Braz. J. vet. Res. anim. Sci.,
São Paulo, v.33, n.1, p.11-14, 1996.

The potentiating effect of chloramphenicol succinate in rat carrageenin-induced acute pleurisy. Inhibition by indomethacin and dexamethasone*

O efeito potenciador do succinato de cloranfenicol sobre a pleurisia aguda induzida pela carragenina. Inibição por indometacina e dexametasona

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SUMMARY

The effect of indomethacin and dexamethasone on PMN cell migration was studied in relation to chloramphenicol in the pleural cavity of rats, 4 hours after local injection of carrageenin or dextran. Pre-treatment of rats with chloramphenicol succinate (30mg/kg), *ip*, every 12h, during 4 days, potentiated the migration of PMN leucocytes to the inflamed cavity, when carrageenin (150mcg) was used as an irritant. However, responses to dextran (100mcg) remained the same. Chloramphenicol pre-treated rats that received indomethacin (2mg/kg, per os, 30min before carrageenin) or dexamethasone (0.25mg/kg, *ip*, 30min before carrageenin) showed inhibition of potentiated cellular responses. Mechanisms of the potentiating effect of chloramphenicol on the acute carrageenin-induced inflammation remains unclear.

UNITERMS: Chloramphenicol; Pleurisy; Carrageenin; Dextran; Indomethacin; Dexamethasone.

INTRODUCTION

Early studies by Laus [6] (1985) showed that dogs bearing enterorraphy and treated with chloramphenicol presented an exacerbation of the local inflammatory response and a delay in the gut healing. The same antibiotic potentiated the rat's paw edema induced by nistatin (Laus [6], 1985) and by carrageenin (Moraes et al.[11], 1993; Lemos et al. [8], 1988), vascular permeability in newformed corneal vessels (Niciporciukas; Malucelli [12], 1990), and carrageenin-induced neutrophil migration in the rat's paw (Moraes et al. [11], 1993). Recent results demonstrate that pretreament of rats with chloramphenicol succinate potentiates the migration of PMN cells to the peritoneal cavity, after local injection of carrageenin. This does not happen with dextran injection, and the effect disappears after local washing-induced depletion of resident macrophages, and following, increased thioglycollate-induced number of local macrophages. These data suggest that chloramphenicol makes easier the peritoneal macrophages release of chemoattractants for PMN cells. This work investigated the effect of indomethacin and dexamethasone on the potentiating effect of chloramphenicol in rat's carrageenin-induced pleurisy.

* Partially suported by the Fundação de Amparo à Pesquisa de São Paulo - FAPESP

MATERIAL AND METHOD

Animals

Male Wistar rats, weighing 180 to 230g, fed with ration and water "ad libitum" were used.

Pretreatment of animals with chloramphenicol succinate

A group of rats was treated with chloramphenicol succinate (30 mg/kg, *ip*, every 12h during 4 days), being pleurisy induced immediately after the last dose of the antibiotic. Control rats received an equivalent volume of physiologic solution 0.9% by the same route, times, and for the same period.

Induction and evaluation of the pleurisy

Pleurisy was induced by local injection of 150mcg of carrageenin or 100mcg of dextran dissolved in 0.2ml of physiologic solution 0.9% according to Velo et al. [18] (1973). Four hours later, animals were anaesthetized with ether and bled by cutting the cervical vessels. Their throracic cavities were exposed and carefully washed with 2.0ml of heparinized phosphate buffered saline PBS (5 IU/ml). The resultant suspensions were harvested with the aid of Pasteur pipettes, transferred to conic tubes and spun at 2.000 rpm for 10min in a clinical centrifuge. Afterwards, cells in the pellet were re-suspended in 2.0ml of heparinized PBS, diluted 1:20 in

pipettes for white blood cells and counted in a Neubauer chamber. Specific countings of the inflammatory cells were done in panchromically stained smears.

Treatment of rats with antiinflammatory drugs

This assay was performed to investigate the possible participation of arachdonates in the potentiating effect of chloramphenicol on the carrageenin-induced pleurisy. So, rats previously treated with the antibiotic, as described before, were given indomethacin (2.0mg/kg) or dexamethasone (0.25mg/kg) half hour before inflammatory stimulus. Two control groups were used: normal rats (untreated) and rats treated with chloramphenicol alone. It should be stressed that doses of the antiinflammatory agents were previously tested in other rats, to establish the intensity of their effects on the pleurisy provoked by carrageenin.

