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DIABETIC RATS ARE HYPOREACTIVE TO NON-STEROIDAL BUT NOT TO STEROIDAL ANTIINFLAMMATORY DRUGS: EFFECT ON CELL MIGRATION*

RATOS DIABÉTICOS SÃO HIPOREATIVOS A DROGAS ANTIINFLAMATÓRIAS ESTEROIDAIS E NÃO ESTEROIDAIS: EFEITO SOBRE A MIGRAÇÃO CELULAR

Flávio Ruas de MORAES [1]; Julieta Rodini Engrácia de MORAES [2]; Edna Akemi MACORIS [3]

SUMMARY

The effect of steroidal antiinflammatory drugs (SAID) and non-steroidal antiinflammatory drugs (NSAID) on carrageenin-induced (300 mcg) pleurisy was studied in diabetic rats (40 mg/kg alloxan, *iv*). Indomethacin (2.0 mg/kg, *po*), pyroxicam (10 mg/kg, *po*) and dexamethasone (0.25 mg/kg, *ip*) significantly inhibited ($P < 0.01$) the accumulation of total leukocytes by 49,57 and 66%, respectively, and of polymorphonuclear cells by 50,60 and 66%, respectively, in normal rats. Diabetes significantly reduced total leukocytes (52%) and polymorphonuclear (58%). Pretreatment of diabetic animals with pyroxicam or indomethacin did not significantly inhibit total leukocyte or polymorphonuclear accumulation, indicating that the drugs were ineffective in the presence of diabetes. Dexamethasone, on the other hand, was as effective in diabetic animals as in the controls. All three drugs were significantly effective in inhibiting increased vascular permeability to carrageenin both in control and in diabetic animals. Untreated diabetic rats displayed a 24% weaker increase in vascular permeability than in controls. At the doses used, NSAID, but not SAID, are ineffective against carrageenin-induced cell migration in diabetic rats. Vascular permeability increase and cellular migration are independent phenomena as far as the effect of antiinflammatory drugs are concerned. Diabetes mellitus can affect NSAID action when cell migration is involved but not when plasma extravasation evoked by inflammatory stimuli is under consideration.

UNITERMS: Inflammation; Diabetes; Antiinflammatory agents; Carrageenin

INTRODUCTION

The response of the microcirculation to noxious stimuli is markedly reduced in diabetic animals (GARCIA LEME et al.[6,7], 1973, 1974; LLORACH et al.[15], 1976; MORAES et al.[16], 1987). This inhibition is not related to increased blood glucose levels, or to hyperosmolarity secondary to hyperglycemia, occuring in the diabetic state (GARCIA LEME et al.[17], 1973; LLORACH et al.[15], 1976). Insulin corrects this condition and potentiates the increase in vascular permeability in normal animals. Therefore, insulin temporarily restores the altered functional condition of endothelial cells in diabetes mellitus (GARCIA LEME et al.[6,7], 1973, 1974) and facilitates the action of vasoactive substances in normal animals (LLORACH et al.[15], 1976). The increased release of endogenous glucocorticoids following the application of a noxious stimulus antagonizes the facilitatory effect of insulin on the microcirculation, thereby adjusting the intensity of the response to the intensity of the noxious stimuli (MORAES; GARCIA LEME[17], 1982).

Diabetic rats also exhibit a lesser intense leukocyte accumulation in carrageenin-induced pleurisy. Pretreatment of animals with insulin restores the number of cells present in the inflammatory exudate (MORAES et al.[16], 1987; PEREIRA et al.[18], 1987). This blockade of cell migration in diabetic rats appears to be due to a plasma protein factor which occupies the C_{5a} receptors on the neutrophil membrane (PEREIRA et al.[18], 1987; SANNOMYIA et al.[21], 1990).

In addition to reduced inflammatory responses, diabetic animals are hyporeactive to non-steroidal antiinflammatory drugs. Indomethacin and pyroxicam are almost ineffective in inhibiting the formation of granulation tissue in the cotton pellet test in diabetic as in control rats (VALLE et al.[24], 1985).

These observations prompted us to investigate the effect of steroidal and non-steroidal antiinflammatory drugs on acute carrageenin-induced pleurisy in diabetic rats.

