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Pharmacological Profile of Rat Pleurisy Induced by *Bothrops jararaca* Venom

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Abstract

Bothrops jararaca venom (30 μg/site) triggered a marked inflammatory reaction in the pleural cavity that was long-lasting and reproducible.

In the first 1 h after pleurisy induction, a significant decrease of total and differential cell count was observed in comparison with control values, despite the gradual enhancement of fluid leakage. A significant increase of cell migration was observed after 3 h of pleurisy induction, due to mononuclear and neutrophil cells that peaked 8 h later and this was followed by a gradual decrease, remaining elevated up to 24 h. In parallel with cell influx, a significant increase of fluid leakage that peaked between 1 and 8 h was observed, being completely abolished after 12 h following pleurisy induction. This inflammatory response was not associated in parallel with significant changes in circulating leucocyte cells and it was significantly inhibited by compound 48/80, cyproheptadine, pyrilamine, dexamethasone, indomethacin and phenidone. Preheating of the venom (100°C) caused a significant decrease of both leakage of fluid and cell migration in the pleural cavity 8 h after pleurisy induction. Previous exposure to the venom (30 μg/site, 5 days before) produced a significant decrease of both cell migration and fluid leakage 4 h after triggering pleurisy with the same dose of the venom. Otherwise, prior daily treatment with the venom (10 μg/site, 4 days) resulted only in marked fluid leakage reduction 1 h after treating the animals with BJV (30 μg/site).

These results show that the venom elicits pro-inflammatory effects in the rat pleural cavity which involve the participation of several mediators, including histamine, 5-hydroxytryptamine and products of arachidonic pathways.

It has been widely recognized that *Bothrops jararaca* venom (BJV) poisoning is often associated with a large variety of symptoms and that inflammation is a common local feature of the effects induced by the snake bites (Rothschild & Rothschild 1979; Fletcher et al 1980; Meier & Stocker 1991). These findings may reflect the diversity and also the different concentrations of substances presented in these venoms. This hypothesis is corroborated by the biochemical and pharmacological data in the literature that reveal a large number of enzymes as well as non-enzymatic compounds of different biological activities which are present and released by this venom (Mandelbaum et al 1982; Meier & Stocker 1991). In concordance with these findings, previous studies from our laboratory have demonstrated that BJV induces a dose- and time-dependent oedema in the rat paw (Trebien & Calixto 1989). These pro-inflammatory effects were primarily mediated by release of cyclooxygenase and lipoxygenase products of arachidonic acid pathways and by activation of both α_1- and α_2-adrenoceptors, while kinins have apparently no participation (Trebien & Calixto 1989). In addition, BJV also caused oedematogenic reaction when administered in the mouse paw, this effect being mediated by arachidonic pathway products, namely metabolites from both cyclooxygenase and lipoxygenase pathways, but not by histamine, 5-hydroxytryptamine (5-HT) or platelet activating factor (Perales et al 1992).

In this study, we further analysed the pro-inflammatory effects induced by BJV using an experimental model of rat pleurisy which permits both a quantitative and qualitative evaluation of cell migration as well as fluid leakage (Vinegar et al 1976; Lo et al 1982). In addition, several drugs known for their potential effects either on the synthesis or at the receptor level of different mediators of the inflammatory process were employed to evaluate the most likely mechanisms involved in the inflammatory response caused by BJV.

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Materials and Methods

Animals

Non-fasted adult male rats, 200–300 g, maintained in a controlled temperature environment (21 ± 1°C), illuminated by daylight supplemented by electric light from 0600 to 1800 h, with water and purina chow freely available, were used.

Induction of pleurisy

Rats were lightly anaesthetized with ether and BJV (final volume of 0.1 mL in sterile saline) was injected into the pleural cavity through the chest skin. The animals were killed with ether at different periods and immediately after opening the thorax the pleural cavity was washed with 2 mL saline plus heparin (10 int. units mL^{-1}) and the volume was collected with a graduated plastic syringe. The total volume collected was then subtracted from the amount of injected saline solution to obtain the real exudate volume. The total number of leucocytes in the exudate (in Türck's solution) was counted in a Neubauer chamber under an optical microscope. A differential count of both mononuclear and neutrophil cells was carried out using May-Greenwald-Giemsa

stain in smear preparations. Since there are no clinical data which provided an estimate of the amount of injected venom, in preliminary experiments we tested different doses of BJV in an attempt to obtain a pro-inflammatory effect dissociated from its action on the coagulation cascade.