Drugs

Carrageenin (Viscarin, Marine Colloids Inc., USA); dextran (MW=78,000) (Sigma Chemical Co., USA); chloramphenicol succinate (Quemicetina, Shering Veterinária, Brazil); indomethacin (Indocid, Prodrome, Brazil); dexamethasone (Decadron, MSD, Brazil) were used in the experiments.

Statistical analysis

Results were compared by Anova, $p<0.01$ being taken statistically significant. To test differences among means, Tuckey test was used (Snedecor; Cochran [17], 1974).

RESULTS

Effect of the pretreatment with chloramphenicol succinate on the pleurisy induced by carrageenin or dextran

It can be seen from Fig. 1a that pretreatment of rats with chloramphenicol led to a statistically significant increase in the number of total leukocytes migrating to the pleural cavity ($p<0.01$), in comparison with the untreated control group.This fact was due to a greater migration of PMN neutrophils ($p<0.01$), since the number of MN cells was similar among groups. This potentiating effect of the antibiotic did not occur when dextran was used as inflammatory stimulus; in this case, there were no statistical differences ($p<0.01$) either in the number of total leucocytes, PMN, or MN cells (Fig. 1b).

Effect of indomethacin and dexamethasone on the carrageenin-induced pleurisy in rats pretreated or not with chloramphenicol

Administration of indomethacin to normal rats (chloramphenicol-untreated) reduced cellular migration to the carrageenin-inflamed site in 50%. Similarly, dexamethasone caused a greater reduction (70%) in the number of migrating cells in the same model, in comparison with normal rats (chloramphenicol-

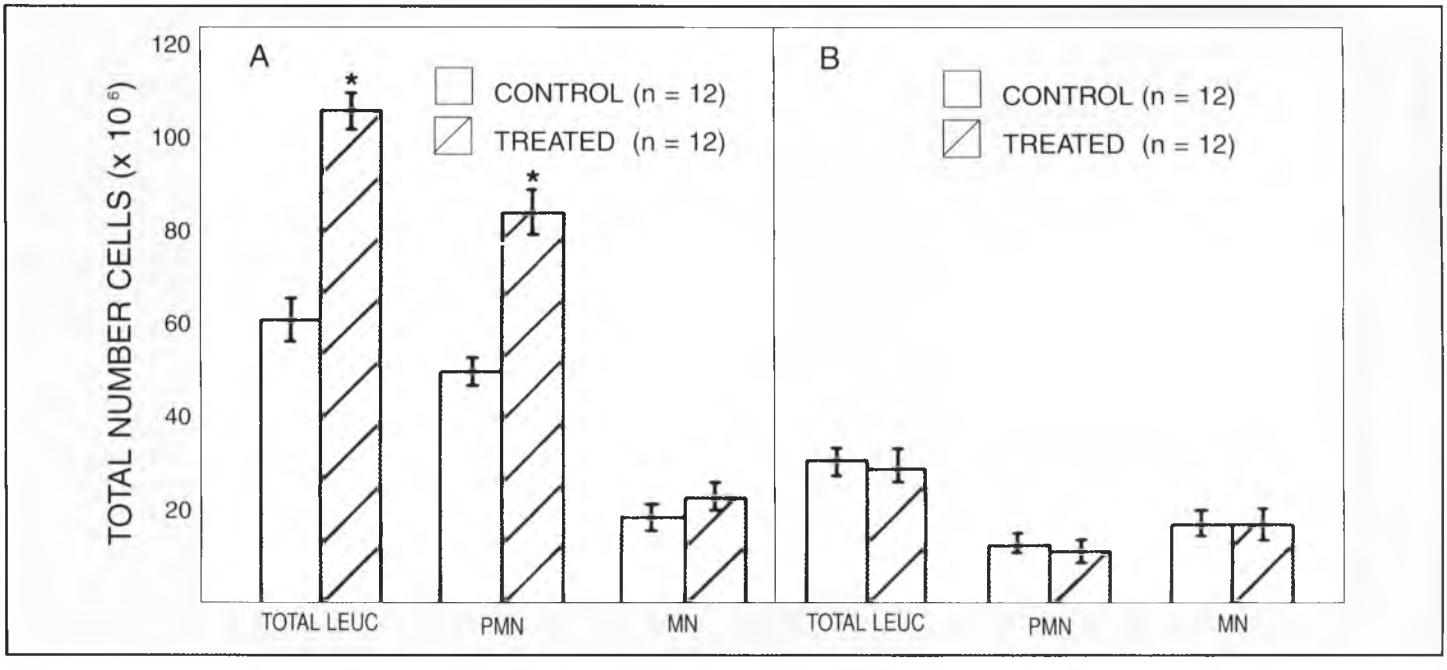

Figure 1

Effect of the pretreatment with chloramphenicol succinate (30mg/kg, *ip*, at 12h interval, for 4 days), on the number of total rats 4h after local injection of A) carrageenin (150mcg) or B) dextran (100mcg). Results are expressed as mean ± SEM. * Significant differences in relation to control group ($p<0.01$). Baseline values for total cells in the pleural cavity (n=7): total leukocytes = (4,8 ± 0.61) x 10^6; PMN = (1.2 ± 0.15) x 10^6; MN = (3.6 ± 0.9) x 10^6.