1 - Professor Adjunto - Faculdade de Ciências Agrárias e Veterinárias da UNESP - Campus de Jaboticabal - SP.
2 - Professora Assistente Doutora - Faculdade de Ciências Agrárias e Veterinárias da UNESP - Campus de Jaboticabal - SP.
3 - Médica Veterinária - Jaboticabal - SP.
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MATERIAL AND METHOD

Animals

Male Wistar rats weighting 180 to 230 g and maintained under ideal housing and feeding conditions were used in this study.

Experimental design

The antiinflammatory doses of pyroxicam, indomethacin and dexamethasone capable of inhibiting 35 to 50% of the inflammatory reaction induced by intrapleural injection of 300 mcg of carrageenin, in normal animals were initially determined. These doses were then administered to diabetic and matching control rats to compare the microcirculatory response and the accumulation of cells in inflammatory exudates, using the pleurisy model of inflammation. Pleurisy was induced by intrapleural injection of carrageenin (300 mcg) and the vascular and cellular components of the response evaluated 4 h later.

Induction of diabetes

Diabetes was induced after 24 h of abstinence from food by the injection of 40 mg/kg alloxan *iv*. Blood glucose levels were determined 4 days later according to KING; GARNER[14] (1947). Rats with blood glucose levels above 200 mg/100 dl were used.

Induction and evaluation of pleurisy

The animals were injected with Evans blue dye (25 mg/kg) given i.v. as a 2.5% solution in 0.45 saline 24 h before carrageenin injection. Pleurisy was then induced by the technique of VELO et al.[26] (1973) using 300 mcg of carrageenin dissolved in 0.1 ml sterilized 0.9% saline solution. The animals were sacrified 4 h later by ether inhalation and the cervical vessels then severed for exsanguination. The chest was opened and exposed and washed with 2.0 ml heparinized 0.1% PBS. The resulting exudate was centrifuged at 2,000 rpm for 10 min in a clinical centrifuge. The supernatant containing the dye was transferred to additional test tubes and the remaining cell pellets were resuspended in 2.0 ml heparinized PBS. Aliquots of the cell suspension were diluted 1:20 with Turk solution and total leukocyte counts performed with the aid of Neubauer chambers. Differential leukocyte counts were made on smears stained panchromatically. The absorbance of Evans blue dye in the supernatant was estimated spectrophotometrically at 620 nm. The final concentration of the dye was determined by a standard graph recording the optical density of serial dilutions of a weighted samples of Evans blue in 0.45% saline.

Statistical analysis

Data were compared by the analysis of variance $p < 0.01$ being taken as statistically significant. To test differences among means, Tukey test was used (SNEDECOR; COCHRAN[22], 1974).

RESULTS

Doses of NSAI and SAI drugs effective in carrageenin pleurisy

Doses of pyroxicam, indomethacin and dexamethasone producing 35 to 50% inhibition in leukocyte accumulation in pleural exudates were initially determined using 300 mcg carrageenin as the noxious stimulus. Results are presented in Tab. 1. The doses of pyroxicam, indomethacin and dexamethasone selected for this assay were 10.0 mg/kg, 2.0 mg/kg and 0.25 mg/kg, respectively.

Effect of pyroxicam, indomethacin and dexamethasone on cell accumulation in pleural exudates

Fig. 1 shows number of cells present in inflammatory exudates of control and diabetic rats 4 h after the injection of carrageenin. Compared to control values, polymorphonuclear determinations made in diabetic rats were markedly reduced (Fig. 2). The number of mononuclear cells were not different in both groups and, as expected, did not differ from basal values found in untreated animals. Accordingly, migration of cells to the pleural cavity during the 4 h interval of time used, refers to polymorphonuclear leukocyte migration. Pyroxicam, indomethacin and dexamethasone significantly reduced polymorphonuclear migration to the pleural cavity in normal animals. In diabetic animals, however, pyroxicam and indomethacin were ineffective to block polymorphonuclear migration. In this group, only dexamethasone was capable of affecting the accumulation of polymorphonuclear leukocytes in pleural exudates. None of the drugs used interfered with the number of mononuclear cells present in the exudates.

Changes in vascular permeability

Fig. 3 shows a significant inhibition in the vascular response of diabetic rats (24%, $p < 0.01$) relative to control rats. Control non-diabetic rats treated with pyroxicam, indomethacin and dexamethasone showed a significant inhibition ($p < 0.01$) of the increase in vascular permeability by

63, 33 and 64%, respectively. Blockade of the same magnitude was observed in diabetic animals, i.e., the antiinflammatory drugs significantly inhibited ($p < 0.01$) the increase in vascular permeability (by 68, 61 and 65%, respectively) when compared to untrated diabetic rats.