In other experiments, after determining the peak of BJV effects on exudate and total leucocyte number and migration pattern of white cells, we evaluated whether the inflammatory reaction induced by BJV was associated with parallel changes in either the leucocyte or platelet number of the peripheral blood. Thus, immediately before killing the animals (4 and 8 h after pleurisy induction) to examine the pleural cavity, blood samples from the tail were collected. Total platelets (in ammonium oxalate 1%) and leucocyte number (in Türck's solution) as well as differential leucocyte number were determined. To evaluate the presence of thermolabile pro-inflammatory agents in the venom, a group of animals was intrapleurally treated with BJV, preheated 30 min before the injection at 100°C for 10 min. After 1 and 8 h, the animals were killed and the inflammatory reaction was analysed as described above. In another set of experiments, we further investigated if the pleural inflammatory response pattern induced by BJV in the experiments cited above was altered after repeated exposure. Thus, a group of animals was challenged 4 days before with the selected dose of BJV that was employed in the above studies, and the studied parameters were analysed on the 5th day, 4 h after triggering pleurisy with the same dose of the venom. In other experiments, the animals were treated daily (4 days) with a dose of the venom that did not cause any measurable inflammatory reaction in the pleural cavity, and the studied parameters were analysed on the 5th day, 1 h after triggering pleurisy with the selected dose of BJV as cited in the experiments above.

To assess the most likely mechanism by which BJV produced its inflammatory effects, different groups of animals were pretreated before inducing pleurisy with different drugs known for their potential effects on the synthesis or effects at the receptor level of several mediators of the inflammatory reaction. Thus, indomethacin (a cyclooxygenase inhibitor: 10 mg kg^{-1}, i.p.), phenidone (a dual inhibitor of cyclooxygenase and lipoxygenase: 20 mg kg^{-1}, i.p.), dexamethasone (the most potent available anti-inflammatory drug: 0.5 mg kg^{-1}, i.p.), pyrilamine maleate (a histamine antagonist: 10 mg kg^{-1}, i.p.) and cyproheptadine (a histamine and 5-HT antagonist: 5.0 mg kg^{-1}, i.p.) were employed. In other experiments, the animals were pretreated with captopril (a kininase II inhibitor: 5 mg kg^{-1}, s.c.) to evaluate the possible participation of kinins in this process. All drugs, except dexamethasone, were administered 1 h before BJV. The different groups of animals were killed 1, 4 or 8 h after the phlogogen administration. Pretreatment of the animals with dexamethasone varied from 6 to 1 h before pleurisy induction according to the period of time chosen to analyse the inflammatory reaction. To further evaluate the role of histamine and 5-HT in this inflammatory response, rats were treated with an intrapleural injection of compound 48/80 (a releaser of both histamine and 5-HT: 12.5 μg/site) and pleurisy with BJV was triggered 5 days after. In other experiments, a group of animals was pretreated with WEB 2086 (PAF-acether antagonist: 15 μg/site), 5 min before inducing pleurisy. The same parameters discussed above were analysed. The choice of the dose, administration pathways and time of pretreatment were based either on data reported in the literature or on preliminary experiments.

Each experimental group included an equivalent number of control animals that received the same intrapleural volume of sterile NaCl (154 mM) and were killed at the same time as their matched treated group. When appropriate, control-treated animals with an intrapleural injection of BJV were also included for comparison.

Statistical analysis

Data are presented as mean ± s.e.m. Differences between groups were determined either by analysis of variance complemented with Dunnett's test or Newman-Keuls test or by unpaired *t*-test when indicated. P less than 0.05 was considered significant.

