo dia de gestação.

Entretanto, à semelhança dos resultados obtidos através das técnicas histoquímicas de SB e FCC, os resultados morfométricos indicam também que o tratamento com I resultam em um maior índice volumétrico ocupado pelas gotículas lipídicas do que qualquer dos sub-grupos que receberam cada uma das três doses distintas deD.

Portanto, esses resultados indicam que a inibição pela I foi maior do que para qualquer dose ministrada no tratamento de D. Provavelmente, a inibição pela I deve ser mais eficaz do que aquela apresentada pela D e, também, estar mais estreitamente relacionada com os lipídios contidos nas células deciduais.

Abstract

Decidualization involves an extensive differentiation and proliferation of the endometrial stromal fibroblasts into large polyploid decidual cells. During this process, several morphological alterations occur such as intracytoplasmic lipid accumulation, hypertrophy, extracellular matrix degradation and cell proliferation. The aim of this study was to analyze the effects of indomethacin (I), a non-steroidal antiinflammatory drug and dexamethasone (D), a synthetic glucocorticoid drug, inhibitors of prostaglandins (PGs) biosynthesis, upon the decidual lipidic bodies. PGs are potent biological lipid mediators that act locally, and are considerated to be pro-inflammatory and have been implicated in mechanisms such as ovulation, menstruation, implantation and decidualization. Female mice on the 5^{th} day of pregnancy (dop) were dividided into two groups: the first group, the animals received either a single sc injection of 0.25 mg I or sesame oil (vehicle) and sacrificed on the 7^{th} dop two hours after the injection has been administered. The animals of the second group received either 0.20, 0.24 or 0.36 mg D doses on the 5^{th}, 6^{th} and 7^{th} or saline solution (vehicle) and sacrificed on the 7^{th} dop two hours after the last injection has been administered. The uteri were dissected, transversely sliced and fixed in 3% $CaCl_2$ – formalin for Sudan Black reaction (SB); or fixed in 3% $HgCl_2$ - formalin for Choline-Containing Phospholipid reaction (CCP). For ultrastructural analysis, the samples were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1M PBS solution, post-fixed with 1% OsO_4 and epoxy resin embedded. The intensity of SB

reaction demonstrated by I-treated-animals was higher than those found in any dose of D treatment. The intensity of SB reaction demonstrated by D was dose-dependent, that is, lower intensity for 0.20mg, medium for 0.24mg, and higher for 0.36mg. A similar pattern was observed for CCP reaction, which showed the highest intensity after I treatment, higher intensity than any D-treated samples. In the same way, the lowest intensity was observed for 0.20mg and the highest intensity was detected for 0.36mg of D-treated animals. Ultrastructural and morphometric analyses confirmed the histochemical results. These results show that the inhibition by I is more effective than any D dose treatment on lipid content of decidual cells.

Introdução

Durante a implantação embrionária em roedores, os fibroblastos do estroma endometrial diferenciam-se em células deciduais. A reação decidual envolve acentuadas mudanças na morfologia das células do estroma endometrial (Psychoyos, 1973; Paria et al., 1993; Grümmer et al., 1999) tais como hipertrofia, poliploidia, formação de junções comunicantes (*gap junctions*) e aderentes, remodelação de matriz extracelular (Zorn et al., 1986) e desenvolvimento de organelas responsáveis pela síntese e secreção de macromoléculas (Kim et al., 1999). As células deciduais acumulam filamentos intermediários, gotículas lipídicas e glicogênio (Welsh & Enders, 1985; Glasser & Julian, 1986).

Estas modificações culminam na transformação de um tecido conjuntivo frouxo em outro com características epitelióides - a decídua (Finn, 1977; Parr & Parr, 1989; Abrahamsohn & Zorn, 1993).

Para que a decidualização ocorra é necessário que o útero esteja hormonalmente preparado (Psychoyos, 1973; Glasser, 1975) e que um estímulo, normalmente produzido pelo blastocisto, seja aplicado ao endométrio durante o processo de implantação embrionária.

A decidualização também pode ser induzida artificialmente (Finn & Keen, 1963; De Feo, 1967; Moulton, 1982; Andrade et al., 1996) em roedores através de vários estímulos, especialmente pela administração intra-uterina de várias espécies de substâncias incluindo óleo e polissacarídeos (Finn & Keen, 1963; De Feo, 1967).

A natureza do estímulo ou dos estímulos que desencadeiam a decidualização é ainda desconhecida e motivo de controvérsias (Paria et al., 1999). Desde a descoberta por Loeb (1908) de que, a traumatização aplicada sobre o útero em estágios apropriados da gestação dava início à reação decidual muitos trabalhos têm sido realizados com a finalidade de desvendar as prováveis substâncias e mecanismos envolvidos.

Acredita-se que células epiteliais do lúmen uterino estejam envolvidas na liberação de um estímulo deciduogênico em resposta ao blastocisto (Cooke et al., 1997; Yang et al., 1997). É provável que estas células liberem prostaglandinas (PGs) através de sua superfície basal e, que por sua vez, estas moléculas atuem diretamente nas células do estroma, ou estimulem a síntese e liberação de outros fatores (IL-1α). Estes fatores, provavelmente, devem estimular a secreção de PGs pelas células do estroma uterino, podendo atuar de maneira autócrina (Alberto-Rincon, 1994).

Lejune et al. (1981) mostraram que, quando a indução da decidualização ocorre em úteros de ratos desprovidos do epitélio de revestimento, o estroma uterino não se decidualiza. Esses autores sugerem que o revestimento epitelial é necessário para a decidualização e que as células epiteliais atuam como transdutores do estímulo decidual, transmitindo sinais às células do estroma as quais iniciam a reação deciduogênica.

A reação decidual, uma vez deflagrada, avança centrifugamente, envolvendo inicialmente as células próximas ao embrião e gradualmente as células mais distante (Abrahamsohn, 1983; Paria et al., 1999), restando no endométrio apenas uma estreita

faixa próxima ao miométrio, que não se decidualiza e que recomporá o endométrio após o parto. (O´Shea et al., 1983).

Como o processo de decidualização desenvolve-se no espaço e no tempo, verifica-se uma regionalização no endométrio, ou seja: região de células deciduais maduras - próximas ao embrião, região de células pré-deciduais, e região de estroma não decidualizado - próximo ao miométrio (Abrahamsohn, 1983; Alberto-Rincon et al., 1989).

Está bem estabelecido que as prostaglandinas (PGs) estão estreitamente relacionadas com o desenvolvimento da decídua (Psychoyos, 1973; Kennedy, 1983; Kennedy et al., 1989; Kennedy, 1990). Este fato foi demonstrado, por esses autores, em vários experimentos quando a decidualização foi bloqueada por inibidores da produção de PGs, como a indometacina (Kennedy et al., 1989). Moulton & Koenig (1986) especulam que o ácido araquidônico é mobilizado a partir de fosfolipídios, pelas células epiteliais uterinas, para produzir PGs durante a implantação.

Estudos de Kennedy (1983, 1985, 1986 e 1990) mostraram que as prostaglandinas também estão envolvidas no aumento da permeabilidade vascular, o qual é essencial para o sucesso da implantação embrionária. Em ratos, quando inibidores de sua síntese estão presentes no período de pré-implantação embrionária, esta é inibida. Sabe-se também que concentrações maiores de PGs são encontradas nos sítios de implantação por ocasião de um iminente processo de nidação embrionária (Jacobs et al., 1994). A permeabilidade aumentada é também uma

característica da resposta inflamatória inicial e tem sido proposto que as PGs mediam esta reação. (Howe et al., 1990; Perkins & Kniss, 1997).

Estudos realizados por Perkins & Kniss (1997) demonstraram que a produção in vitro de PGs por células amnióticas humanas pode ser induzida pela adição de TNF-α, que aumenta a expressão do gene da enzima prostaglandina sintase-2 (PGHS-2), também conhecida como ciclooxigenase-2 (COX-2). Romero et al. (1991) demonstraram que a decídua humana é uma fonte rica em TNF-α bioreativo, que induz a produção de PGs pelas células amnióticas e deciduais (Mitchell et al., 1990).

Nesta mesma linha de raciocínio, Jackson et al. (1993) demonstraram através de estudos de Northern Blot em duas linhagens celulares, fibroblastos pulmonares e células endoteliais – ambas em cultura - que tanto TGF-β como IL-1β, induzem a transcrição de mRNA para PLA_2 e de COX-1, que por sua vez, estão envolvidas na cascata biossintética de PGs. Estas citocinas apresentam ação estimuladora quando atuam separadamente e seus efeitos são potencializados quando agem sinergisticamente (Jackson et al., 1993).

Duas classes de enzimas são importantes na síntese das PGs. A primeira constituída pelas fosfolipases A (PLAs) liberam o substrato, o ácido araquidônico, à partir dos fosfolipídios para a ação subseqüente da prostaglandina sintase (PGHS) ou COX, constituem a segunda classe (Hansen et al., 1999).

Há pelo menos duas isoformas de PLA_2, incluindo uma molécula de baixo peso molecular (14 kDa) que se refere à isoforma secretória ($sPLA_2$) e, a de alto peso molecular que permanece no citoplasma, e portanto é denominada de PLA_2

citoplasmática ($cPLA_2$) (Xue et al., 1996). Duas isoformas da enzima prostaglandina sintase, ou ciclooxigenase, foram clonadas e seqüenciadas (Smith, 1989), uma constitutiva, COX-1 (70 kDa), sua função, provavelmente, seria produzir prostaglandinas, que por sua vez, estariam envolvidas em funções celulares ditas importantes para a manutenção celular (housekeeping functions), tais como coordenar a ação de hormônios circulantes (Smith, 1989) e regular a homeostase vascular (Maede et al., 1993); e uma isoforma indutiva, COX-2 (72-74 kDa), que está associada à elevada produção de PGs durante a inflamação e sua super-expressão está relacionada com a tumorogenicidade (Dubois et al., 1996, Oshima et al., 1996).

Lyons-Giordano et al. (1993) mostraram que IL-1 induz a síntese de ambos os mRNA para $sPLA_2$ e COX-2 em condrócitos humanos de cartilagem sinovial (Angel et al., 1994). Entretanto, Li et al. (1992), mostraram que IL-α induz a liberação de PGE_2 em fibroblastos humanos pelo aumento da expressão e da atividade de fosfolipase A_2 citosólica ($cPLA_2$) sem afetar a expressão de COX.

Xue et al. (1996) analisaram os efeitos indutórios de IL-1β sobre as células amnióticas humanas e, concluíram que esta citocina apresenta efeito estimulador para a síntese de mRNA para $cPLA_2$, bem como para COX-2, e conseqüentemente, o aumento da atividade de ambas as enzimas e, também, o acréscimo de PGE_2 produzida.

Além de as PGs estarem relacionadas ao processo de decidualização do endométrio, sabe-se também que estes lipídios exercem papel importante durante toda a gestação e também no período do parto, aumentando a contratilidade do miométrio,

dando início ao parto e à sua manutenção em humanos (Schatz et al., 1987; Olson et al., 1990).

O endométrio não decidualizado produz PGs e a modificação do tipo de PG que está sendo sintetizada ao longo da gestação parece estar envolvida, entre outras ações, no reconhecimento imunológico do embrião durante a implantação (O'Neill et al., 1993). As PGs atuam, também, como mediadores da febre, dor, e de outros processos inflamatórios bem como em funções fisiológicas e homeostáticas (Perkins & Kniss, 1997).

Em trabalhos realizados em nosso laboratório, verificamos através de reações histoquímicas para detecção de fosfolipídios contendo colina (PCC) (Hadler & Silveira, 1978) que a administração de indometacina e de dexametasona, esta última, apresentando ação inibitória de maneira dose-dependente (Ishihara et al., 1995) em animais prenhes altera o padrão dos lipídios encontrados nas células deciduais.

Alberto-Rincon et al., (1994) observaram que os fosfolipídios contendo colina encontram-se sob a forma de gotículas que se distribuem ao redor do núcleo. Esses fosfolipídios presentes em membranas celulares ou em corpos lipídicos citoplasmáticos (Dvorak et al., 1992; Alberto-Rincon et al., 1994), que contêm colina em suas moléculas, são sugeridos como prováveis precursores dos mediadores da decidualização, como as PGs, e que poderiam atuar como indutores parácrinos da reação decidual (Alberto-Rincon et al., 1994).