untreated). These effects were seen particularly on the number of PMN cells (Fig. 2a). Fig. 2b shows that pretreatment of the animals with chloramphenicol markedly potentiated cellular response ($p<0.01$), in relation to the control group. Previous administration of indomethacin abolished the exacerbation of cell migration in rats pretreated with the antibiotic, being the number of cells similar to that observed in the control group, but in a lesser extent ($p<0.01$) when compared to that seen in rats given the antibiotic alone. On the other hand, dexamethasone reduced in 70% the accumulation of total inflammatory cells in the pleural cavity, in comparison with the control group. This effect was mainly due to a reduction in the migrating PMN cells. The number of migrating MN cells did not show any difference among the several groups ($p<0.01$), irrespective of pretreatment with chloramphenicol and/or antiinflammatory drugs (Fig. 2a, 2b).

DISCUSSION

These results show that pretreatment of rats with chloramphenicol by the intraperitoneal route leads to a greater accumulation of inflammatory cells in the pleural cavity, 4 hours after the local injection of carrageenin, but not after dextran, in relation to a non-treated control group of animals. This potentiating effect was due to a major migration of PMN cells, since the number of PMN cells in the inflamed site did not change at all among groups. Similar results were seen by Moraes et al. [11] (1993) in the peritonitis induced by carrageenin, but not by dextran, in rats pretreated with chloramphenicol succinate. It was demonstrated that the potentiating effect of the antibiotic on the number of PMN cells was due to participation of macrophages. In the present work, it was also shown that chloramphenicol exerted its potentiating effect in the pleural cavity through, at least in part, a systemic way, since it was administered intraperitoneally. Moraes et al. [10] (1986) and Niciporciukas; Malucelli [12] (1990) observed such effect in the rat's paw edema and corneal vessels after *ip* and *sc* administration, respectively, of the same antibiotic. The inflammatory reaction induced by carrageenin is multimediated and involves the participation of several mediators of edematogenous response (Rothschild; Gascon [16], 1966; Crunkhorn; Meacok [4], 1971; Di Rosa et al.[5], 1971; Leme et al. [7], 1974; Moncada et al. [9], 1974). In addition, carrageenin produces "in situ" mediators, such as plasmatic proteins, lysosomal enzymes, complement-derived products, arachdonate metabolites and other chemoattractants and permeability factors (Bailey [1], 1988; Bliven; Otterness [2], 1988). On the other hand, dextran interacts with mast cell receptors in the tissues and elicits an anaphylactoid type of reaction characterized by a vascular response mediated particularly by histamine and serotonine (Parrat; West, 1957 [13,14,15]; Bonacorsi; West [3], 1963). The different mechanisms involved in the response to these irritants may explain the existence of a potentiating effect of