Drugs

Indomethacin, dexamethasone, compound 48/80 (condensation product of *N*-methyl-*p*-methoxy-phenethylamine with formaldehyde), pyrilamine maleate, cyproheptadine hydrochloride, phenidone and captopril were purchased from Sigma (St Louis, USA), heparin was purchased from Cristália Laboratório (São Paulo, Brazil), BJV was kindly supplied by the Butantan Institute (São Paulo, Brazil), WEB 2086 (3-4-(2 chloro-phenyl)-9-methyl-6H-thieno-(3, 2*f*) (1, 2, 4)-triazolo(4, 3-*a*)(1,4)-diazepine-2-yl)-1-(4-morpholynil-l-propranone), was obtained from Instituto Oswaldo Cruz (Rio de Janeiro, Brazil) and sterile saline solution (NaCl 154 mM) was obtained from different commercial sources. Drugs were diluted in water, except BJV, compound 48/80 (sterile saline solution, 154 mM), indomethacin (5% $NaHCO_3$) and WEB 2086 (sterile saline plus HCl 0.1 M).

Results

The intrapleural administration of 30 μg of BJV resulted in a time-related inflammatory effect. Higher venom doses (50 and 100 μg/cavity) resulted in significant local haemorrhagic effects, whereas low doses (0.3 and 1.0 μg/cavity) did not cause expressive or reproductive inflammatory effects that could be expressed through the analysed parameters (results not shown). Thus, all further experiments were performed by administering 30 μg of the venom into the pleural cavity.

As shown in Fig. 1, fluid accumulation in the pleural cavity was detected within the first 15 min and it peaked between 1 and 8 h ($P < 0.01$), being completely abolished after 12 h. Changes in the cellular content of the harvest exudate ran almost in parallel with fluid accumulation, but with distinct features. In the first hour after pleurisy induction, a marked decrease in the total cell number in comparison with control animals was observed ($P < 0.05$). A significant increase of cell migration was observed after 3 h of pleurisy induction, due to mononuclear and neutrophil cells that peaked at 8 h. This late effect was long lasting (up to 24 h after pleurisy induction), since it remained elevated ($P < 0.05$) even when no more exudate could be detected in the pleural cavity. Differential cell count revealed that mononuclear leucocytes were significantly decreased in the first hour ($P < 0.01$), whereas neutrophils gradually increased in

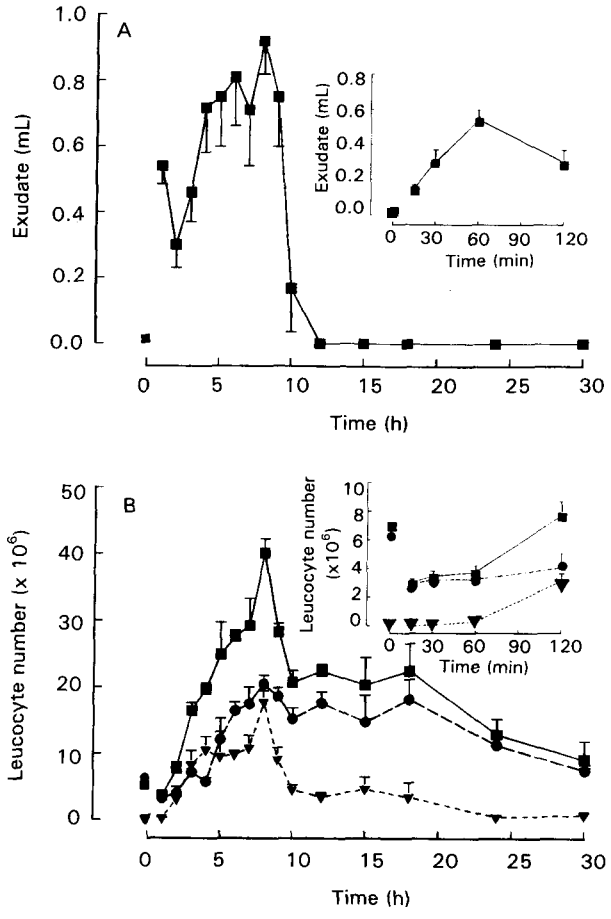

FIG. 1. Time-course of BJV (30 μg/site)-induced pleurisy in rats: A: exudate volume (■) and B) total leucocyte (■), neutrophil (●) and mononuclear cells (▼). Insets show the same parameters in the first 2 h. Each point represents the mean of 4–10 animals per group and the vertical lines the s.e.m.

the pleural cavity at 2 h, peaking at 8 h ($P < 0.01$) and remaining elevated up to 18 h ($P < 0.05$). It is interesting to note that 24 h later, the total cellular content was still elevated, the mononuclear leucocytes being predominant at this time. In addition, 30 h after pleurisy induction, both total and differential leucocyte numbers were completely normal, indicating that there was a full resolution of this inflammatory process. Eosinophil content in the pleural cavity did not significantly change after the administration of BJV (results not shown).