Como as PGs são derivadas dos fosfolipídios (Rana & Hokin, 1990; Dvorak et al., 1992), o estudo da distribuição e caracterização dos lipídios no endométrio é certamente um ponto de interesse.

Objetivos

Como as prostaglandinas (PGs) são derivadas dos fosfolipídios, o estudo da distribuição dos lipídios no endométrio é certamente um ponto de interesse. No presente trabalho analisou-se, através de técnicas histoquímicas, ultraestruturais e morfométricas a distribuição dos lipídios acumulados no interior das células do estroma endometrial decidualizadas submetidas à ação de drogas inibidoras da biossíntese de PGs não-esteróides como a Indometacina e esteróides como a Dexametasona (Goodman & Gilman, 1987; Perkins & Kniss, 1997), sendo que ambas as drogas são amplamente utilizadas pela clínica médica. Fez-se necessário o estudo da ação desses inibidores sobre fosfolipídios para melhor compreensão destes com os eventos relacionados aos processos que regem a decidualização do endométrio de roedores, uma vez que os lipídios estocados no ambiente uterino têm despertado pouco interesse durante o processo de decidualização.

Histochemical and ultrastructural morphometric analyses of mouse decidual lipids upon indomethacin and dexamethasone effects.

By Vidsiunas, A. K.; Mello, J. & Alberto-Rincon, M. C.

Laboratory of Cytochemistry and Immunocytochemistry, Department of Histology and Embryology, IB/UNICAMP,
postal code 6109, Campinas-São Paulo, 13083-970
Brazil.

Running head: Indomethacin and dexamethasone effects on mouse decidual lipids.

All correspondences should be addressed to:
Prof. Maria do Carmo Alberto-Rincon
Department of Histology and Embriology, IB/UNICAMP,
Postal code 6109, Campinas-São Paulo, 13083-970
Brazil.

Phone: 55-19-3788-7801
Fax: 55-19-3289-3124
e-mail: arincon@unicamp.br

Key words: Indomethacin, Dexamethasone, Decidualization, Decidual cells, Prostaglandins.

Abstract

During decidualization several alterations occur such as intracytoplasmic lipid accumulation and cell proliferation. The aim of this study was to analyze the dexamethasone (DEX) and indomethacin (INDO), inhibitors of prostaglandins biosynthesis effects upon the decidual lipidic bodies. Female mice received daily, either a single sc injection of 0.25mg INDO on the 5^{th} or a single dose of 0.20, 0.24 0.36mg DEX on the 5^{th}, 6^{th} and 7^{th} days of pregnancy (dop). Control animals received just their vehicles (sesame oil or saline) as described above for each drug treatment. All animals were sacrificed on the 7^{th} dop two hours after the last injection has been administered. The uteri were dissected, transversely sliced and fixed in 3% $CaCl_2$ – formalin for Sudan Black reaction (SB); or fixed in 3% $HgCl_2$ - formalin for Choline-Containing Phospholipid reaction (PCC). For ultrastructural analysis, the samples were fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.1M PBS solution, post-fixed with 1% OsO_4 and epoxy resin embedded. The intensity of SB reaction demonstrated by INDO-treated-animals was higher than those found in any dosage of DEX treatment. The intensity of SB reaction demonstrated by DEX was dosage-dependent, that is, lower intensity for 0.20mg, medium for 0.24mg, and higher for 0.36mg. A similar pattern was observed for PCC reaction, which showed highest intensity in INDO treatment, higher than any DEX-treated samples. In the same way, the lowest intensity was observed for 0.20mg and highest intensity was detected for 0.36mg of DEX-treated animals. Ultrastructural and morphometric analyses confirmed the histochemical results shown by decidual cells on the 7^{th} dop. These results show the inhibition by INDO being more effective than any DEX dosage treatment on lipid content of decidual cells.

Introduction

The endometrium undergoes several morphological changes during the estral/menstrual cycle and pregnancy. Under the influence of hormones and the developing embryo, changes in the local synthesis and metabolism of a variety of bioactive substances occur [Kim et al., 1999].

Implantation of mammalian blastocyst into the endometrium involves a complex series of precisely synchronized physiological and cell biological events that prepare the developing blastocyst and the endometrium for interacting with one another [Psychoyos, 1973; Paria et al., 1993; Grümmer et al., 1999].

The establishment of successful pregnancy requires precise coordination between the receptive uterus and activated state of blastocyst, and depends upon the interaction among factors that are under the control of progesterone and estradiol [Psychoyos, 1973; Glasser & Clark, 1975]. However, the nature of the interaction between the two ovarian steroid hormones that trigger the decidualization process remains unknown [Paria et al., 1999].

The attachment of blastocyst trophectoderm to the uterine luminal epithelium occurs in the mouse and is followed by stromal cell proliferation and differentiation into decidual cells at the site of blastocyst apposition [Paria et al., 1999].

Decidualization process occurs in uterine stromal cells that transform into decidual cells. Some morphological alterations take place in decidual cells such as gap junctions, polyploidy, hypertrophy, organelles responsible for macromolecular synthesis and their secretion, intermediate filaments accumulation, lipid bodies and glycogen inside cytoplasm [Welsh & Anders, 1985; Glasser & Julian, 1986; Abrahamsohn & Zorn, 1993].

Once decidual reaction has triggered, it evolves centrifugally from cells situated next to the embryo and gradually reaches other cells, however not so far from the conceptus [Abrahamsohn, 1983; Paria et al., 1999]. After decidualization, just a thin layer of endometrium remains not decidualized. This layer is near to the myometrium and will reconstitute a new endometrium after pregnancy [O´Shea et al., 1983].

Three distinct areas can be delimited in the endometrium after decidualization has occurred. The first region, next to the embryo is named mature decidua (MD) the second area, not so distant from the conceptus and juxtapose to the previous one is referred to pre-decidua (PD); and finally, the third and last area is called non-decidualized stroma (NDS) and is located near to myometrium.

Lejune et al. [1981] have demonstrated that rat uteri lacking epithelial coverage were not able to carry on the decidualization process after artificial induction. Uterine stromal cells, in that case, do not decidualize. These authors report the importance of the epithelial coverage of the uterus for the decidualization of the uterine stroma.

There are evidences supporting the communication between uterine epithelium and stroma and vice-versa, that is, signals produced by stromal cells that influence epithelial cells functions [Cooke et al., 1997]. Frequent communication between stroma and epithelium, via cellular surface receptors attached to adenyl cyclase enzyme, represent an important event for both embryo implantation and the beginning of deciduzlization reaction [Yang et al., 1997].

Prostaglandins (PGs), which are potent lipid mediators that act locally, are considered to be pro-inflammatory [Tischfield, 1997]. They have been implicated in such mechanisms as ovulation [Olofsson & Leung, 1996; Tsafriri & Chun, 1997],

menstruation [Downie et al., 1974], implantation [Kennedy, 1977; Chakraborty et al., 1996; Lim et al., 1997], and decidualization [Parr et al., 1988; Kennedy, 1990; Lim et al., 1997]. In rodents, PGs are implicated as important mediators of endometrial vascular permeability, which is evident at the site of blastocyst implantation [Psychoyos, 1973; Kennedy, 1990].

Prostaglandin synthesis occurs in the chorion, amnion and decidua whereas prostaglandin degradation occurs in the chorion and decidua [Brown et al., 1998]. The decidua produces both PGE_2 and $PGF_{2\alpha}$ from a population of macrophage-like cells [Norwitz et al., 1991] and also from stromal cells [Ishihara et al., 1992].

Among the different stimuli that the uterus receives during the pre-implantation period, it is believed that PGs produced by blastocyst nidation could trigger the decidualization process that takes place in the endometrium stroma.

Alberto-Rincon et al. [1994] have demonstrated that choline-containing phospholipids were observed in droplet-shaped arrangements around the decidual cell nucleus. Additionally, these authors also suggest these phospholipids could be the precursors in PGs synthesis. Phospholipids also could act as paracrine mediator of decidualization process [Alberto-Rincon et al., 1994].

To evaluate the importance of PGs on decidualization process, we administererd either INDO, a non-steroidal anti-inflammatory drug (NSAID) that inhibits both COX-1 and COX-2; or indiscriminately DEX, a synthetic glucocorticoid and anti-inflammatory drug that inhibits PLA_2s enzymes [Perkins & Kniss 1997]. Histochemistry and ultra-structural techniques were employed to analyze the lipid commitment to the PGs synthesis in decidual cells treated with those anti-inflammatory drugs.

Material and Methods

Animals

Eighty-four (84) pregnant albino Swiss mice (*Mus musculus*) aged 8 weeks were obtained from the Central Animal House of the State University of Campinas, UNICAMP. The animals were maintained under conditions of controlled temperature of 22 to 23°C and 12 hours of light. Females were placed in the presence of a male at the proportion of 3:1 and checked every morning for the presence of a vaginal plug indicating the occurrence of mating. The presence of a vaginal plug was considered to correspond to the first day of the pregnancy (dop).

Treatments

The animals were divided into two groups of treatments.

a) First group: twenty two animals on the fifth day of pregnancy (treated and control). Each female in the treated animal group received intraperitoneally, on the 5^{th}, 0.25 mg INDO on the 5^{th} (Sigma Chemical Co., St. Louis, MO, USA) diluted in 0.04 mL sesame oil [Kennedy, 1977], and control females received the same volume (0.04 mL) of sesame oil;

b) Second group: sixty two animals on the fifth day of pregnancy (treated and control). Each female in the treated animal group received, daily, on the 5^{th} , 6^{th} and 7^{th} dop, a subcutaneous (s.c.) injection of either 0.20 mg DEX (physiological dose), or 0.24 mg DEX (anti-inflammatory dose), or 0.36 mg DEX (immunosuppressive dose) diluted in 0.02 mL

saline solution, and control females received the same volume (0.02 mL) of saline solution on the 5^{th} , 6^{th} and 7^{th} dop.

Embryo implantation sites

All females were sacrificed on the seventh day of pregnancy, two hours after the last injection has been administered, under ether inhalation anesthesia. Laparotomy was performed and the uterus was removed. The embryo implantation sites were removed and placed in different fixatives according to the technique to be performed.

Histochemical procedures (n = 53 animals, 11 for INDO treatment and 42 for DEX treatment)

The material fixed either in formalin containing 3% calcium chloride or in formalin containing 3% mercury bichloride was washed in distilled water for 15 minutes and frozen in CO_2. Transverse sections (12μm-thick) from uterine horns were obtained using a freezing microtome (Jung Heidelberg, Germany). The 12μm-thick transverse sections were then submitted either to the Sudan Black technique for total lipid detection [McManus & Mowry, 1960] or to histochemical reaction for the detection of choline-containing phospholipids [Hadler & Silveira, 1978], respectively.

Scoring the intensity of the different reactions for lipid detection

To quantify the intensity of the reactions for the detection of total lipids and choline-containing phospholipids, we used a scale ranging from + (weak) to +++++(highly intense) reaction.

Processing for transmission electron microscopy (T.E.M.) (n = 31 animals, 11 for INDO treatment and 20 for DEX treatment)

The material to be examined by electron microscopy was fixed by immersion in 2% glutaraldehyde in 0.5 M phosphate buffer saline, pH 7.2, and submitted to 35 pulses of 1 second at medium potency in a model NN 7809-BH microwave oven (Panasonic of Brasil Ltda., Brazil). The material was then postfixed in 1% osmium tetroxide in a microwave oven, submitted to 35 pulses of 1 second each, and embedded in epoxy resin.

Ultrathin 60 nm sections were obtained with a Super New ultramicrotome (Reichert-Jung), deposited on a 300 mesh copper grid and contrasted with 2% uranyl acetate in citrate buffer.

Determination of the relative volume (volume fraction) occupied by lipid droplets [Weibel, 1979]

The ultrathin sections were observed on an electron microscope (Leo 906, Zeiss) and the fields of interest were photographed at 1,670 X magnification in areas covering two to three decidual cells containing lipid droplets. The negatives were enlarged approximately 5 times.

The electronmicrographs were submitted to morphometric analysis to obtain the volume index occupied by the lipid droplets of the decidual cells using a test system containing 1,517 regularly spaced points. Using the ratio for the calculation of relative volume indicated below, seven randomly electronmicrographs were taken to each treatment, that is, sesame oil and INDO, and to saline and to the three differnt DEX doses and counted for the determination of the volume fraction occupied by lipid droplets:

$Ppi = p/P$

Ppi = volume fraction or relative volume;

P = total number of points in the decidual cells;

p = number of points in the lipid droplets, or in the cytoplasm, or in the nucleus.