DISCUSSION

The present results show that alloxan-diabetic rats present marked reduction of PMN, in pleural exudates during the 4 hour carrageenin pleurisy when compared to control rats. This finding agrees with previous observations (MORAES et al.[16], 1987); PEREIRA et al.[18], 1987; SANNOMYIA et al.[21], 1990). The inhibition of polymorphonuclear cell migration is apparently associated with the presence of a protein in plasma of diabetic animals, capable of interacting with C_{5a} receptors on the neutrophil surface and block the activity of the chemoattractant. This seems to be a unique circumstance, since responses to the formulated tripeptide (FMLP) or to leukotriene B4 remain unchanged (SANNOMYIA et al.[21], 1990).

Pyroxicam, indomethacin or dexamethasone administration to normal rats inhibited PMN to the pleural cavity. In contrast, pyroxicam and indomethacin had no effect on leukocyte accumulation in alloxan-diabetic animals. This was observed with doses of the drugs capable of inhibiting 35-50% of the response in normal animals. Nevertheless, dexamethasone remained active in the group of alloxan-treated rats. The failure of NSAI drugs to effect leukocyte migration in diabetic rats might be interpreted as a physiopathological condition, in which the diabetic state is capable of influencing the action of drugs, though the underlying mechanism remains unclear. Thromboxane A2 and B2 production is increased in experimental diabetes (HARRISON et al.[8], 1978; VALENTOVIC; LUBAWY[23], 1982) and in diabetic patients (ZIBOH et al.[27], 1979; QUILLEY; McGIFF[19], 1985; BOURA et al.[2], 1986; HUI et al.[12], 1989). In contrast, prostacyclin production is decreased in both experimental and clinical diabetes (HARRISON et al.[8,9], 1978, 1980; QUILLEY; McGIFF[19], 1985; DOLLERY et al.[4], 1979; JOHNSON et al.[13], 1979). These report show that in diabetes mellitus, disorders in the arachdonic acid metabolism can occur, leading to an unbalance in the metabolite production. This metabolic disorder might, at least partially, be associated with the reduction of the antiinflammatory effects of pyroxicam and indomethacin in alloxan-diabetic rats as presently reported. This reduction can occur because an unbalance in arachdonic acid metabolism might be related to a lower production of eicosanoids importants for the inflammatory development. Similar effects were observed by VALLE et al.[24] (1985) who implanted cotton pellets in subcutaneous tissue of alloxan-diabetic rats and observed that pyroxicam and indomethacin, but not dexamethasone, were ineffective in reducing granulation tissue formation in these animals.

Dexamethasone was equally effective in normal and diabetic rats and blocked leukocyte accumulation during the carrageenin-induced pleurisy.

Accordingly, different mechanisms are involved when one considers the antiinflammatory effects of pyroxicam, indomethacin and dexamethasone and the response of diabetic subjects to these drugs.

According to VANE[25] (1976), non-steroidal antiinflammatory drugs (NSAID) act by inhibiting the cyclooxigenase pathway of the arachdonic acid (AA) metabolism. Low doses of indomethacin, aspirin and flurbiprofen supress the prostaglandin production, inhibiting the vascular permeability increase and enhance PMN migration. In contrast, high doses of the same drugs inhibit the cellular migration due to a nonspecific inhibition of AA peroxidation (HIGGS et al.[10], 1980). Glucocorticoids inhibit eicosanoids synthesis by blocking the phospholipase A2 which releases arachdonate from membrane phospholipids (RUSSO-MARIE et al.[20], 1979; FLOWER[5], 1988). In addition, glucocorticoids block the release of different cytokines by macrophages (DINARELLO[3], 1984; BEUTLER; CERAMI[1], 1986) and block the leucocyte-endothelial adherence (HIGGS et al.[11], 1981; FLOWER[5], 1988).

Contrary to what was observed with leukocyte migration, pyroxicam, indomethacin and dexamethasone were capable of blocking vascular permeability increases in alloxan-diabetic animals.

This finding suggest that plasma leakage and cellular migration in inflammation are independent phenomena when the effects of antiinflammatory drugs are concerned. Diabetes mellitus affects non-steroidal antiinflammatory drug action when cell migration is involved but not when plasma extravasation evoked by inflammatory stimuli is involved.