This acute inflammatory effect evoked by BJV in the pleural cavity did not result in parallel changes in the total number of leucocytes in the peripheral blood (control non-treated group = $6506.0 \pm 524.0\,mm^{-3}$ and BJV-treated group: 7100.0 ± 62.3 and $6093.0 \pm 853.6\,mm^{-3}$, 4 and 8 h after pleurisy induction). Differential leucocyte count of peripheral blood smears obtained at 4 and 8 h following pleurisy induction also did not significantly differ from that of control (non-treated animals) (results not shown). The platelet number, however, was significantly increased 4 h after pleurisy (control = $81000.0 \pm 5.7\,mm^{-3}$, BJV-treated group = $200000.0 \pm 16.8\,mm^{-3}$, $P < 0.01$), being normal 8 h thereafter (BJV-treated group = $85200.0 \pm 9.9\,mm^{-3}$).

Preheating of BJV at 100°C for 10 min resulted in a significant decrease of both fluid leakage and cell content in the pleural cavity (Table 1). Following 1 h of pleurisy induction, the animals exposed to the pre-heated venom presented a slight but non-significant decrease of the number of leucocytes, associated with a marked decrease in the volume leakage ($P < 0.01$) (Table 1). At 8 h after pleurisy induction, a marked decrease either in the total ($P < 0.01$) or differential ($P < 0.01$) leucocyte numbers (Table 1) was observed. In other experiments, animals previously exposed to a full dose of BJV (30 μg/site), when rechallenged 5 days after with the same dose of the venom, presented a significant decrease 4 h after pleurisy induction of both total and differential leucocyte number, due to neutrophils ($P < 0.01$) as well as exudate volume in the pleural cavity (Table 2). On the other hand, daily treatment of the animals with a dose of BJV (10 μg/site, 4 days), which did not cause any measurable inflammatory reaction resulted

Table 1. Effect of preheating of BJV at 100°C, for 10 min, on the development of 1 and 8 h BJV-induced pleurisy (30 μg/site) in comparison with treated groups with the venom. A. Parameters evaluated 1 h after pleurisy induction. B. Parameters evaluated 8 h after pleurisy induction.

A. 1 h after pleurisy induction Cell number (×10⁶)	BJV treated (1 h)	BJV (pre-heated 100°C)
Total leucocytes	3·5 ± 0·4	2·2 ± 0·5
Mononuclear leucocytes	3·2 ± 0·6	2·0 ± 0·5
Neutrophils	0·3 ± 0·02	0·2 ± 0·001
Exudate volume (mL)	0·54 ± 0·03	0·08 ± 0·001**
B. 8 h after pleurisy induction Cell number (×10⁶)	**BJV treated**	**BJV (pre-heated 100°C)**
Total leucocytes	40·2 ± 2·1	12·3 ± 3·0**
Mononuclear leucocytes	22·2 ± 1·3	7·0 ± 0·5**
Neutrophils	17·7 ± 1·8	0·2 ± 0·01**
Exudate volume (mL)	0·9 ± 0·1	0·01 ± 0·0**

(Mean ± s.e.m. n = 4–6 animals per group). **$P < 0.01$.

Table 2. Effect of prior intrapleural administration of BJV on the development of BJV-induced pleurisy (30 μg/site) in comparison with the same parameter obtained in animals exposed once to the venom. A. Only exposed once to BJV (30 μg/site) or previously exposed to BJV (30 μg/site) and rechallenged 5 days after with the same dose of venom. Studied parameters were analysed 4 h after pleurisy induction. B. Only exposed once to BJV (30 μg/site) or animals were treated daily with BJV (10 μg/site, 4 days) and pleurisy (BJV: 30 μg/site) was triggered on the 5th day. Studied parameters were analysed 1 h after pleurisy induction. Results are expressed as millions of cells.