Statistical analysis

The fractions occupied by the lipid droplets, as well as the values obtained for the thickness of the different layers of the sites of embryo implantation, were compared by analysis of variance (ANOVA), one way. All treatments were compared with their respective control values, and $p < 0.05$ was considered to be significant.

Results

Sudan Black dissolution for total lipids detection (SB)

Indomethacin treatment (0.25 mg):

The animals that have received just the vehicle, sesame oil (Fig. 1a), the sudanophily was weak (+) and revealed the presence of intracytoplasmic total lipid bodies distributed either around the nucleus (perinuclear) or near the cellular poles. The SB staining intensity observed for MD cells was also weak (+) and diffuse; PD and NDS cells were not stained by SB (-) (Table 1).

Sudanophily was the most intense for those animals which have received indomethacin. MD has shown maximum staining intensity (+ + + + +) and moderate intensity for PD (+ +) (Fig. 1b). (Table 1).

In all cases, the intensity for the sudanophily was shown as several sized-droplets of lipids which occupied either perinuclear or polar position of the cell. However, in some cases, these droplets have filled all the cytoplasm content, except from the nuclear region itself, which appeared as a negative image (Fig. 1b).

Dexamethasone treatment

Physiological dose (0.20 mg):

The distribution pattern of intracytoplasmic lipid bodies of those cells under saline treatment (Fig. 2a) was similar of those presented by physiological dose of

DEX treatment, that is, the lipid bodies surrounded the nucleus and the intensity for SB dissolution weak for MD (+) and absent for PD (-). (Table 1).

Anti-inflammatory dose (0.24 mg):

The intensity for SB dissolutions shown by saline and physiological dose were slightly lower than those observed for anti-inflammatory dosage. MD cells have shown more lipid bodies distributed inside the cytoplasm and moderate sudanophily (+ +) and PD cells have shown a weaker sudanophily. The NDS has not shown SB lipid detection (-). (Table 1).

Immunosuppressive dose (0.36 mg):

MD and PD cells have shown the most intense SB staining intensity among all the three groups that have received the respective doses of DEX. The prior, MD cells have shown middle intensity for SB (+ + +) while PD have presented moderate intensity (+ +) (Fig. 2b). The NDS has not shown SB lipid detection (-). (Table 1).

Marked differences between indomethacin and dexamethasone treatments were observed. The SB intensity shown in MD and PD cells under INDO treatment was far greater than any dose of DEX one. (Table 1).

Choline-Containing Phospholipid detection (CCP)

Indomethacin treatment (0.25 mg):

MD cells from the control group (Fig. 3a) have presented weak staining intensity for CCP (+) and both PD and NDS have not shown any detection for

CCP (-). MD cells from animals which have received INDO (Fig. 3b) presented maximal staining intensity for CCP (+ + + +). A moderate CCP staining intensity was observed for PD cells (+ +) and the distribution pattern of the purple droplets followed the same pattern described above (Table 2).

Nevertheless, some decidual cells did not show purple-stained lipid bodies, just few of them were stained by CCP reaction.

Dexamethasone treatment

Physiological dose (0.20 mg):

As observed in SB, there is no marked difference for the CCP staining intensity for both control (Fig. 4a) and physiological dosage of DEX. The distribution pattern was similar, lipid bodies were either distributed around the nucleus or were located at poles. A moderate CCP staining intensity was observed for MD cells (+ +) and no detection for PD and NDS. (Table 2).

Anti-inflammatory dose (0.24 mg):

Middle CCP staining intensity for cells from MD was observed in the group treated with anti-inflammatory dosage (+ + +), weak for PD (+) and absent for NDS (-). A similar distribution pattern of lipid bodies around the nucleus and at poles was also observed (Table 2).

Immunosuppressive dose (0.36 mg):

MD region has presented vivid CCP staining intensity (+ + + +) (Fig. 4b), PD had weak intensity (+) and no detection was observed for NDS region (-) (Table 2).

As observed for SB, CCP intensity was greater for INDO treatment than any DEX dose administered. More decidual cells and purple-stained lipid bodies inside these cells were observed in INDO-treated group than DEX groups (Table 1 and Table 2).

Transmission Electron Microscopy (T.E.M.)

Indomethacin treatment (0.25 mg):

Decidual cells from control group (Fig. 5a) have presented fewer lipid bodies dispersed throughout cytoplasm than those presented by INDO-treated group cells (Fig. 5b). These lipid bodies were either isolated from one another or clustered forming bigger droplets.

Dexamethasone treatment

Physiological dose (0.20 mg):

Decidual cells from control group (Fig. 6a) have presented fewer lipid bodies dispersed throughout cytoplasm than those presented by DEX-treated group cells. These lipid bodies were either isolated from one another or clustered forming bigger droplets. The distribution pattern and the morphology of these lipid bodies were similar to those in control group.

Few lipid bodies were observed around the cytoplasm of decidual cells from physiological dosage following the same pattern as described above. Nevertheless, a slight, but not significant, augmentation of the number of these bodies was evaluated.

Anti-inflammatory dose (0.24 mg):

Decidual cells that received anti-inflammatory dosage of DEX have shown greater accumulation of lipid bodies inside their cytoplasm and the distribution pattern followed the same pattern already described previously. More lipid bodies were observed in this group (anti-inflammatory) than the two previous one (control and physiological dose).

Immunosuppressive dose (0.36 mg):

Decidual cells that received DEX immunosuppressive dose (Fig. 6b) have shown even greater accumulation of lipid bodies inside their cytoplasm than control, DEX physiological and DEX anti-inflammatory doses. The lipid distribution pattern followed the same described previously. Even more lipid bodies were observed in this group (DEX immunosuppressive dose) (Fig. 6b) when compared to the two previous one (DEX physiological and DEX anti-inflammatory doses).

Nevertheless no DEX dose has presented as many lipid bodies inside decidual cells cytoplasm as INDO dose injected, that is, the number of lipid bodies shown for INDO treatment was greater than any dosage administered for DEX treatment.

Relative Volumetric Lipid Fraction Evaluation

To calculate the significancy between the INDO and DEX treatments, and within DEX different doses, all Ppi obtained had their respective ASIN (arch-sine)

values estimated and ANOVA-one way performed after that. Below follows the comparisons between groups.

Indomethacin treatment (0.25 mg):

Control group (sesame oil) vs. Indomethacin treatment (0.25 mg):

Volumetric lipid fraction occupied by lipid bodies in decidual cells from animals that have received sesame oil is low (Ppi = 0.025 or 2.5%) and that treated with INDO was 30 % increased (Ppi =0.032 or 3.2%). This increase is significant by ANOVA (5 %) (Table 3).

Dexamethasone treatment

Control group (saline solution) vs. Physiological dose (0.20 mg):

When control group that has received saline solution is compared with 0.20 mg DEX-treated group, no significant difference between them was observed. Similar Ppi values were estimated, that is $Ppi_{saline\ solution} < Ppi_{physiological\ dose}$ (0.008 < 0.010) or (0.8% < 1.0 %), P > 0.05 (Table 3).

Control group (saline solution) vs. Anti-inflammatory dose (0.24 mg):

Comparing control group with anti-inflammatory dose (DEX 0.24 mg) treated group, a significant difference between them was observed, that is $Ppi_{saline\ solution} < Ppi_{anti\text{-}inflammatory\ dose}$ (0.008 < 0.012) or (0.8% < 1.2%), P < 0.05 (Table 3).

Control group (saline solution) vs. Immunosuppressive dose (0.36 mg):

A greater significant difference was observed when saline-treated group was compared with 0.36 mg DEX-treated one. $Ppi_{saline\ solution} < Ppi_{immunosuppressive\ dose}$ (0.008 < 0.028) or (0.8% < 2.8%), $P < 0.05$ (Table 3).

Physiological dose (0.20 mg) vs. Anti-inflammatory dose (0.24 mg):

Apparently, there was no significant difference between physiological dose (DEX 0.20 mg) and anti-inflammatory dose (DEX 0.24 mg) treatments. The Ppi values for both dosages were similar, $Ppi_{physiological\ dose} = 0.010$ and $Ppi_{anti\text{-}inflammatory\ dose} = 0.012$, respectively (1.0% < 1.2%). $P > 0.05$ (Table 3).

Physiological dose (0.20 mg) vs. Immunosuppressive dose (0.36 mg):

Camparing 0.20 mg DEX-treated group with 0.36 mg DEX-treated one, there was significant difference between them. $Ppi_{physiological\ dose} = 0.010$ and $Ppi_{immunosuppressive\ dose} = 0.028$, respectively (1.0% < 2.8%). $P < 0.05$ (Table 3).

Anti-inflammatory dose (0.24 mg) vs. Immunosuppressive dose (0.36 mg):

A significant difference between 0.36 mg DEX-treated group was compared with 0.24 mg DEX-treated one. $Ppi_{anti\text{-}inflammatory\ dose} = 0.012$ and $Ppi_{immunosuppressive\ dose} = 0.028$, respectively (1.2% < 2.8%). $P < 0.05$ (Table 3).

Discussion

The present study examines the effects of two well established anti-inflammatory agents, indomethacin (INDO), a non-steroidal anti-inflammatory [Dubois et al., 1998; Hay & Belleroche, 1998; Smith, 1998; Vane & Botting, 1998] and anti tumorigenic [Connolly et al., 1996; Reich & Martin, 1996] agent and dexamethasone (DEX), a powerful synthetic drug for treatment of human diseases [Hay & Belleroche, 1998; Ray et al., 1999], upon the mice decidual lipid content by assessing endometrial response to the both drugs by histochemical, ultrastructural and morphometric analyses.

The histochemical results indicate that a single injection of the non-steroidal anti-inflammatory drug, INDO when administered, provokes changes in uterine lipids and choline-containing phospholipids accumulations. Similarly, the synthetic glucocorticoid, DEX, induced a dose-dependent inhibitory effect on decidual cells.

When DEX treatment is analysed, it was observed that the greater lipid and phospholipid accumulations were detected after the immunosuppressive dose (0.36mg) has been injected. Our results are in accordance with Xue et al. [1996] and demonstrate that prostaglandin synthesis and the decidual lipid content and their response to DEX displayed significant variation during different doses, with maximal levels attained at the higher dose injected [Xue et al., 1996].

Phospholipase A_2 (PLA_2) and cyclooxygenases (COX) are two groups of enzymes critical for the generation of potent lipid mediators, PGs and thromboxane A_2 [Smith et al., 1996; Leslie, 1997; Tischfield, 1997]. PLA_2 represent a family of enzymes that catalyze the hydrolysis of glycerophospholipids at sn-2 position, liberating free fatty acids, among which is arachidonic acid (AA) [Dennis, 1997]. AA

released by PLA_2 is further converted to bioactive eicosanoids by other enzymes such as COXs [Rys-Sikora et al., 2000] and lipooxygenases.

Prostaglandins (PGs) are produced by the sequential release of AA from membrane [Rys-Sikora et al., 2000] and intracellular phospholipids [Alberto-Rincon et al., 1994] by cellular phospholipases (PLs), followed by the enzymatic peroxydation and cyclooxygenation by COX [Rys-Sikora et al., 2000]. PGs contribute to many inflammatory pathophysiologic states among many other roles [Kast, 2000], including ovulation [Olofsson & Leung, 1996; Tsafriri & Chun, 1996], menstruation [Downie et al., 1974], implantation [Chakraborty et al., 1996; Lim et al., 1997] and decidualization [Parr et al., 1988; Kennedy, 1990; Lim et al., 1997].

Mammalian tissues normally contain more than one PLA_2 isoform, which can be subdivided into several groups based on their structures and enzymatic characteristics [Dennis et al., 1997; Leslie, 1997; Tischfield, 1997]. There are two main PLA_2 isoform classes, secretory PLA_2 s ($sPLA_2$s) have low molecular-mass (~14kDa) and was originally isolated from inflammatory fluids and inflammatory cells [Rys-Sikora et al., 2000]. The other main PLA_2 isoform is the cytosolic PLA_2 ($cPLA_2$s, or group IV PLA_2) and is a ubiquitously distributed 85-kDa enzyme, whose activation is regulated by post-receptor transmembrane signaling [Leslie, 1997].

PLA_2s can be induced by pro-inflammatory cytokines and are generally believed to play a role in cellular inflammatory responses [Tischfield, 1997].