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RESUMO

Estudou-se o efeito de drogas antiinflamatórias esteroidais (DAIE) e não-esteroidais (DAINE) sobre a pleurisia induzida pela carragenina (300 mcg) em ratos diabéticos (aloxana, 40 mg/kg, *iv*). Em ratos normais, indometacina (2,0 mg/kg, *vo*), piroxicam (10 mg/kg, *vo*) e dexametasona (0,25 mg/kg, *ip*) inibiram significativamente ($P < 0,01$) o acúmulo de leucócitos totais (49%, 57% e 66%, respectivamente) e polimorfonucleares (50%, 60% e 66%, respectivamente). Ratos diabéticos sofreram redução no acúmulo de leucócitos totais (52%) e polimorfonucleares (58%). O tratamento de animais diabéticos com indometacina ou piroxicam não interferiu significativamente com o acúmulo de leucócitos totais ou polimorfonucleares, indicando que as drogas foram inefetivas em presença de diabetes. Por outro lado, a dexametasona foi efetiva tanto em animais normais quanto em diabéticos. Estes sofreram inibição significativa do aumento de permeabilidade vascular (24%) em comparação com animais normais. As três drogas inibiram o aumento de permeabilidade vascular à carragenina em ratos diabéticos e controles. Nas doses utilizadas DAINE mas não DAIE foram inefetivas em bloquear o acúmulo de leucócitos em ratos diabéticos. Considerando-se o efeito de drogas antiinflamatórias os resultados sugerem que o aumento de permeabilidade vascular e a migração celular sejam fenômenos independentes. O diabetes mellitus parece interferir na ação das DAINE quanto à migração celular mas não quando se considera o extravasamento plasmático induzido pelo estímulo inflamatório.

UNITERMOS: Inflamação; Diabetes; Agentes antiinflamatórios; Carragenina

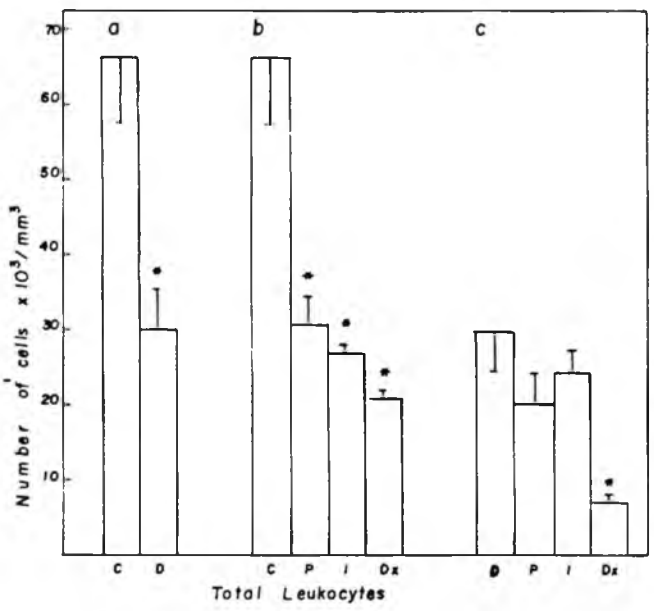

FIGURE 1

a, Inhibition of total leukocyte migration in carrageenin-induced pleurisy in control (C) and diabetic (D) rats, *b*, effect of pyroxicam (P), indomethacin (I) and Dexamethasone (Dx) on normal rats; *c*, effect of the same drugs on diabetic rats. Each column represents mean values (± SE) - N = 14. * significant difference in relation to control and non-treated diabetic groups ($P < 0,01$). Baseline values for total leukocytes: 2.2 ± 0.52 x 10^3 / mm^3.

