Braz. J. vet. Res. anim. Sci.,
São Paulo, v. 35, n. 6, p. 243-245, 1998.

Chloramphenicol restores the polymorphonuclear accumulation in carrageenin-induced pleurisy in diabetic rats

CORRESPONDENCE TO:
Julieta Rodini Engrácia de Moraes
Departamento de Patologia Veterinária
Faculdade de Ciências Agrárias e Veterinárias da UNESP
Campus de Jaboticabal
Rod. Carlos Tonanni, km 5
14870-000 – Jaboticabal – SP
e-mail: jrmoraes@fcav.unesp.br

1 - Departamento de Patologia da Faculdade de Ciências Agrárias e Veterinárias da UNESP, Jaboticabal – SP

Succinato de cloranfenicol restaura o acúmulo de células polimorfonucleares na pleurisia induzida pela carragenina em ratos diabéticos

Julieta Rodini Engrácia de MORAES[1]; Flávio Ruas de MORAES[1]; Jair Rodini ENGRÁCIA FILHO[1]

SUMMARY

We investigated the effect of chloramphenicol succinate (30 mg/kg, every 12 h for 4 days, ip) on the reduced polymorphonuclear leukocytes (PMN) accumulation in carrageenin-induced pleurisy (150 mg) in diabetic (alloxan, 40 mg/kg, IV) rats (Wistar, males, 180-320 g, n = 12). Chloramphenicol produced an increase ($p<0.05$) on PMN count in pleural exudate of 36% in normal animals. In the animals non-treated with chloramphenicol the diabetes state reduced ($p<0.05$) the accumulation of PMN cells by 45%. In diabetic animals treated with chloramphenicol the number of PMN in pleural exudate was similar to the one observed in the control group. Total and differential counts of leukocytes in the peripheral blood were similar between groups before and 4 h after the carrageenin-injection.

UNITERMS: Chloramphenicol; Diabetes; Pleurisia; Neutrophils.

INTRODUCTION

Chloramphenicol succinate was shown to potentiate the paw edema induced by nystatin[3] and carrageenin[5], and to increase vascular permeability of newly formed corneal vessels[8]. Data from our laboratory showed that chloramphenicol can potentiate polimorphonuclear leukocytes (PMN) accumulation induced by carrageenin, but not by dextran, in the peritoneal cavity of rats, and that macrophages are involved in this phenomenon[6,7].

Diabetic rats exhibit a less intense leukocyte accumulation in carrageenin-induced pleurisy. Pretreatment of these animals with insulin restores the number of cells present in the inflammatory exudate[4,9]. This blockage of cell migration in diabetic rats is associated with a plasmatic proteic factor that interacts with neutrophil receptors for complement-derived chemoattractants[9,10].

In this work we investigated the effects of treatment with chloramphenicol on the cellular influx elicited by carrageenin in diabetic rats.

MATERIAL AND METHOD

Animals

Male Wistar rats weighing 180-230 g, housed under standard conditions and given water and food "ad libitum" were used in the present study.

Induction of diabetes

After 12 hours fasting, diabetes was induced by the injection of 40 mg/kg alloxan, IV. After the administration of alloxan, animals were allowed free access to food and water. Five days thereafter the presence of diabetes was verified by blood glucose concentrations[2]. Only rats with blood glucose levels above 200 mg/dcl were used.

Treatment with chloramphenicol

Normal and diabetic rats were treated with chloramphenicol succinate, 30 mg/kg, by intraperitoneal injection, every 12 h for 4 days. The pleurisy was induced in 5th day.

Induction of pleurisy

The pleurisy was induced by carrageenin (150 *u*g) in control animals (NN); chloramphenicol-treated animals (NC); diabetic animals (DD) and diabetic animals treated with chloramphenicol (DC). In all animals the carrageenin-pleurisy was induced according to Velo *et al.*[12]. All animals were sacrificed four hours later by ether inhalation and exsanguination. The chest was opened, exposed and washed with 2.0 ml heparinized 0.1% phosphate buffer saline (PBS). The resulting exudate was centrifuged at 1000 rpm for 5 min in a clinical centrifuge. The supernatant was despised and the remaining cell pellet was resus-

pended in 2.0 ml heparinized 0.1% PBS. Aliquots of the cell suspension were diluted 1:20 with Turk solution and total leukocyte counts were performed in a Neubauer chamber. Differential leukocyte counts were made on smears stained panchromatically.