A.	BJV treated (30 μg/site)	BJV pretreated (30 μg/site)
Total leucocytes	19·7 ± 0·7	8·5 ± 1·2**
Mononuclear lucocytes	9·0 ± 0·7	4·7 ± 1·4
Neutrophils	10·6 ± 0·7	2·5 ± 0·5**
Exudate volume (mL)	0·7 ± 0·1	0·04 ± 0·02**
B.	**BJV treated (30 μg/site)**	**BJV (treated daily with 10 μg/site)**
Total leucocytes	3·5 ± 0·4	4·1 ± 1.1
Mononuclear leucocytes	3·0 ± 0·4	3·3 ± 0·9
Neutrophils	0·2 ± 0·09	0·3 ± 0·2
Exudate volume (mL)	0·5 ± 0·05	0·1 ± 0·01**

Mean ± s.e.m.(n = 4–6 animals per group). **$P < 0.01$ Compared with matched group only exposed to BJV.

in a significant reduction of fluid leakage, but not in the cell content when the studied parameters were analysed 1 h after pleurisy induction (BJV: 30 μg/site) (Table 2).

Table 3 shows that the pretreatment of the animals with different drugs resulted in distinct effects against BJV-induced pleurisy. Following 1 h of pleurisy induction, it was observed that indomethacin, phenidone, dexamethasone, pyrilamine and cyproheptadine all significantly inhibited fluid accumulation in the pleural cavity ($P < 0.01$). At this time, phenidone and dexamethasone significantly potentiated

Table 3. Effect of the pretreatment of the animals with several drugs on the development of 1, 4 and 8 h *Bothrops jararaca* venom-induced pleurisy in the rat.