COX or prostaglandin H synthase (PGHS) converts AA to an unstable intermediate PGH_2, which is further metabolized to PGs such as PGE_2, $PGF_{2\text{-}\alpha}$ and PGD_2, and thromboxane A_2 by their respective synthases [Takano et al., 2000].

Although COX-1 is expressed constitutively in a wide range of tissues, COX-2 is inducible by growth factors, tumor promoters and cytokines [Kim et al., 1999]. COX-1 is important for the housekeeping functions [Kim et al., 1999] and is active in many cells [Smith et al., 1994]; it is the only isoform present in platelets [Funk et al., 1994] and is an important intermediary in cellular homeostasis [De Witt, 1991].

On the other hand, COX-2 has been mentioned to lead to various pathological changes in body issues [Herschman, 1996; Dubois et al., 1998] when its expression is increased, for instance, breast, lung and colon cancer have been reported [Kargman et al., 1995; Hwang et al., 1998; Wolff et al., 1998]. COX-2-deficient mice should present defects in ovulation, fertilization, implantation and decidualization [Lim et al., 1997].

Different physiological mechanisms have been proposed to regulate the expression of the COX isozymes [Broussard et al., 1997]. The COX-1 isozyme is constitutively produced and believed to be involved in daily regulation of prostaglandin formation for normal gastric and vascular homeostasis [De Witt et al., 1993]. The COX-2 isozyme has been associated with immediate response situations where prostaglandins are needed to be produced at rapid pace in instances such as inflammation, ovulation, possibly luteolysis [De Witt et al., 1993].

Since DEX has been shown to specifically suppress production of the COX-2 isozyme in mitogen stimulation rats [Lee et al., 1992], it was hypothesized that uterine endometrial COX-2 production by mice receiving anti-inflammatory and immunosuppressive DEX doses would be suppressed, while maintaining some level of COX-1 production. This could explain why lipids and choline-containing phospholipids were detected by SB and CCP reactions, respectively, herein.

At molecular level, the cyclooxygenase isozymes are differentially regulated at the transcriptional and expression levels [Prigen-Tessier et al., 1996]. Glucocorticoids, directly, either down-regulate the transcription and expression of COX-2 [Hay & Belleroche, 1998] or inhibit COX-2 production by increased lipocortin generation [Flower & Rothwell, 1994].

Consequently, these may reflect inhibition of PG synthesis in the lumbar spinal cord following intraplantar Freund's complete adjuvant (FCA) in parallel with inhibition of oedema in rats [Hay & Belleroche, 1998]. These same authors have shown that DEX inhibited almost completely the FCA-induced PGs such as PGE_2 and $PGF_{2\alpha}$ biosynthesis by spinal cords of rats.

Additionally, previous evidence has shown that glucocorticoids inhibit PLA_2 mRNA transcription and as a result, prostanoids, leukotrienes and platelet-activated factor (PAF) have their production blocked. Besides, glucocorticoids may induce the formation of an anti-inflammatory mediator, lipocortin-1, that inhibits the PLA_2 activity [Taranto, 1997].

Bany & Kennedy [1999] observed a similar down-regulation mechanism of COX-2 gene. These authors have observed that DEX diminished the prostaglandin production and the COXs activities in uterine endometrial stromal cells, which in turn, was induced by IL-1-α [Bany & Kennedy, 1995]. However, in certain cells corticosteroids paradoxically act by stimulating the expression of COX-2 [Prigen-Tessier et al., 1996].

The amount of both COX-2 mRNA and COX-2 isozyme present in endometrial cells increase during decidualization [Bany & Kennedy, 1999] and when DEX is present, both mRNA and enzyme do not have their concentration increased in a spatio-temporal manner following decidualization.

In accordance with Bany & Kennedy [1999], we did not observe any delay of decidualization reaction even after 2 hours DEX has been injected on the seventh day of pregnancy. This experimental approach could somehow affect the decidual reaction, but it did not.

A similar inhibitory effect happened to the INDO treatment, that is, a similar inhibitory pattern was observed at SB, CCP reactions. It was observed greater lipid and phospholipid accumulations inside decidual cells under INDO treatment.

Differently from DEX and the other glucocorticoids, INDO and the other NSAIDs share a similar mechanism of PG inhibition, they inhibit covalently COX-1 and –2 enzymes and as a result the enzymatic activities of both isozymes and the PGs synthesis are irreversibly interrupted. These drugs acetilate Ser 530 and Ser 516 amino acids residues of COX-1 and –2, respectiveky [Prigen-Tessier et al., 1996]. In the present, INDO treatment has shown more effective inbitory effect upon the PG synthesis inhibition than any DEX dose administered. Probably, a hypothesis for this could be explained by the similar, but different manners thereby COX-1 and –2 are blocked.

The ultrastructural and morphometric data confirmed the histochemical results. The maximal intensity presented by, the two first histochemical reactions, SB and CCP, and was confirmed by the statistical analysis of the intracytoplasmic lipid content of mice decidual cells.

INDO treated cells showed the greatest inhibitory effect when compared with any of three administered DEX doses. Inhibiting PGs synthesis by DEX or other glucocorticoids depend on the dose administered and even in higher dosages of them [Xue et al., 1996], the inhibitory effects, characterized by higher SB and CCP intensities, are not similar to those observed by INDO inhibition.

Supplementary, it was also observed that endometrial growth and differentiation during decidualization and pregnancy normally exhibit local hyperemia and increased vascular permeability [Moulton, 1982], events that are regulated by estrogen and prostaglandins. Some of these effects were also observed, some of the animals have their uterine horns and embryo implantation sites diminished in size (data not shown) [Campbell, 1978; Stewart et al., 1983].

A likely explanation for that is the restricted availability of endometrial substrates of phospholipid (pools from plasmatic membrane or cytoplasmic ones) to prostaglandin formation by decidual cells under either INDO or DEX treatment. These anti-inflammatory drugs, somehow, may restrain the conversion of the substrates into prostaglandins. Consequently, lipid and choline-containing phospholipid accumulation is observed inside mouse decidual cells.

Besides of these examples of hormonally mediated DEX actions in utero, there is a scarcity of physiological pathway incorporating the decidual tissue [Spencer et al., 1998]. In an in vitro system of decidual cells, prostaglandins and COX-2 (the enzyme that controls prostaglandins biosynthesis) were both adversely affected by DEX [Ishihara et al., 1995; Perkins & Kniss, 1997]. In another decidually related pathway, DEX inbited implantation by affecting lysossomal fluidity and movement [Szego, 1976; Brasitus et al. 1987; Dudeja et al., 1987; Hicks et al., 1994].

Functionally, the anti-deciduogenic action of DEX was apparently mediated by modulating hypertrophy, hyperplasia and the mobilization of these decidual substrates during remodelling and differentiation of the endometrial stromal cells and the extracellular matrix.

Based on the results of the present study is concluded that the different inhibitory effects showed by INDO and DEX could be explained, in part, by their

different molecular mechanisms of action; and due to other mediators such as cytokines, growth factors, hormones that could stimulate the COX–2.

In summary, the findings define an endometrial substrate repertoire for decidual growth which are affected in a spatio-temporal manner by INDO and DEX.

At present, RP-HPLC is being employed to evaluate the presence of such lipid mediators, PGs, at embryo implantation sites under the same INDO and DEX treatments and we expect to confirm our results obtained herein.

Abbreviations

Indomethacin (INDO), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), Dexamethasone (DEX), Cyclooxygease-1 (COX-1), Cyclooxygease-2 (COX-2), Prostaglandin H synthase (PGHS), Phospholipases (PLs), Phospholipase A_2 (PLA_2), cytosolic Phospholipase A_2 ($cPLA_2$), secretory Phospholipase A_2 ($sPLA_2$), Arachidonic Acid (AA), Prostaglandins (PGs), Prostaglandin D (PGD), Prostaglandin E_2 (PGE_2), Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), day of pregnancy (dop), Sudan Black (SB), Choline-Containing Phospholipid (CCP), Transmission Electronic Microscopy (TEM), Reverse Phase-High Performance Liquid Chromatography (RP-HPLC), Mature deciua (MD), Pre-decidua (PD), Non-decidualized stroma (NDS), subcutaneous (s.c.), Phosphate-buffered saline (PBS), Analisys of Variance (ANOVA), Arch-sine (ASIN), Freund's complete adjuvant (FCA), platelet-activated factor (PAF), Interleukin-1β (IL-1β).

Tables

Table 1.

Region	Sesame Oil (control group)	INDO Treatment (0.25mg)	Saline Solution (control group)	DEX Treatment (0.20mg)	DEX Treatment (0.24mg)	DEX Treatment (0.36mg)
MD	+	++++	+	+	++	+++
PD	-	++	-	-	+	++
NDS	-	-	-	-	-	-

Reaction Intensity scale:

- : absence +++ : middle

+ : weak ++++ : intense

++ : moderate +++++ : maximal intensity of the reaction

Table 2.

Region	Sesame Oil (control group)	INDO Treatment (0.25mg)	Saline Solution (control group)	DEX Treatment (0.20mg)	DEX Treatment (0.24mg)	DEX Treatment (0.36mg)
MD	+	+++++	+	++	+++	++++
PD	-	++	-	-	+	+
NDS	-	-	-	-	-	-

Reaction Intensity scale:

- : absence +++ : middle

+ : weak ++++ : intense

++ : moderate +++++: maximal intensity of the reaction

Table 3.

Treatment	Ppi	Percentage (%)
Sesame Oil (control group)	0.025	2.5
INDO (0.25mg)	0.032	3.2
Saline Solution (control group)	0.008	0.8
DEX (0.20mg)	0.010	0.9
DEX (0.24mg)	0.012	1.2
DEX (0.36mg)	0.028	2.8

Table Legends

Tabela 1. Sudan Black (SB) dilution for total lipids detection. SB intensities for regions of uterine stroma, mature decidua (MD), pre-decidua (PD) and non-decidualized stroma (NDS) of pregnant mice at 7th dop treated either with indomethacin (INDO) or dexamethasone (DEX).

Table 2. Choline-Containing Phospholipids (CCP) reaction for choline-containing phospholipids detection. CCP intensities for regions of uterine stroma, mature decidua (MD), pre-decidua (PD) and non-decidualized stroma (NDS) of pregnant mice at 7th dop treated either with indomethacin (INDO) or dexamethasone (DEX).

Table 3. Ppi calculated for the pregnancy mice, which have received either INDO or DEX treatment on the 5th, 6th and 7th dop and sacrificed 2 hours later the last dose has been administered.

Picture Legends

Fig. 1a. Photomicrography of control animal uterus on the 7th dop treated with sesame oil. SB reaction showing total lipids bodies (arrow heads) inside decidual cells (MD). 400x.

Fig. 1b. Photomicrography of uterus on the 7th dop treated with 0.25 mg (INDO). SB reaction showing total lipids bodies (arrow heads) inside decidual cells (MD). 400x.

Fig. 2a. Photomicrography of control animal uterus on the 7th dop treated with saline solution. SB reaction showing total lipids bodies (arrow heads) inside decidual cells (MD). 500x.

Fig. 2b. Photomicrography of uterus on the 7th dop treated with 0.36 mg (DEX). SB reaction showing total lipids bodies (arrow heads) inside decidual cells (MD). 500x.

Fig. 3a. Photomicrography of control animal uterus on the 7th dop treated with sesame oil. CCP reaction showing phospholipid containing choline bodies (arrow heads) inside decidual cells (MD). 400x.

Fig. 3b. Photomicrography of uterus on the 7th dop treated with 0.25 mg (INDO). CCP reaction showing phospholipid containing choline bodies (arrow heads) inside decidual cells (MD). 400x.

Fig. 4a. Photomicrography of control animal uterus on the 7th dop treated with saline solution. CCP reaction showing phospholipid containing choline bodies (arrow heads) inside decidual cells (MD). 400x.