Leucogram assay

Before and 4 h after the carrageenin inoculation, blood samples were taken from the retroorbital plexus of the rats. Total and differential cell counts were performed and the results reported as the number of neutrophils per ml of blood.

Statistical analysis

The results were analyzed statistically by analysis of variance of the means and by the Tukey's test[11].

RESULTS

Pretreatment with chloramphenicol caused a significant increase ($p<0.05$) in total leukocyte number in response to intrapleural injection of carrageenin in relation to the control group.

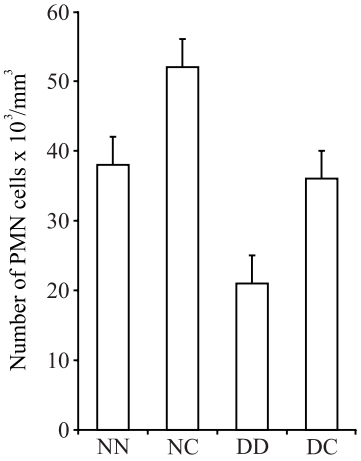

Figure 1

Effect of chloramphenicol succinate on neutrophil migration 4 h after carrageenin (150 mm) injection into the pleural cavity of in normal rats (NN); chloramphenicol-treated rats (30 mg/kg, ip, every 12 h for 4 days) (NC); diabetic rats (DD) and diabetic rats treated with chloramphenicol (30 mg/kg, ip, every 12 h for 4 days) (DC). Each bar is the mean ± SEM for 12 animals. $p<0.05$ compared to normal group (Tukey's test).

This increase was the result of an increase in polymorphonuclear (PMN) (Fig. 1) migration since the number of mononuclear (MN) cells did not differ statistically between groups. Compared with control values, total cells and PMN (Fig. 1), but not mononuclear, leukocytes were markedly reduced in diabetic rats ($p<0.05$). The treatment of diabetic rats with chloramphenicol produced a restoration of the cell counts. The number of total cells, PMN (Fig. 1) and mononuclear leukocytes was not different ($p<0.05$) between DC and NN groups.

The results showed that total and differential counts of circulating leukocytes in the peripheral blood were not different between NN, NC, DD and DC groups (Tab. 1).

DISCUSSION

Earlier results showed that chloramphenicol potentiates the edema and neutrophil migration induced by carrageenin in the rat paw[5] and increases vascular permeability of newly formed corneal vessels[8]. The same antibiotic increases the PMN migration in the rat peritoneal[6] and pleural[7] cavities stimulated with carrageenin, but not with dextran, and the macrophages are involved in this phenomenon[6]. The data showed here are in concordance with our previous results concerning the increase in PMN counts in the pleural cavity four hours after stimulation with carrageenin in chloramphenicol-treated rats when compared with control animals.

Results of the present work show a reduction ($p<0.05$) in total and PMN leukocyte counts in the pleural exudate of diabetic rats compared with non-diabetic rats. These results agree with that reported by Garcia Leme[1]. In contrast, diabetic animals treated with chloramphenicol succinate (DC) and the control group (NN) did not show any difference ($p<0.05$) between total and PMN leukocyte counts. So, the antibiotic treatment appears to restore the cellular migration inhibited in diabetic state. This phenomenon was not dependent on the number of circulating leukocytes because total and differential counts in the peripheral blood were not different between NN, DD, NC and DC groups.

The failure of cellular migration in a diabetic state is a physiopathological condition and the underlying mechanism involved in the potentiation of PMN response in normal animals or the restoration of this response in diabetic animals remains unclear.

ACKNOWLEDGEMENTS

We thank Miss FA Ardisson and Mrs. MIY Campos for their technical assistance.

Table 1

Blood leukocyte counts (x10³/mm3) in controls (NN), chloramphenicol-treated animals (NC), diabetic rats (DD) and diabetic animals treated with chloramphenicol (DC) before (0) and 4 hours after the noxious stimulation (n = 12). Jaboticabal – SP, 1994.

time	Lymphocytes		Monocytes		Neutrophils		Eosinophils	
	0	4	0	4	0	4	0	4
NN	7.2 ± 0.4	6.9 ± 0.6	1.3 ± 0.1	1.4 ± 0.2	4.9 ± 0.2	4.8 ± 0.2	0.1 ± 0.04	0.1 ± 0.04
NC	7.4 ± 0.4	7.2 ± 0.7	1.3 ± 0.1	1.3 ± 0.1	4.5 ± 0.1	6.0 ± 0.1	0.09 ± 0.06	0.1 ± 0.05
DD	6.8 ± 0.6	7.1 ± 0.5	1.7 ± 0.1	1.3 ± 0.1	4.6 ± 0.4	4.1 ± 0.3	0.1 ± 0.05	0.1 ± 0.03
DC	7.1 ± 0.3	6.9 ± 0.5	1.5 ± 0.2	1.3 ± 0.1	4.6 ± 0.2	4.3 ± 0.2	0.1 ± 0.05	0.1 ± 0.05