Groups	Doses	Total leucocytes	Neutrophils	Mononuclears	Exudate
1 h[a]					
Control[b]	–	3·5 ± 0·4	0·21 ± 0·09	3·0 ± 0·4	0·5 ± 0·05
Indomethacin	10·0 mg kg^{-1}, i.p.	4·7 ± 0·7	0·15 ± 0·05	3·5 ± 0·7	0·1 ± 0·05**
Phenidone	20·0 mg kg^{-1}, i.p.	1·8 ± 0·2**	0·03 ± 0·02	1·2 ± 0·4	0·1 ± 0·04**
Dexamethasone	0·5 mg kg^{-1}, p.o.	1·2 ± 0·2**	0·01 ± 0·01	1·1 ± 0·2	0·1 ± 0·04**
Pyrilamine	10·0 mg kg^{-1}, i.p.	2·4 ± 0·5	0·05 ± 0·05	1·9 ± 0·5	0·05 ± 0·05**
Captopril	5·0 mg kg^{-1}, s.c.	2·1 ± 0·3	0·03 ± 0·03	2·2 ± 0·3	0·5 ± 0·05
Cyproheptadine	5·0 mg kg^{-1}, i.p.	2·7 ± 0·3	0·09 ± 0·04	1·9 ± 0·2	0·1 ± 0·05**
Compound 48/80[c]	12·5 μg/site	2·5 ± 0·2	0·13 ± 0·02	1·7 ± 0·2	0·2 ± 0·03
WEB 2086[d]	15·0 μg/site	2·3 ± 0·2	0·13 ± 0·02	1·7 ± 0·2	0·6 ± 0·06
4 h[a]					
Control[b]	–	19·7 ± 0·7	10·6 ± 0·7	9·0 ± 0·7	0·7 ± 0·1
Indomethacin	10·0 mg kg^{-1}, i.p.	18·8 ± 1·0	9·1 ± 1·4	9·4 ± 1·2	0·2 ± 0·1**
Phenidone	20·0 mg kg^{-1}, i.p.	14·0 ± 1·2*	7·2 ± 1·2	6·5 ± 1·1	0·2 ± 0·1**
Dexamethasone	0·5 mg kg^{-1}, p.o.	8·7 ± 1·2**	3·9 ± 0·6**	4·5 ± 0·7	0·4 ± 0·04
Pyrilamine	10·0 mg kg^{-1}, i.p.	20·2 ± 0·8	12·9 ± 1·7	7·2 ± 1·4	0·5 ± 0·1
Captopril	5·0 mg kg^{-1}, s.c.	19·4 ± 0·5	11·6 ± 1·0	7·3 ± 1·3	0·7 ± 0·1
Cyproheptadine	5·0 mg kg^{-1}, i.p.	7·3 ± 2·2**	5·0 ± 1·0	2·2 ± 0·4**	0·3 ± 0·05**
Compound 48/80	12·5 μg/site	10·6 ± 1·1**	3·5 ± 1·0**	7·0 ± 0·7	0·2 ± 0·1**
WEB 2086[d]	15·0 μg/site	16·6 ± 3·4	11·7 ± 1·8	4·9 ± 0·6	0·4 ± 0·1
8 h[a]					
Control[b]	–	40·2 ± 2·1	17·7 ± 1·8	22·2 ± 1·3	0·9 ± 0·1
Indomethacin	10·0 mg kg^{-1}, i.p.	16·4 ± 0·9**	7·3 ± 1·1**	9·0 ± 0·6**	0·1 ± 0·1**
Phenidone	20·0 mg kg^{-1}, i.p.	18·5 ± 5·3**	7·9 ± 3·7**	10·3 ± 2·7**	0·3 ± 0·1**
Dexamethasone	0·5 mg kg^{-1}, p.o.	5·8 ± 0·6**	1·2 ± 0·3**	4·6 ± 0·2**	0·0**
Pyrilamine	10·0 mg kg^{-1}, i.p.	23·6 ± 3·6**	7·2 ± 0·7**	15·7 ± 4·7	0·1 ± 0·1**
Captopril	5·0 mg kg^{-1}, s.c.	35·4 ± 0·6	14·2 ± 1·5	18·9 ± 1·5	0·7 ± 0·04
Cyproheptadine	5·0 mg kg^{-1}, i.p.	19·3 ± 1·7**	15·2 ± 0·8*	6·7 ± 0·9	0·3 ± 0·7**
Compound 48/80[c]	12·5 μg/site	17·9 ± 2·7**	5·4 ± 0·6**	2·4 ± 0·7**	0·2 ± 0·09**
WEB 2086[d]	15·0 μg/site	32·7 ± 2·1	19·7 ± 1·3	12·5 ± 1·9	1·4 ± 0·2**

All animals were injected with *Bothrops jararaca* venom (30 μg/site). Values represent the mean ± s.e.m. (n = 4–10 animals per group). Statistically significant difference between treated and control animals is indicated by asterisk. *$P < 0.05$, **$P < 0.001$. [a]Time after intrapleural injection of BJV, [b]only treated with BJV, [c]pretreated with compound 48/80 five days before BJV, [d]pretreated with WEB 2086, 5 min before BJV. All other experimental groups were treated 1 h before, except for dexamethasone (see text).

the decrease of total cell content in the harvest fluid of pleural cavity ($P < 0.01$), whereas the other studied drugs had no effect. In addition, 4 h after triggering the pleurisy with BJV, phenidone, dexamethasone, cyproheptadine and compound 48/80 were shown to be effective in reducing cell migration in the pleural cavity ($P < 0.05$), whereas indomethacin, dexamethasone, cyproheptadine and compound 48/80 inhibited fluid accumulation. Even after 8 h following pleurisy induction by the BJV, all drugs except captopril and WEB 2086 significantly inhibited both fluid accumulation and cell migration ($P < 0.01$). At this time, the most potent drugs were dexamethasone, followed by indomethacin, compound 48/80, phenidone, cyproheptadine and pyrilamine (Table 3).

Discussion

These results show that the intrapleural injection of BJV triggered a marked inflammatory reaction that was well reproducible, time-dependent and long-lasting, being completely reverted by about 30 h after pleurisy induction. Since this inflammatory reaction revealed a distinct profile in relation to leucocyte content and fluid leakage, associated with the fact that it was inhibited by different drugs, it is suggested that different inflammatory mediators take part in this process.