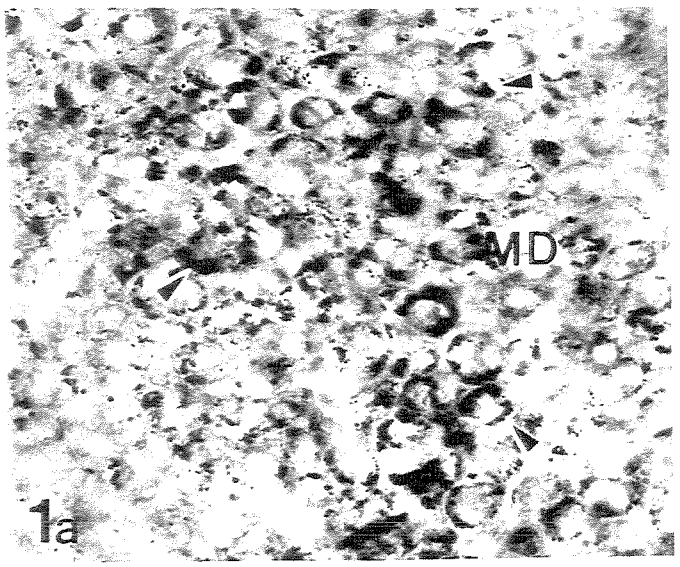
Fig.4b. Photomicrography of uterus on the 7th dop treated with 0.36 mg (DEX). CCP reaction showing phospholipid containing choline bodies (arrow heads) inside decidual cells (MD). 400x.

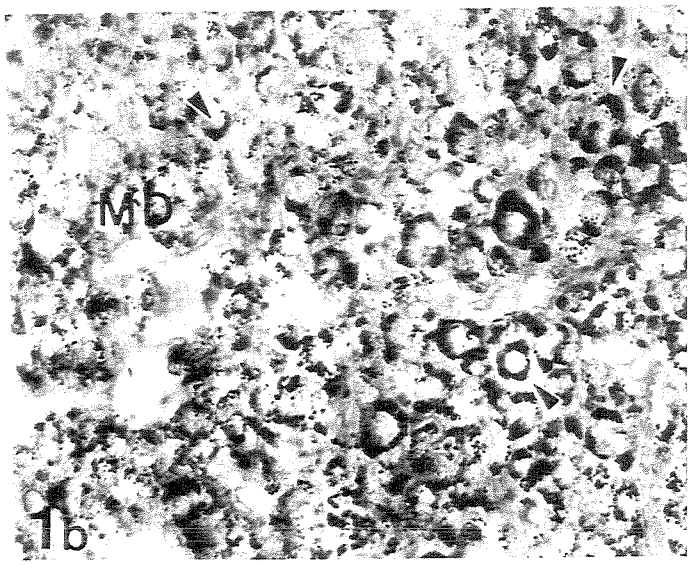
Fig. 5a. Electronmicrography of decidual cell on the 7th dop treated with with sesame oil showing lipid body (star) inside. 20,160x.

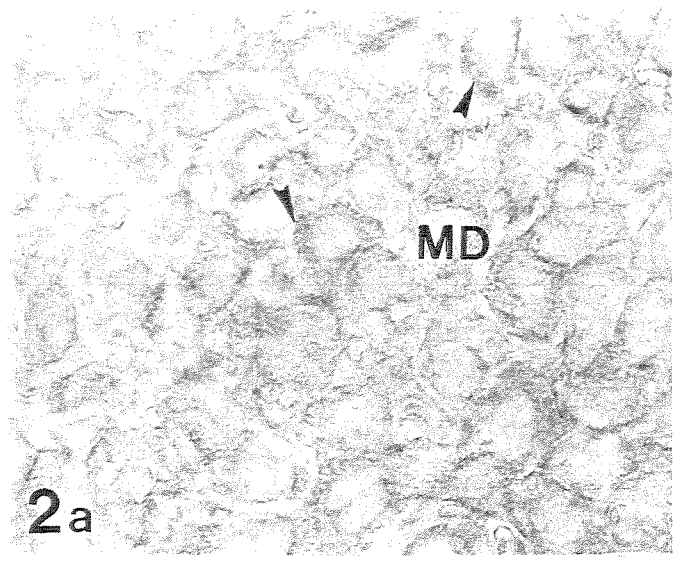
Fig. 5b. Electronmicrography of decidual cell on the 7th dop treated with 0.25 mg (INDO) showing lipid bodies (star) inside. 17,280x.

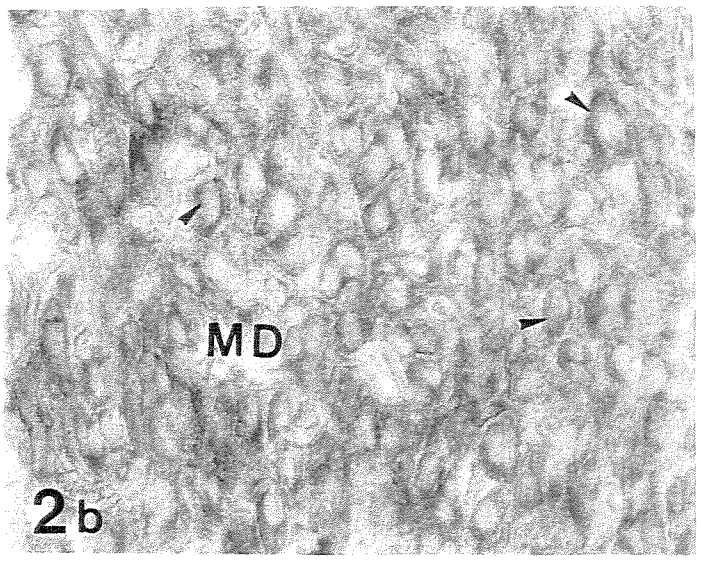
Fig. 6a. Electronmicrography of decidual cell on the 7th dop treated with saline solution showing lipid bodies (star) inside. 20,667x.

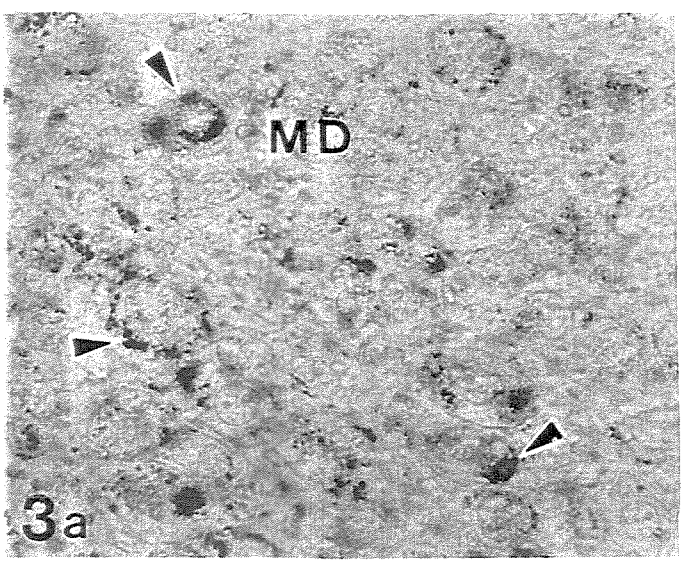
Fig. 6b. Electronmicrography of decidual cell on the 7^{th} dop treated with 0.36 mg (DEX) showing lipid bodies (star) inside. 31,000x.