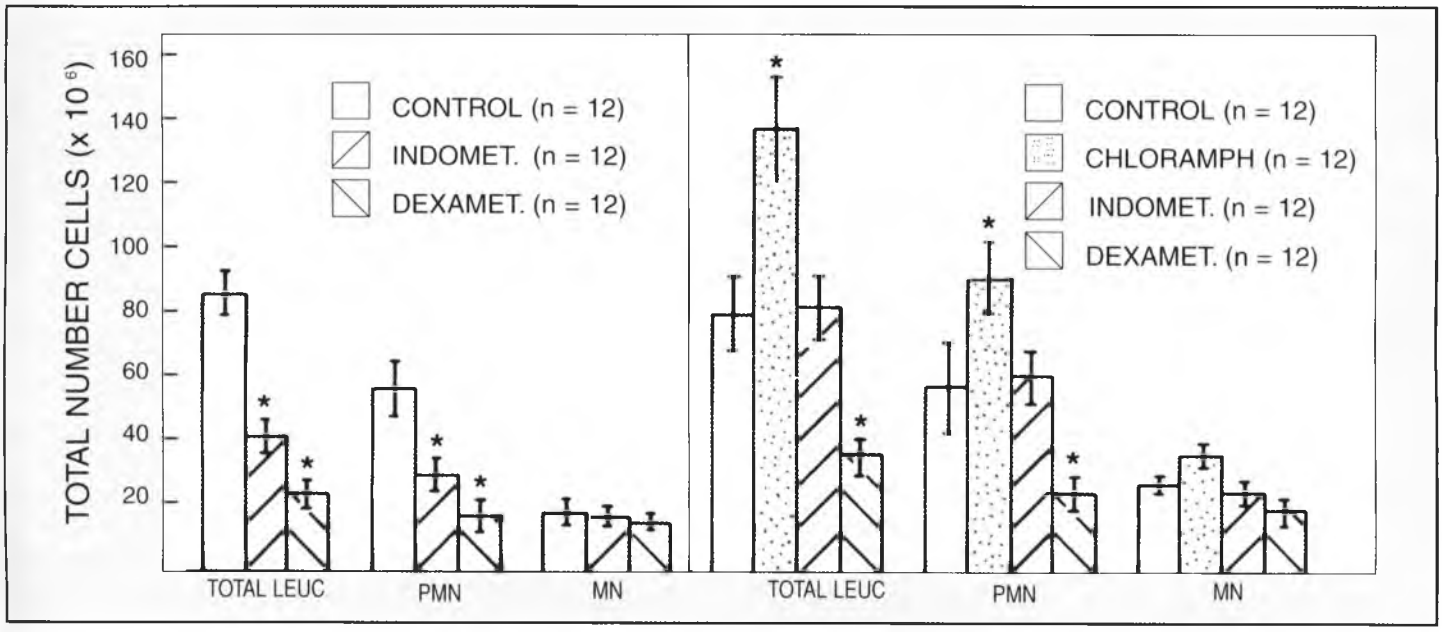

Figure 2
Effect of indomethacin (2mg/kg) and dexamethasone (0.25mg/kg) on the number of total, PMN and MN inflammatory cells migrated to the pleural cavity 4h after local injection of carrageenin (150mcg) in rats without (A) or with (B) chloramphenicol succinate (30mg/kg, *ip*, at 12h interval, 4 days). Results are expressed as mean ± SEM. * Significant differences in relation to control group ($p<0.01$).

the chloramphenicol, but only when carrageenin is used as inflammatory stimulus. Recent results from this laboratory have suggested that resident macrophages may be involved in the potentiating effect of chloramphenicol on the migration of PMN cells to the peritoneal cavity, in rats pretreated with the antibiotic, as a response to the local injection of carrageenin. This effect was enhanced after increase in the number of macrophages elicited by tioglycollate injected prior to carrageenin, and was abolished after depletion in the number of resident macrophages through washing of the peritoneal cavity before the inflammatory stimulus (Moraes et al. [11], 1993). In this work, administration of indomethacin (2.0mg/kg) or dexamethasone (0.25mg/kg) blocked 50% and 70%, respectively, the cell migration to the inflamed site, in comparison with control animals. The same dose of indomethacin was effective in blocking the potentiating effect of chloramphenicol, being the number of migrated cells similar to that of control rats (non-treated with the antibiotic). Dexamethasone was effective in chloramphenicol-treated rats as much as in those of the control group. Finnaly, although chloramphenicol can potentiate the carrageenin-induced acute inflammation, the involved mechanism still remains unclear.

RESUMO

Neste trabalho estudaram-se os efeitos da indometacina e dexametasona sobre a migração das células polimorfonucleares (PMN), produzida pelo cloranfenicol 4h após a aplicação intrapleural de carragenina ou dextrano. Demonstrou-se que o pré-tratamento de ratos com cloranfenicol (30mg/kg, *ip*, a cada 12h, por 4 dias) potenciou a migração de PMN para a cavidade inflamada, quando a carragenina (150mg) foi utilizada como estímulo inflamatório, enquanto a resposta ao dextrano (100mcg) não se alterou, em comparação com animais não tratados com o antibiótico. Ratos que receberam cloranfenicol e foram tratados com indometacina (2,0mg/kg, per os, 30 min antes da aplicação de carragenina) ou dexametasona (0,25mg/kg, *ip*, 30 min antes da aplicação de carragenina) apresentaram inibição das respostas celulares potenciadas. O mecanismo pelo qual o cloranfenicol produz aumento da resposta inflamatória permanece obscuro.

UNITERMOS: Cloranfenicol; Pleurisia; Carragenina; Dextrano; Indometacina; Dexametasona.

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Recebido para publicação em 12/04/94
Aprovado para publicação em 16/06/95