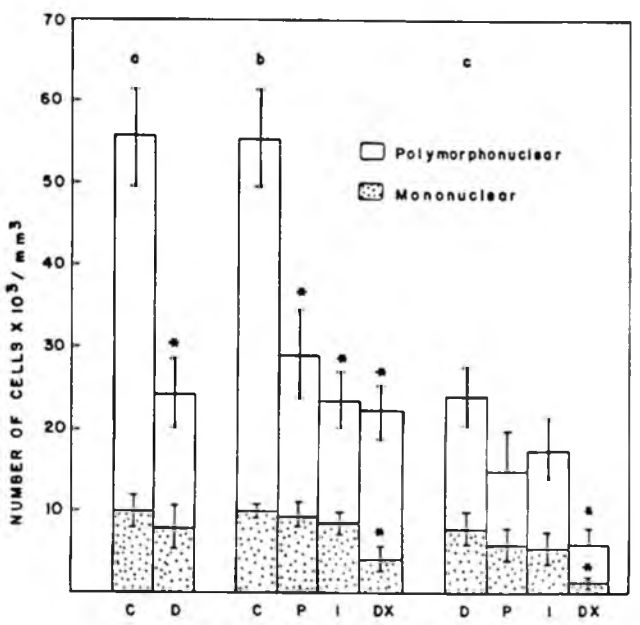

FIGURE 2

a, Inhibition of polymorphonuclear and mononuclear migration in carrageenin-induced pleurisy in control (C) and diabetic (D) rats; *b*, effect of pyroxicam (P), indomethacin (I) and dexamethasone (Dx) on control (normal) rats; *c*, effect of the same drugs in diabetic rats. Each column represents mean values (± SE) - N = 14. * significant difference in relation to the control and non-treated diabetic groups $P < 0,01$. Baseline values for polymorphonuclear and mononuclear leukocytes: 0.75 ± 0.68 x 10^3 / mm^3; 1.3 ± 0.5 x 10^3 / mm^3.

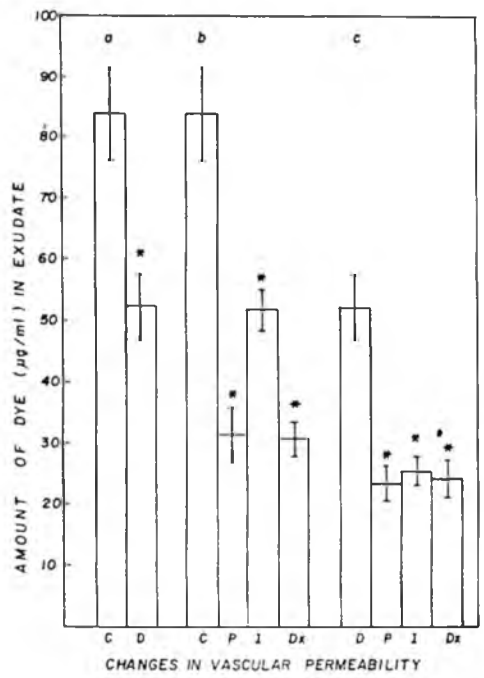

FIGURE 3

a, Changes in vascular permeability in control (C) and diabetic (D) rats; *b*, effect of pyroxicam (P), indomethacin (I) and dexamethasone (Dx) on control rats; *c*, effect of the same drugs on diabetic rats. Each column represents mean values (± SE) - N = 14. * significant difference in relation to control and non-treated diabetic groups P < 0.01. The baseline value for vascular permeability: 8.3 ± 0.41 mcg/ml of dye (n = 12).

TABLE 1

Effect of pyroxicam, indomethacin and dexamethasone on leukocyte accumulation in rat carrageenin induced-pleurisy 4 h later local injection of the irritant. Results are men ± SE. n = 12. Jaboticabal - SP, março a abril de 1991.

Treatment	Cells x 10^3/mm^3		
Carrageenin (300 mcg)	Total	PMN	MN
Untreated	65.0 ± 8.1	54.1 ± 6.3	10.9 ± 1.4
Pyroxicam 10.0 mg/kg	32.7 ± 6.2	28.8 ± 5.6	6.2 ± 2.0
Pyroxicam 20.0 mg/kg	21.1 ± 8.5	17.0 ± 4.2	5.3 ± 0.8
Indomethacin 2.0 mg/kg	28.3 ± 2.0	21.0 ± 2.4	6.1 ± 0.8
Indomethacin 4.0 mg/kg	21.3 ± 3.1	12.9 ± 3.4	8.8 ± 1.0
Dexamethasone 0.25 mg/kg	22.5 ± 1.9	19.2 ± 2.6	5.3 ± 0.7
Dexamethasone 0.50 mg/kg	12.7 ± 1.5	7.1 ± 1.3	5.8 ± 1.1

All drugs and its doses had a significant difference ($p < 0.01$) in comparison with control values.

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