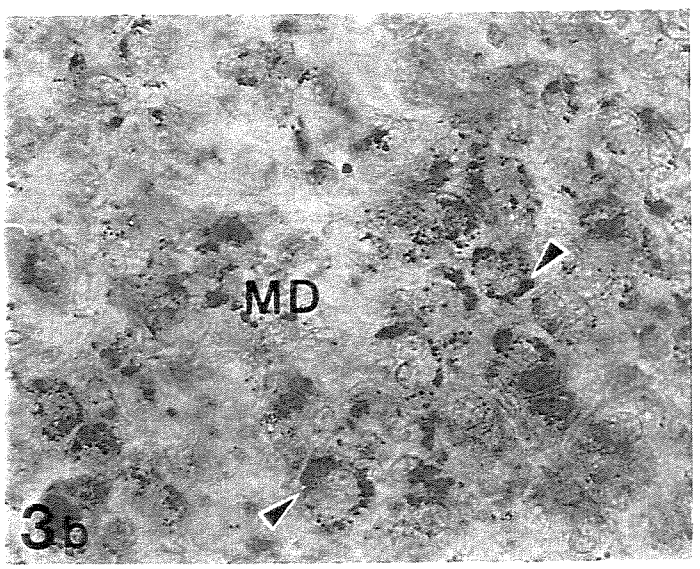

MD
1a

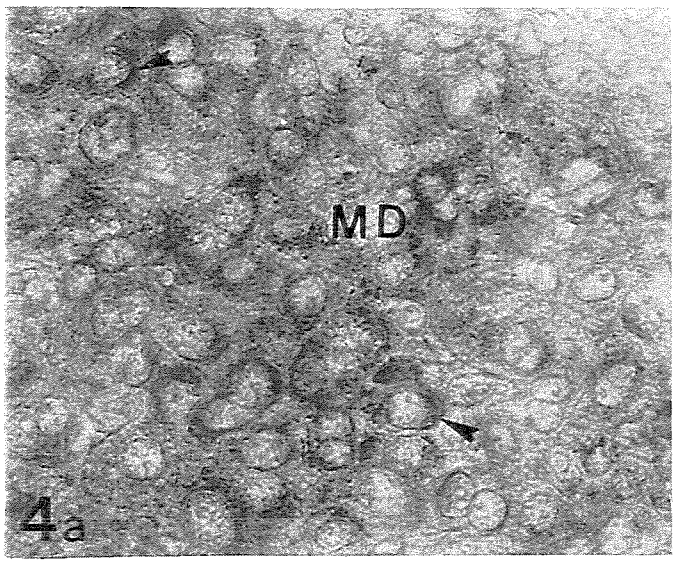

MD
1b


MD
2a

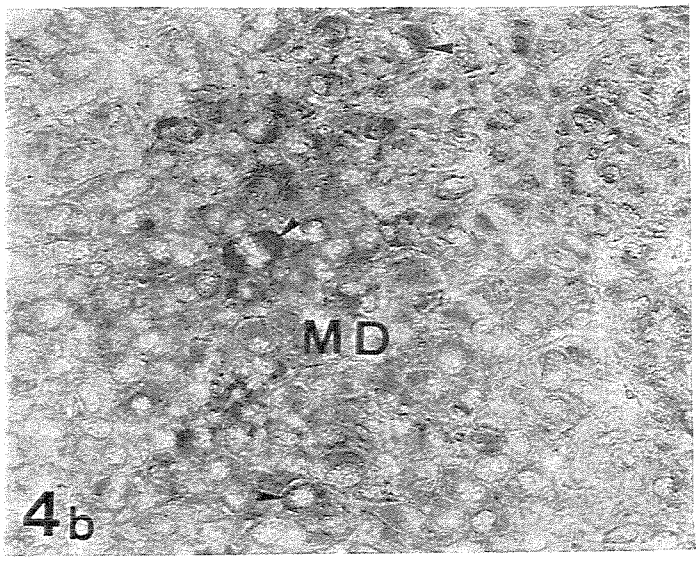

MD
2b


MD
3a

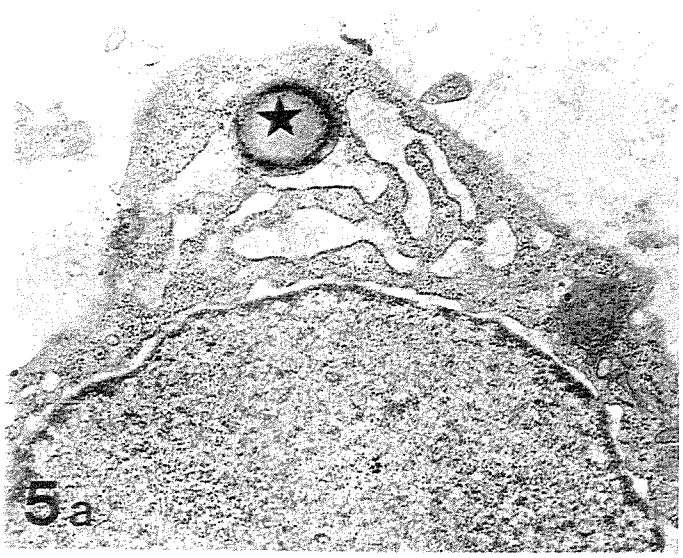

MD
3b



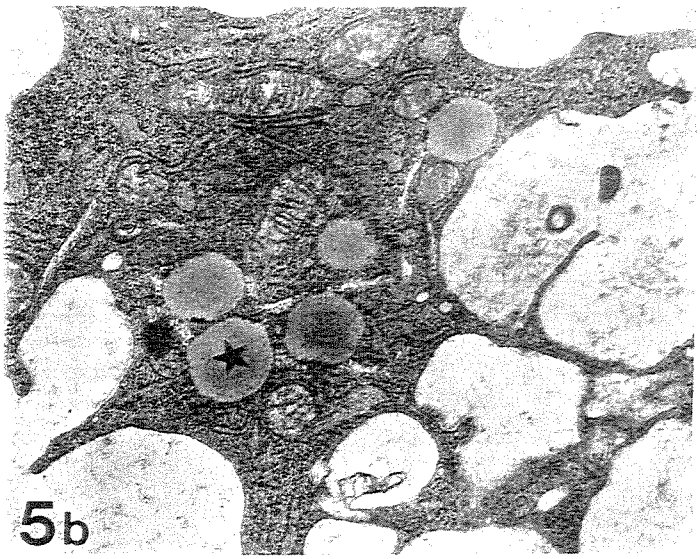
4a



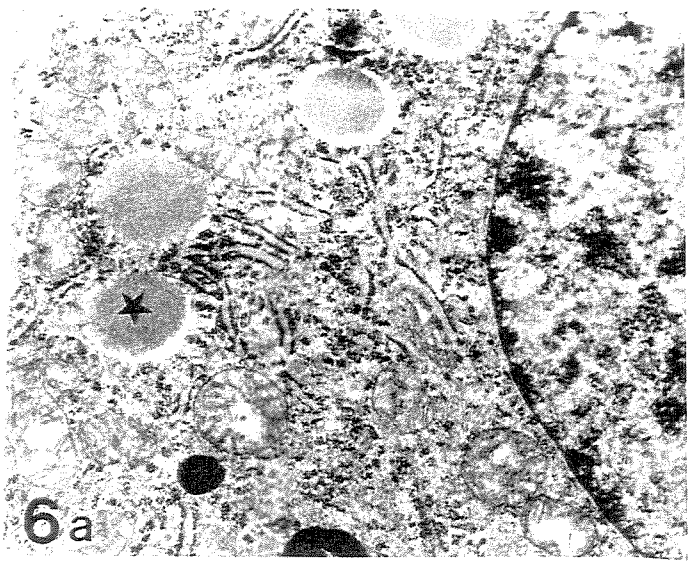
4b



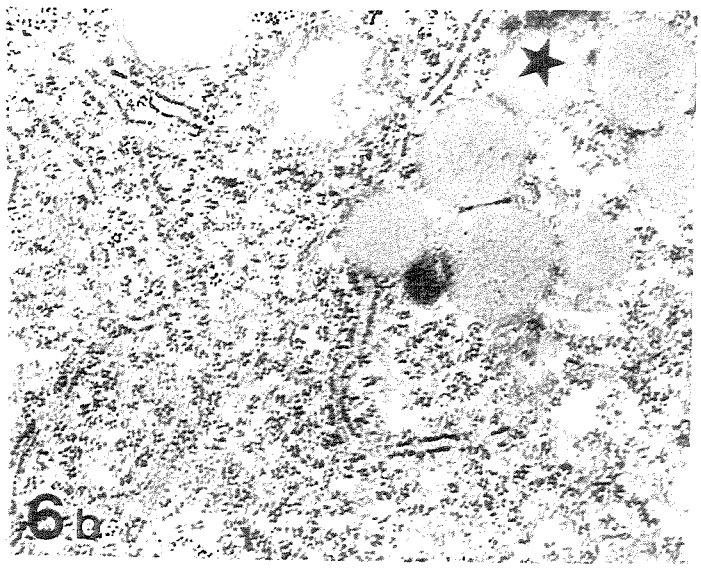
5a



5b



6a



6b

References

Abrahamsohn, P. A. (1983) U ultrastructural study of the mouse antimesometrial decidua. Anat Embryol 166: 263-274.

Abrahamsohn, P.A., Zorn, T.M.T. (1993) Implantation and decidualization in rodents. J Exp Zool 266: 603-628.

Alberto-Rincon, M.C.; Abrahamsohn, P.A.; Zorn, T.M.T. (1989) Increase of collagen fibrils of the endometrium during decidualization. Am J Anat 186: 417-429.

Alberto-Rincon, M.C.; Paiva, C.S.; Abrahamsohn, P.A.; Zorn, T.M.T. (1994) Histochemical demonstration of phospholipid containing choline in the cytoplasm of murine decidual cells. Acta Anat 150: 119-26.

Andrade, C. G. T. J.; Abrahamsohn, P. A.; Gondinho, F.; Samuel, E.; Zorn, T. M. T. (1996) Death and replacement of uterine epithelial cells during oil-induced deciduoma development in the mouse. The Anat Record 244: 316-326.

Banny, B.M., Kennedy, T.G. (1995) Inter leukin-1α regulates prostaglandin production and cyclooxygenase activity in sensitized endometrial stromal cells in vitro. Biol Reprod 53: 126-132.

Banny, B.M., Kennedy, T.G. (1999) Role of interleukin-1 in the regulation of cyclooxygenase gene expression in rat endometrial stromal cells. J Reprod Fertil 115: 125-131.

Brasitus, T.A., Dudeja, P.K., Dahiyaha, R., Halline, A. (1987) Dexamethasone-induced alterations in lipid composition of fluidity of rat proximal-small-intestinal brush-boarders membranes. Biochem J 248: 455-461.

Brown, N.L., Alvi, S.A., Elder, M.G., Bennett, P.R., Sullivan, H.F (1998) Interleukin-1β and bacterial endotoxin change the metabolism of prostaglandins E_2 and $F_{2\alpha}$ in intact fetal membranes. Placenta 19: 625-630.

Broussard, J.R., Rocha, A., Sirois, J., Roussel, J.D., Thibodeaux, J.K., Godke, R.A, Hansel, W. (1997) Effects of dexamethasone administration to diestrus cows on systemic progesterone, estrogen and uterine cyclooxygenase production. Animal Reprod Sci 47: 263-271.

Campbell, P.S. (1978) The mechanism of inhibition of uterotrophic responses by acute dexamethasone pretreatment. Endocrinol 103: 716-723.

Chakraborty, I., Das, S.K., Wank, J., Dey, S.K. (1996) Developmental expression of the cyclooxygenase-1 and cyclooxygenase –2 genes in the prei-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. J Mol Endocrinol 16: 107-122.

Connoly, J.M., Liu, X-H., Rose, P.D. (1996) Dietary linoleic acid-stimulated human breast cancer cell growth and metastasis in nude mice and their suppression by indomethacin, a cyclooxygenase inhibitor. Nutr Cancer 25: 231-240.

Cooke, P.S., Buchanan, D.L., Young, P., Setiawant, T., Brody, J., Korach, K.S., Taylor, J.A., Lubahn, D.B, Cunha, G.R. (1997) Stromal estrogen receptors mediate mitogenic effects on uterine epithelium. Proc Natl Acad Sci USA 94: 6535-6540.

Dennis, E.A. (1997) The growing phospholipase A_2 superfamily of signal transduction enzymes. Trends Biochem Sci 22: 1-2.

De Witt, D.L. (1991) Prostaglandin endoperoxyde synthase: regulation of enzyme expression. Biochem Biophys Acta 1083: 121-134.

De Witt, D.L., Maede, E.A., Smith, W.L. (1993) PGH synthetase isoenzyme selectivity: The potential for safer non-steroidal anti-inflammatory drugs. Am J Med 95 (suppl. 2A): 2A-40S – 2A-44S.

Downie, J., Poyser, N.L., Wunderlich, M. (1974) Levels of prostaglandins in human endometrium during the normal mesntrual cycle. J Physiol 236: 465-472.

Dubois, R.N., Abramson, S.B., Crofford, R.L., Gupta, R.A., Simon, L.S., Van de Putte, L.B.A., Lipsky, P.E. (1998) Cyclooxygenase in biology and disease. FASEB J 12: 1063-1073.

Dudeja, P.K., Foster, E.S., Brsitus, T.A. (1987) Modulation of rat distal colonic brush-boarder membrane Na^+-H^+ exchange by dexamethasone: role of lipid fluidity. Biochim Byophis Acta 905: 485-493.

Flower, R.J, Rothwell, N.J. (1994) Lipocortin-1: Cellular mechanisms and clinical relevance. Trends Pharmacol Sci 15: 71-76.

Funk, C.D., Funk, L.B., Kennedy, M.E., Pomg, A.S., Fitzgerald, G.A. (1994) Human platelet/erythroleukemya cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. FASEB J 5: 2304-2312.

Glasser, S.R., Clark, J.H.A. (1975) Determinant role for progesterone in the development of uterine sensitivity to decidualization and ovo-implantation; in: Merket, C.L., Papaconstantinos, J. (eds.): The 33rd Symposium of the Society for Developmental Biology: The Developmental Biology of Reproduction. Academic Press, New York, pp 311-354.

Glasser, S. R., Julian, J. A.(1986) Intermediate filament protein as a marker of uterine stromal cell decidualization. Biol. Reprod. 35: 463-476.

Grümmer, R., Traub, O., Winterhager, E. (1999) Gap junction connexin genes cx 26 and cx 43 are differentially regulated by ovarian steroid hormones in rat endometrium. Endocrinol 140(6): 2509-2516.

Hadler, W.A., Silveira, S.R. (1978) Histochemical technique to detect choline-containing lipids. Acta Histochem 63: 265-270.

Hay, C.H., Belleroche, J.S. (1998) Dexamethasone prevents the induction of COX-2 mRNA and prostaglandin in lumbar spinal cord following intraplantar FCA in parallel with inhibition of oedema. Neuropharmacol 37: 739-744.

Herschman, H. R. Prostaglandin synthase–2 (1996) Prostaglandin Synthase 2. Biochem. Biophys Acta 1299: 125-140.

Hicks, J.J, Duran-Reyes,G, Díaz-Flores, M. (1994) Effect of dexamethasone as an inhibitor of implantation and embryo development in rat; lysosomal role. Contraception 50: 581-589.

Hwang, D., Scollard, D., Byrne, J., Levine, E. (1998) Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Acad Sci USA 94: 455-460.

Ishihara, O., Sullivan, M.H., Elder, M.G. (1992) Interleukin-1β stimulates decidual stromal cell cyclooxygenase and prostaglandin E_2 production. Prostaglandins 44: 43-52.

Ishihara, O., Matsuoka, K., Kinoshita, K., Sullivan, M.H., Elder, M.G. (1995) Interleukin-1β-stimulated PGE_2 production from early first trimester human decidual cells is inhibited by dexamethasone and progesterone. Prostaglandins 49: 15-26.

Kargman, S., O'Neill, G. Vickers, P., Evans, J., Mancini, J. (1995) Expression of prostaglandin G/H synthase-1 and –2 protein in human colon cancer. Cancer Res 55: 2556-2559.

Kast, R.E. (2000) Non-steroidal anti-inflammatory drugs might also be pro-inflammatory by increasing tumor necrosis factor. Biomed & Pharmacother 54: 168-168.

Kennedy, T.G. (1977) Evidence for a role of prostaglandin in the initiation of blastocyst implantation ir rat. Biol Reprod 16: 286-291.

Kennedy, T.G. (1990) Eicosanoids and blastocyst implantation; in: Mitchell, M D. (ed) Eicosanoids and Reprduction. CRC Press, Boca Raton, pp123-138.

Kim, J.J., Wang, J., Bambra, C., Das, S.K., Fazleabas, A.T. (1999) Expression of cyclooxygenase-1 and –2 in the baboon endometrium during the menstrual cycle and pregnancy. Endocrinol 140(6): 2672-2678.

Leslie, C.C. (1997) Properties and regulation of cytosolic phospholipase A_2. J Biol Chem 272: 16709-16712.

Lejune, B., Van Hoeck, J., Leroy, F. (1981) Transmitter role of the luminal uterine epithelium in the induction of decidualization in rats. J Reprod Fert 61: 235-240.

Lim, H., Dey, S.K. (1997) Prostaglandin E_2 receptor subtype EP_2 gene expression in the mouse uterus coincides with differentiation of the luminal epithelium for implantation. Endocrinol138: 4599-4606.

Lim, H., Paria, B.C., Das, S.K., Dinchuck, J.E., Langerbach, R., Trzaskos, J.M., Dey, S.K. (1997) Multiple female reproductive failures in cyclooxygenase-1 and –2 deficient mice. Cell 91: 197-208.

McNamus, J.F.A. (1960) Staining methods: Histological and histochemical. Hober, New York, pp 143-162.