As described in other models, 1 h after pleurisy induction a low leucocyte count was observed, which is probably due to aggregation and adherence to cells on the serosa of the cavity, as previously described (Born & Planker 1979; De Brito 1989). In addition, the gradual enhancement of cell migration after 3 h that peaked 8 h later and remained elevated up to 24 h thereafter may constitute indirect evidence that the injected venom is still acting on the cells, or may even represent their late response to the venom. Together, these results indicate that a time-dependent release of different mediators may be occurring in a similar manner to that described in the pleurisy induced by other agents (Capasso et al 1975; Berckenkopf & Planker 1987; Hori et al 1988). In addition, the fact that changes in the pleural cavity elicited by BJV were not associated in parallel with systemic modifications in the total and differential leucocyte concentrations as assessed by analysis of peripheral blood smears indicates that this process was primarily limited to its site of injection. This is not valid for the platelets that significantly increased 4 h after pleurisy induction. We have no clear explanation for these findings, but it is likely that the increased number of platelets may not represent a real enhancement in the number of these cells in the circulation, but most probably reflects the activation of platelet aggregation, as it occurs in other conditions (Lastória et al 1987).

The findings that pyrilamine maleate, compound 48/80 and cyproheptadine were able to inhibit BJV-induced fluid accumulation or cell migration at distinct periods of time provide indirect evidence that histamine or 5-HT are the possible mediators involved in this inflammatory response. However, in spite of the limitations in interpreting the above data, it may be concluded that histamine and 5-HT are not the unique agents involved in this process, since pleurisy induced by these autachoids resulted in a monophasic enhancement of mononuclear cells which is barely associated with fluid leakage (Martins et al 1989; Henriques et al 1991). In addition, we cannot ignore the fact that products of arachidonic acid pathways also take part in this process, since dexamethasone, phenidone and indomethacin also inhibited some parameters analysed in the pleurisy induced by BJV. However, the marked inhibition caused by dexamethasone compared with the other studied drugs in both cell migration and fluid leakage certainly is not exclusively linked to its inhibitory effects on the arachidonic acid cascade, but is also due to its action on other steps of the inflammatory process such as nitric oxide synthesis or cytokine effects (Barnes & Adcock 1993). Otherwise, we must conclude that neither captopril nor WEB 2086 interfered with either fluid leakage or total and differential cell accumulation in the pleural cavity, discarding the possibility that bradykinin and PAF-acether are involved in this inflammatory process. Taken together, these data provide evidence that histamine, 5-HT and eicosanoids are most likely the main mediators released in the early and late phases of the pleurisy elicited by BJV.

In spite of the differences in the models and methodological approaches, there are some similarities between BJV-induced pleurisy (present study) and paw oedema in rats (Trebien & Calixto 1989) and mice (Perales et al 1992). First, the dose and time-course profiles of the pleural leakage and of the oedematogenic effect in the paw are quite similar. Second, preheating of the BJV at 100°C almost completely abolished the fluid leakage in the pleural cavity and significantly reduced the paw oedema induced by the venom. In contrast, repeated exposure of the venom before the analysis of the studied parameters in both models in the rat revealed opposite effects. Whereas no interference was observed in the rat paw oedema induced by BJV, this procedure resulted in marked reduction of fluid leakage with no change in the cellular content of the pleural cavity after pleurisy induction by the venom. Furthermore, comparison of our results with the literature data where the effect of the crude venom from different snakes was tested (Tan et al 1991) reinforces the hypothesis that most probably histamine and phospholipase A_2-dependent pathway products may be responsible, at least in part, for this inflammatory process.

In conclusion, the intrapleural injection of BJV caused a marked and long-lasting inflammatory reaction in the pleural cavity of rats. Although, based on our experimental data, it is not possible to determine the exact nature of the inflammatory mediators that participate in this process, our results seem to indicate that the pleurisy induced by BJV involves the participation of several mediators, namely histamine, 5-HT and products of arachidonic acid pathways. In addition, since the pleurisy induced by BJV presents distinct features in comparison with the known inducers of pleurisy (De Brito 1989), this model may constitute another important tool in improving evaluations of the inflammatory process and in testing potential anti-inflammatory drugs.

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