RESUMO

Investigou-se o efeito do succinato de cloranfenicol (30 mg/kg, a cada 12 h, durante 4 dias, IP) sobre o acúmulo de leucócitos polimorfonucleares (PMN) na pleurisia induzida pela carragenina (150 mg) em ratos (Wistar, machos, 180-230 g, n = 12) diabéticos (40 mg/kg de aloxana, IV). O antibiótico produziu aumento de 36% no número de PMN ($p<0,05$) migrados para a cavidade pleural de animais normais. O estado diabético provocou redução de 45% dos PMN ($p<0,05$) acumulados no exsudato pleural de animais não tratados com o antibiótico. Por outro lado, animais diabéticos tratados com succinato de cloranfenicol apresentaram resposta de PMN que não diferiu estatisticamente do observado em animais controle, não tratados. A contagem total e diferencial dos leucócitos circulantes realizada antes e 4 h depois da aplicação da carragenina não diferiu estatisticamente entre os grupos.

UNITERMOS: Cloranfenicol; Diabetes; Pleurisia; Neutrófilos.

REFERENCES

1- GARCIA LEME, J. **Hormones and inflammation**. Boca Raton, Florida : CRC Press, 1989. p.238.

2- KING, E.S.; GARNER, R.J. Colorimetric determination of glucose. **Journal of Clinical Pathology**, v.1, n.4, p.30-3, 1947.

3- LAUS, J.L. **Estudo experimental comparativo de enterorrafias e dos efeitos do cloranfenicol sobre a cicatrização intestinal**. São Paulo, 1985. 72p. Dissertação (Mestrado). Faculdade de Medicina Veterinária e Zootecnia, USP.

4- MORAES, F.R.; BECHARA, G.H.; MORAES, J.R.E. Effect of alloxan diabetes and adrenalectomy on carrageenin-induced pleurisy in the rat. **Brazilian Journal of Medical and Biological Research**, v.20, n.1, p.47-53, 1987.

5- MORAES, F.R.; MELITO, I.; MORAES, J.R.E.; LAUS, J.L. The pro-inflammatory effect of chloramphenicol on the acute inflammation. **Ars Veterinaria**, v.2, n.2, p.205-9, 1986.

6- MORAES, J.R.E.; MORAES, F.R.; BECHARA, G.H. Participation of macrophages in chlorampehicol-potentiated carrageenin-induced peritonitis in rats. **Brazilian Journal of Medical and Biological Research**, v.26, n.5, p.497-507, 1993.

7- MORAES, J.R.E.; MORAES, F.R.; BECHARA, G.H. The potentiation effect of chloramphenicol succinate in rat carrageenin-induced acute pleurisy. Inhibition by indometacina and dexamethasone. **Brazilian Journal of Medical and Biological Research**, v.33, p.11-4, 1996.

8- NICIPORCIUKAS, M.C.; MALUCELLI, B.E. Chloramphenicol induces an increase on the vascular permeability of newly formed blood vessels. *In*: INTERNATIONAL CONGRESS OF INFLAMMATION, Barcelona, 1990. **Annals**. p.313.

9- PEREIRA, M.A.; SANNOMIYA, P.; GARCIA LEME, J. Inhibition of leukocyte chemotaxis by a factor in alloxan diabetic rat plasma. **Diabetes**, v.36, n.11, p.1307-14, 1987.

10- SANNOMIYA, P.; PEREIRA, M.A.; GARCIA LEME, J. Inhibition of leukocyte chemotaxis by a serum factor in diabetes mellitus: selective depression of cell response mediated by complement derived chemoatractants. **Agents and Actions**, v.30, n.3-4, p.369-76, 1990.

11- SNEDECOR, G.W.; COCRHAN, W.G. **Statistical methods**. 6.ed. Ames : Iowa State University Press, 1974. p.294.

12- VELO, G.P.; DUNN, C.J.; GIROUD, J.P.; TIMSIT, J.; WILLOUGHBY, D.A. Distribuition of prostaglandins in inflammatory exudate. **Journal of Pathology**, v.111, p.49-158, 1973.

Received: 10/04/1997
Accepted: 22/07/1998