Moulton, B.C. (1982) Progesterone and estrogen control of response of rat uterine lysosomal cathepsin D activity to deciduogenic stimulus. Endocrinol 110: 1197-1201.

Norwitz, E.R., Starkey, P.M., Lopez-Bernal, A., Turnbull, A. C. (1991) Identification by flow cytometry of the prostaglandin-producing cell populations of term human decidua. J Endocrinol 131: 327-334.

Olofsson, J.I., Leung, P.C. (1996) Prostaglandins and their receptors: implications for ovary physiology. Biol Signals 5: 90-100.

O'Shea, J., Kleinfeld, R. G.; Morrow, H. A. (1983) Ultrastructure of decidualization in the pseudo-pregnant rat. Am J Anat 166: 271-298.

Paria, B.C, Huet-Hudson, Y.M., Dey, S.K. (1993) Blastocyst's state of activity determines the "window" of implantation in the mouse receptive uterus. Proc Natl Acad Sci USA 90: 10159 – 10162.

Paria, B.C., Tan, J., Lubahn, D.B., Dey, S.K., Das, S.K. (1999) Uterine decidual response occurs in estrogen receptor-α- deficient mice. Endocrinol 140(6): 2704-2710.

Parr, M.B., Parr,E.L., Munaretto, K., Clark, M.R., Dey, S.K. (1988) Immunohistochemical localization of prostaglandin synthase in the rat uterus and embryo during the peri-implantation period. Biol Reprod 38: 333-343.

Perkins, D.J., Kniss, D.A (1997) TNF-α Promotes Sustained Cyclooxygenase-2 Expression: Attenuation by Dexamethasone and NSAIDs. Prostaglandins 54: 727-743.

Prigent-Tessier, A., Pageaux, J-F., Fayard, J-M., Lagarde, M., Laugier, C., Cohen, H (1996). Arachidonic acid up-regulates and prostaglandin E_2 down-regulates the expression of pancreatic-type phospholipase A_2 and prostaglandin-endoperoxyde synthase 2 in uterine stromal cells. Eur J Biochem (241): 872-878.

Psychoyos, A. (1973) Endocrine control of egg implantation; in: Greep R. O., Astwood, E. B., Geiger, S.R. (eds): Handbook of Physiology. American Physiological Society, Washington DC, pp 187-215.

Ray, D.W., Suen, C.-S., Brass, A., Soden, J., White, A. (1999) Structure/Functions of the human glucocorticoid receptor: Tyrosine 735 is important for transactivation. Mol Endocrinol 13(11): 1855-1863.

Reich, R., Martin, G.R. (1996) Identification of arachidonic acid pathways required for the invasive and metastatic activity of malignant tumor cells. Prostaglandins 51: 1-17.

Rys-Sikora, K., Konger, R.L., Schoggins, J.W., Malaviya, R., Pentland, A.P. (2000) Coordinate expression of secretory phospholipase A_2 and cyclooxigenase-2 in activated human keranitocytes. AJP- Cell Physiol 278(4): C822 – C833.

Smith, T. J. (1998) Cyclooxygenases as the principle targets for the actions of NSAIDs. Rheum Clinics N Amer 24: 501-523.

Smith, W.L., Maede, E.A., DeWitt, D.L. (1994) Interactions of PGH synthase isozymes-1 and –2 with NSAIDs. Ann NY Acad Sci 744: 50-57.

Smith, W.L., Garavitto, R.M., DeWitt, D.L. (1996) Prostaglandin endoperoxyde H synthases (cyclooxygenases)-1 and –2. J. Biol Chem 271: 33157-33160.

Spencer, F., Chi, L., Zhu, M.-X. (1998) Dexamethasone-induced changes in endometrial growth and inducible nitric oxide synthase during decidualization in rats. Clin Exp Pharmacol Physiol 25: 240-245.

Steawrt, P.J., Zaloudek, C.J., Inmann, M.M., Webster, R.A (1983) Effects of dexamethasone and indomethacin on estrogen-induced uterine growth. Life Sci 33: 2349.

Szego, C.M., Seeler, B.J., Smitl, R.E. (1976) Lysosomal cathepsin B1: partial charecterization in rat preputial gland and recompartmentation in response to estradiol-17β. Eur J Biochem 69: 463.

Taranto, G. (1997) Sistema Endócrino; in Rang, H. P., Dale, M. M. & Ritter, J. M. (eds): Farmacologia. Rio de Janeiro, Editora Guanabara Koogan S. A., p.350.

Takano, T., Panesar, M., Papillon, J., Cybulsky, A.,V. (2000) Cyclooxygenases-1 and –2 couple to cytosolic group II phospholipase A_2 in COS-1 cells. Prostaglandins & other Lipids Mediators 60: 15-26.

Tischfield, J.A. (1997) A reassessment of the molecular weight phospholipases gene family in mammals. J Biol Chem 272: 17247-17250.

Tsafriri A., Chun, S.Y. (1996) Ovulation; in: Adashi, E Y, Rock, J A, Rosenwaks, Z (eds) Reproductive Endocrinology, Surgery and Technology. Lippincott-Raven, Phipaldelphia, pp 235-249.

Vane, J.R., Botting, R.M. (1998) Mechanism of action of anti-inflammatory drugs; in Szczeklik, A., Gryglewski, R. J., Vane, J. R. (eds): Eicosanoids, Aspirin, and Asthma, Lung Biology in Health and Disease. Vol.114. Marcel Dekker, New York , pp. 1-23.

Weibel, E.R. (1979) Sterological methods: Practical methodos for biological morphometry Vol-1. Academica Press, New York.

Welsh, A.O., Enders, A.C. (1985) Ligfht and electron microscopic examination of mature decidual cells of rat with emphasis on the antimesometrial decidua and its degeneration. Am J Anat 172: 1-29.

Wolff, H., Saukkonen, K., Anttila, S., Karjalainen, A., Vaininio, H., Ristinaki, A. (1998) Expression of cyclooxygenase–2 in human lung cancer. Cancer Res 58: 4997-5001.

Xue S., Donna, S.M., Bennet, P.R., Myatt, L. (1996) Induction of both cytosolic phospholipase A_2 and prostaglandin H synthase-2 by interleukin-1 β in WISH cell is inhibited by dexamethasone. Prostaglandins 51: 107-124.

Yang, Z.M., Das, S.K., Wang, J., Sugimoto, Y., Ichicawa, A., Dey, S.K. (1997) Potential sites of prostaglandin actions in the periimplantation mouse uterus: differentiatial expression and regulation of prostaglandin receptor genes. Biol Reprod 56: 368-379.

Considerações Finais

Neste trabalho nos propusemos a estudar os efeitos de duas drogas de ação antinflamatórias inibidoras da síntese de prostaglandinas sobre o conteúdo lipídico das células deciduais de camundongos fêmeas prenhes, bem como a sua distribuição. Tanto a indometacina, uma droga não-esteróide, como a dexametasona, um glicocorticóide sintético, são antiinflamatórios amplamente utilizados na clínica médica.

Tendo em mente que as respostas primordiais uterinas à implantação do blastocisto, tais como acúmulo de líquidos, aumento da permeabilidade vascular e infiltração de eosinófilos assemelham-se em muitos aspectos a uma resposta inflamatória, (Howe et al., 1990), estamos analisando os efeitos da indometacina e da dexametasona injetadas somente após o período em que a implantação se sucedeu, uma vez que o uso de esteróides inibem tais respostas (Xue et al., 1996).

Inibidores da síntese de prostaglandinas (PGs) têm sido usados para estudo das relações entre essas substâncias e o seu possível papel na implantação embrionária (Li et al., 1997). Em roedores, está bem estabelecido o importante papel destas substâncias, citando como exemplos a PGE_2 e a $PGF_{2\alpha}$, que atuam na decidualização do estroma uterino (Jacobs et al., 1994).

O balanço adequado entre as concentrações de ambos os prostanóides no ambiente uterino parece ser imprescindível para o sucesso da implantação do blastocisto ao endométrio e também da manutenção da gestação (Li et al., 1997).

Contudo, estes autores afirmam que em doses inflamatórias, os glicocorticóides podem inibir a síntese de PGs (atuando de forma dose-dependente) que por sua vez é mediada por IL-1 e TNF.

As prostaglandinas constituem uma família de lipídios bioativos derivados dos fosfolipídios (Dvorak et al., 1992). Esses fosfolipídios presentes em membranas celulares ou em corpos lipídicos citoplasmáticos (Dvorak et al., 1992; Alberto-Rincon et al., 1994) sofrem ação de fosfolipases e originam araquidonil-fosfolípides, que após uma cascata de reações enzimáticas dão origem as PGs (Rana & Hokin, 1990). Dentre os inibidores de PGs podemos incluir as substâncias antiinflamatórias de origem não-esteróide e as de origem esteróide (Goodman & Gilman, 1987; Perkins & Kniss, 1997).

Sabe-se que as prostaglandinas (PGs) estão relacionadas com o desenvolvimento da decídua (Kennedy, 1983; Kennedy et al., 1989), o que foi demonstrado por estes autores em experimentos nos quais promoveram o bloqueio da decidualização por inibidores de síntese de PGs, com o uso da indometacina.

Os resultados obtidos através da técnica de Sudan Black, e também da técnica para a detecção de fosfolipídios contendo colina (FCC), em úteros tratados com indometacina ou dexametasona mostraram diferença quanto a intensidade para cada uma das reações. Esperávamos que ambos os tratamentos apresentassem resultados semelhantes por se tratarem de drogas inibidoras da síntese de PGs, onde a indometacina bloqueia a ação da última enzima da cascata, a PG sintase (também denominada de ciclooxigenase - COX), e a dexametasona inibe a primeira, a

fosfolipase A_2 (PLA_2). Desta forma, haveria acúmulo dos respectivos substratos que poderiam ser, indiretamente, evidenciados através das reações histoquímicas.

Entretanto, o tratamento com indometacina mostrou uma maior sudanofilia e também uma maior intensidade para a reação de FCC quando comparado com o tratamento com dexametasona, o que está correlacionado com a intensidade da reação para a detecção dos lipídios totais contidos em gotículas citoplasmáticos das células deciduias e dos fosfolipídios contendo colina, respectivamente.

Assim, houve maior intensidade tanto para SB como FCC quando o útero foi submetido ao tratamendo com indometacina quando comparado com qualquer uma das três doses administradas de dexametasona (fisiológica, antiinflamatória ou imunosupressora). Dentro do grupo tratado com dexametasona, foi observado que a intensidade para ambas as reações foi dose-dependente, isto é, quanto maior foi a dose maior foi a intensidade.

Estes resultados estão de acordo com os resultados obtidos por Xue et al. (1996) que relataram que em dosagens fisiológicas, os glicocorticóides não afetam a síntese de PGs. Contudo, estes autores afirmam que em dosagens antiinflamatórias, os glicocorticóides podem inibir a síntese de PGs que por sua vez é mediada por IL-1 e TNF.

Além das técnicas histoquímicas de SB e FCC, a análise ultra-estrutural e morfométrica de eletromicrografias para a estimação dos índices de fração volumétirca ocupada pelas gotículas lipídicas no interior das células deciduais foram usadas. Através da microscopia eletrônica de transmissão, os resultados obtidos pela

microscopia fotônica foram confirmados. Houve presença maior de gotículas lipídicas no interior de células deciduais que receberam indometacina do que em qualquer célula do mesmo tipo que tenha recebido uma das três diferentes doses de dexametasona.

É bem provável que a quantidade de PGs existente nas células deciduais, antes do efeito inibitório das drogas, pudesse ser o suficiente para que o processo de decidualização do estroma uterino se promovesse após ter ocorrido a implantação embrionária.

Concluímos que dose fisiológica de dexametasona não teve praticamente efeito algum quando comparado ao grupo controle, isto é, ambos apresentaram resultados semelhantes quanto às intensidades para as reações de SB e FCC. Além disso, pudemos observar que doses mais elevadas do glicocorticóide, antiinflamatória e imunosupressora, apresentaram efeito inibitório sobre a mobilização de lipídios destinados, provavelmente, à síntese de prostaglandinas.

Os resultados obtidos através de técnicas histoquímicas, ou seja, maior acúmulo de gotículas lipídicas no interior das células deciduais, foram comprovados pela microscopia eletrônica, através da qual foi possível visualizar as gotículas lipídicas dispersas pelo citoplasma, bem como a quantificação destas por morfometria.

Contudo, ainda se faz necessário o estudo minucioso da natureza bioquímica dos lipídios e fosfolípides que se acumulam no interior das células deciduais

submetidas às ações de ambas as drogas de ação antiinflamatória, indometacina e dexametasona.

Bibliografia

ABRAHAMSOHN, P. A. U ultrastructural study of the mouse antimesometrial decidua. Anat. Embryol. 166: 263-274, 1983.

ABRAHAMSOHN, P. A. & ZORN, T. M. T. Implantation and decidualization in rodents. J. Exp. Zool. 266: 603-628, 1993.

ALBERTO-RINCON, M. C.; ABRAHAMSOHN, P. A.; ZORN, T. M. T. Increase of collagen fibrils of the endometrium during decidualization. Am. J. Anat. 186: 417-429, 1989.

ALBERTO-RINCON, M. C.; PAIVA, C. S.; ABRAHAMSOHN, P. A.; ZORN, T. M. T. Histochemical demonstration of phospholipid containing choline in the cytoplasm of murine decidual cells. Acta Anat. 150: 119-26, 1994.

ANDRADE, C. G. T. J.; ABRAHAMSOHN, P. A.; GONDINHO, F.; SAMUEL, E.; ZORN, T. M. T. Death and replacement of uterine epithelial cells during oil-induced deciduoma development in the mouse. The Anat. Record. 244: 316-326, 1996.

COOKE, P. S., BUCHANAN, D. L., YOUNG, P., SETIAWAN T., BRODY, J., KORACH, K. S., TAYLOR, J. A., LUBAHN, D. B., CUNHA, G. R. Stromal

estrogen receptors mediate mitogenic effects on uterine epithelium. Proc Natl Acad Sci USA 94: 6535-6540, 1997.

DE FEO . Decidualization; in Wynn RM (ed): Cellular Biology of the Uterus. Amsterdam, North-Holland, 191-226, 1967.

DUBOIS, R. N., RADHIKA A., RADDY, B. S., ENTINGH, A. J. Increased COX-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology 110: 1259-1262, 1996.

DVORAK, A. M., MORVAN, E.; SCHLEIMER, R. P.; YCON, S. W., LICHTENSTEIN, L. M., WELLER, P. F. Ultrastructural immunogold localization of prostaglandin endoperoxidase synthetase (cyclooxygenase) to non-membrane-bound cytoplasmic lipid bodies in human lung mast cells, alveolar macrophages, type II pneumocytes and neutrophils. J. Histochem. Cytochem. 6: 759-69, 1992.

FINN, C. A., KEEN, P. M. The Induction of deciduomata in the rat. J. Embryol. Morphol. 11:673-683, 1963.

FINN, C. A.. The implantation reaction; in Wynn RM (ed): Biology of the Uterus. New York, Plennum Press, 245-308, 1977.

GLASSER, S. R., CLARK, J. H. A. Determinant role for progesterone in the development of uterine sensitivity to decidualization and ovo-implantation; in: Merket, C.L., Papaconstantinos, J. (eds.): The 33rd Symposium of the Society for Developmental Biology: The Developmental Biology of Reproduction. Academic Press, New York, pp 311-354, 1975.

GLASSER, S. R., JULIAN, J. A. Intermediate filament protein as a marker of uterine stromal cell decidualization. Biol. Reprod. 35: 463-476, 1986.

GOODMAN, L. S., GILMAN, L. S. As Bases farmacológicas da Terapeútica. 7 ed, Editora Guanabara Koogan, 1987.

GRÜMMER, R., TRAUB, O., WINTERHAGER, E. Gap junction connexin genes cx 26 and cx 43 are differentially regulated by ovarian steroid hormones in rat endometrium. Endocrinol 140(6): 2509-2516, 1999.

HADLER, W. A., SILVEIRA, S. R. Histochemical technique to detect choline-containing lipids. Acta Histochem 63: 265-270, 1978.

HANSEN, W. R., KEELAN, J. A., SKINNER, S. J. M., MITCHELL, M. D. Key enzymes of prostaglandin byosynthesis and metabolism. Coordinate regulation of

expression by cytokines in gestational tissues: a review. Prostaglandins & Lipid Mediators 57: 243-257, 1999.

HERRMANN, F., LINDERMANN, A, GAUSS, A, and MERTELSMANN, R. Eur. J. Immunol. 20: 2513-2516, 1990.

HOWE, R. S., LEE, Y. H., FISCHKOFF, S. A., TEUSCHER, C., LYTTLE, R.: Glucocorticoid and progestin regulation of eosinophil chemotactic factor and complement C3 in the estrogen treated rat uterus. Endocrinology 126: 3193-3199, 1990.

ISHIHARA, O., MATSUOKA, K., KINOSHITA, K., SULLIVAN, M. H. F., ELDER, M. G. IL-1β-stimulataed PGE2 procuction from early first trimester human decidual cells is inhibited by dexamethasone and progesterone. Prostaglandins 49: 15-26, 1995.

JACKSON, B. A., GOLDSTEIN, R. H., ROY, R., COZZANI, M., TAYLOR, L., POLGAR, P. Effects of TGF-β and IL-1β on expression of COX 1 and 2 and PLA2 mRNA in lung fibroblasts and endothelial cells in cuture. Biochem. Biophys. Res. Comm. 197: 1465-1474, 1993.

JACOBS, A. L., HWANG, D., JULIAN, J., CARSON, D. D. Regulated expression of prostaglandin endoperoxide synthase-2 by uterine stroma. Endocrinology135: 1807-15, 1994.

KENNEDY, T. G. Prostaglandin E2 adenosine 3'5'-monophosphate and changes in endometrial vascular permeability in rat uteri sensitized for the decidual cell reaction. Biol. Reprod. 29: 1069-76, 1983.

KENNEDY, T. G. Evidence for the involvement of prostaglandins throughout the decidual cell reaction in the rat. Biol. Reprod. 33: 140-6, 1985.

KENNEDY, T. G. Prostaglandins and uterine sensitization for the decidual cell reaction. Ann. NY Acad. Sci. 476: 43-8, 1986.

KENNEDY, T. G., SQUIRES, P.M., YEE, G.M. Mediators involved in decidualization; in Yoshinaga, K. (ed): Blastocyst Implantation. Boston, Adams, 135-143, 1989.

KENNEDY, T. G. Eicosanoids and blastocyst implantation; in: Mitchell, M. D. (ed) Eicosanoids and Reprduction. CRC Press, Boca Raton, pp123-138, (1990).

KIM, J. J., WANG, J., BAMBRA, C., DAS, S. K., FAZLEABAS, A. T. Expression of cyclooxygenase-1 and –2 in the baboon endometrium during the menstrual cycle and pregnancy. Endocrinol 140(6): 2672-2678, 1999.

LEJUNE, B., VAN HOECK, J., LEROY, F., Transmitter role of the luminal uterine epithelium in the induction of decidualization in rats. J. Reprod. Fert .61: 235-240, 1981.

LI, W., BOOMSMA, R. A., VERHAGE, H. G. Immunocytochemical analysis of estrogen and progestin receptors in uteri of steroi-treated and pregnant cat. Biol. Reprod. 47: 1073-1081, 1992.

LI, X. J., YU, H., KOIDE, S. S. Effect of mifepristone and antiestrogens on uterine $PGF_{2\alpha}$ and PGE_2 concentrations in ovariectomized and pregnant rats. Prostaglandins 53: 187-197, 1997.

LOEB, L. The experimental production of the maternal placenta and formation of the corpus luteum. J. AM. Med. Assoc. 50: 1897-1901, 1908.

LYONS-GIORDANO, B., PRATTA, M. A, GALBRAITH, W., DAVIES, G. L., ARNER, E. C. Interleukin-1 differentially modulates chondrocyte expression of cycklooxygenase-2 and phospholipase A2. Exper. Cell Res. 206: 58-62, 1993.

MAEDE, E. A., SMITH, W. L., DE WITT, D. L. Differential inhibition of Prostaglandin Endoperoxide Synthase (Cyclooxygenase) Isozymes by Aspirin and Other Non-steriodal Anti-inflammtory Drugs. The J. Biol. Chem. 268: 6610-6614, 1993.

MITCHELL, M. D., EDWIN, S. S., ROMERO, R. J. Prostaglandin biosynthesis by human decidual cells: Effects of inflamatory mediators. Prostaglandins Leukotrienes Essential fatty Acids 41: 35-38, 1990.

MOULTON, B. C. Progesterone and estrogen control of response of rat uterine lysosomal cathepsin D activity to deciduogenic stimulus. Endocrinology 110: 1197-1201, 1982.

MOULTON, B. C., KOENIG, B. B. Hormonal control of phospholipid methylation in uterine epithelial cells during sensitivity to deciduogenic stimuli. Endocrinology: 118: 244-249, 1986.

OLSON, D. M., ZAKAR, T., POTESTIO, F. A, Control of Prostaglandin Production in Human Amnion. NIPS 5: 259-263, 1990.

O'NEIL, L. A., BARRETT, M. L., LEWIS, G. P. Iduction of cyclooxygenase by interleukin-1 in reumatoid synovial cells. FEBS Lett. 212: 35-39, 1987.

O'NEIL, P. G., FORD-HUTCHINSON, A. W. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. FEBS 330 (2): 156-160, 1993.

O´SHEA, J., KLEINFELD, R. G.; MORROW, H. A. Ultrastructure of decidualization in the pseudo-pregnant rat. Am. J. Anat. 166: 271-298, 1983.

OSHIMA, M., DINCHUCK, J. E., KARGMAN, S. L., OSHIMA, L., HANCOCK, B., KWONG, E., TRZASKOS, J. M., EVANS, J. F., TAKETO, M. M. Suppresion of intestinal polyposis in APC-delta716 knockout mice by inhibition of COX-2. Cell 87: 803-809, 1996.

PARIA, B. C., HUET-HUDSON, Y. M., DEY, S. K. Blastocyst´s state of activity determines the "window" of implantation in the mouse receptive uterus. Proc Natl Acad Sci USA 90: 10159 – 10162, 1993.

PARIA, B. C., TAN, J., LUBAHN, D. B., DEY, S. K., DAS, S. K. Uterine decidual response occurs in estrogen receptor-α- deficient mice. Endocrinol 140(6): 2704-2710, 1999.

PARR, M.B., PARR, E.R. The implantation reaction; in Wynn RM, WP Jollie (eds): Biology of the Uterus. New York, Plennum Press, 233-237, 1989.

PERKINS, D. J., KNISS, D. A. TNF-α Promotes Sustained Cyclooxygenase-2 Expression: Attenuation by Dexamethasone and NSAIDs. Prostaglandins 54: 727-743, 1997.

PSYCHOYOS, A. Endocrine control of egg implantation. In: Greep, R.O (ed.) Handbook of Physiology, sect. 7, vol 2, part 2. American Physiology Society, Washington DC, 187, 1973.

ROMERO, R., MAZOR, M., MANOGUE, K. Human decidua: A source of tumor necrosis factor. Eur. J. Obstet. Gynecol. Reprod. Biol. 41: 123-127, 1991.

SCHATZ, F., MARKIEWICZ, L., GURPIDE. Differential effects of estradiol, arachidonic acid and A23187 on prostaglandin $F_{2\alpha}$ output by epithelial and stromal cells of human endometrium. Endocr. 120: 1465-, 1987.

SMITH, W L. The eicosanoids and their biochemical mechanisms of action. Biochem. J. 259: 315-324, 1989.

WELSH, A. O, ENDERS, A. C. Ligfht and electron microscopic examination of mature decidual cells of rat with emphasis on the antimesometrial decidua and its degeneration. Am. J. Anat. 172: 1-29, 1985.

XUE, S., DONNA, S. M., BENNET, P. R., MYATT, L. Induction of both cytosolic phospholipase A_2 and prostaglandin H synthase-2 by interleukin-1 β in WISH cell is inhibited by dexamethasone. Prostaglandins 51: 107-124, 1996.

YANG, Z. M., DAS, S. K., WANG, J., SUGIMOTO, Y., ICHIKAWA, A., DEY, S. K. Potential sites of prostaglandin actions in the peri-implantation mouse uterus: differential expression and regulation of prostaglandin receptor genes. Biol. Reprod. 56: 368-379, 1997.

ZORN, T. M. T., BECILACQUA, E. M A. F. ABRAHAMSOHN, P. A. Collagen remodeling during decidualization in the mouse. Cell Tissue Res. 244: 443-448, 1986.