ANTI-INFLAMMATORY EFFECTS OF PRASEODYMIUM, GADOLINIUM AND YTTERBIUM CHLORIDES

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Anti-inflammatory effects of chloride salts of praseodymium, gadolinium and ytterbium were investigated, using various experimental inflammatory models in rats. The lanthanide salts administered by oral route showed no significant effect, but when injected intraperitoneally they significantly inhibited the carrageenin-induced oedema, proportional to their doses ranging from 15 to 75 mg/kg. They also reduced nystatin-induced oedema and vascular permeability response to histamine and serotonin. Pronounced inhibitory effect of lanthanide salts at the dose of 50 mg/kg, i.p., was observed in histamine- and serotonin-induced changes in vascular permeability. Repeated administration of lanthanide salts in the dose of 20 mg/kg for 13 d significantly inhibited arthritis development. The same dose of these salts for a 6-d period similarly reduced granuloma formation. However, praseodymium, gadolinium and ytterbium chlorides showed no significant difference among themselves and their anti-inflammatory effects were smaller than those from phenylbutazone.

Keywords—lanthanide salt; praseodymium; gadolinium; ytterbium; anti-inflammatory effect

INTRODUCTION

Pharmacological studies of lanthanides have been made since 1920, when Doerr\(^1\) described their germicidal action. Haley et al.\(^2\) and other authors\(^3\)–\(^8\) have shown the toxicity of several lanthanides in animals. Anti-coagulant activity was accurately investigated,\(^8\)–\(^13\) and their effect similar to that of dicumarol is antagonized by vitamin K.\(^9\) More recently, Jancsó\(^14\) has reported that pyrocatechol sodium disulphonate of neodymium and praseodymium exert a specific inhibitory effect upon some inflammatory processes.

The purpose of this paper was to investigate comparatively the anti-inflammatory effects of the chloride salts of three lanthanides, praseodymium, gadolinium and ytterbium.

MATERIALS AND METHODS

Animals — Male Wistar rats weighing 180±10 g were used. They were fasted overnight before experiments.

Carrageenin-Induced Oedema — The inhibitory activity of praseodymium, gadolinium or ytterbium chlorides on the carrageenin-induced paw oedema was studied in comparison with phenylbutazone sodium. Thirty minutes after treatment with one of the lanthanide chlorides, 0.1 ml of 1% carrageenin was injected into the sub-planter area of the left hind paw of unanaesthetized rats. An equal volume of saline was injected into the right hind paw. At various intervals of time, the volume of each paw, up to tibio-tarsal articulation was determined by plethysmograph method of Winder et al.\(^15\)

Nystatin-Induced Oedema —Oedema was produced according to the model described by Schiatti et al.\(^16\) i.e., 0.1 ml (42500 U) of 8.5% nystatin suspension was injected into the plantar area of the left hind paw of unanaesthetized rats. An equal volume of saline was injected into the right hind paw. Six hours later, the animals were injected intraperitoneally with one of the lanthanides at the dose of 50 mg/kg. The paw volume

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was determined by plethysmograph method as described above 0, 6, 8, 10, 12, 24 and 48 h after the nystatin injection.

Measurement of Increased Vascular Permeability — The vascular permeability responses were studied with Evans blue according to the method of Wilhelm et al.17 with a slight modification. At 30 min after treatment of animals with the lanthanide chlorides, 25 mg/kg of Evans blue dye in 2.5% aqueous solution was administered intravenously. Five minutes later, each animal received intracutaneous injection 4 times in the abdominal wall. These intracutaneous injections consisted of two different amounts (0.5 and 1.0 μg) of histamine or serotonin in saline solution, at a constant volume of 0.1 ml plus 2 control injection of 0.1 ml of saline. Thirty minutes later, the animals were killed and their skins were stripped from the subcutaneous tissue. The fragments containing the stained area were cut and immersed in formamide to extract the exuded dye.18 The estimation of the dye concentrations was made using a Varian Techtron UV-visible Spectrophotometer, Model 634 S.

Mycobacterial Adjuvant-Induced Arthritis — The arthritis syndrome was induced with slight modifications of method of Newbould.19 An aliquot of 0.1 ml of mycobacterial adjuvant was injected into the plantar surface of the right hind paw. Two control groups were used. One of them was injected with 0.1 ml of saline and the other with 0.1 ml of lanoline/mineral oil. At various time intervals after injection, the swelling was measured by the plethysmograph method of Winder et al.15 Results were expressed as percent values of oedema in relation to the initial volume of the right paw. Three groups of 8 rats were injected intraperitoneally with 20 mg/kg of lanthanide chlorides, one group for treatment with phenylbutazone sodium (20 mg/kg) and two other groups for saline solution as a control once a day, 24 h before adjuvant injection and during a 13-d period.

Cotton Pellet Granuloma — Dental cotton rolls (Johnson and Johnson Inc.) were cut to 5 mm-sections and lots of 4 pellets weighing 160 mg were sterilized and implanted subcutaneously in four symmetrical positions of the abdomen under ether anaesthesia.20 The animals were treated daily with either saline, phenylbutazone sodium (20 mg/kg) or lanthanide chlorides (20 mg/kg) intraperitoneally for 6 d. On day 7, the animals were killed using chloroform. The granuloma was removed and dried overnight at 60°C and weighed. The difference between the initial pellet weight and the final dry weight was evaluated.

Drugs — Praseodymium chloride, gadolinium chloride and ytterburn chloride were synthesized at the Department of Inorganic Chemistry, the Institute of Chemistry, University of São Paulo. Phenylbutazone sodium was supplied by De Angeli; carrageenin by Sigma; nystatin by Squibb; histamine by Carlo Erba; serotonin creatinine sulfate, Evans blue and formamide by Merck, Darmstadt.

Statistical Assessment — Analysis of variance with one way classification was used. Sequential differences among means were calculated at a level of $p < 0.05$, using the Tukey contrast analysis.21

RESULTS

Carrageenin-Induced Oedema

Chloride salts of praseodymium, gadolinium and ytterbium definitely inhibited the carrageenin-induced paw oedema. Like phenylbutazone sodium, the lanthanide chlorides reduced the paw’s volume proportional to the doses administered (Fig. 1), ranging from 15 to 75 mg/kg (i.p.). However this effect was smaller than that of phenylbutazone sodium.

Linear regressions of phenylbutazone sodium and lanthanide chlorides obtained from dose-response relationships showed high correlation coefficient ($r$). The comparison of parameters of linear regression among pairs showed: a) no significant difference among praseodymium, gadolinium and ytterbium chlorides; b) significant difference between phenylbutazone sodium and lanthanide chlorides with respect to their linear coefficient (Fig. 1).

The lanthanide chlorides when administered
FIG. 1. Linear Regressions of Dose–Response Relationships of Phenylbutazone Sodium, and Praseodymium, Gadolinium and Ytterbium Chlorides on Carrageenin-Induced Oedema

Each point is the mean of 6 animals. Correlation coefficient (r) and parameters (a and b) of linear regression: praseodymium chloride (○ --- ), $r = 0.93$, $y = -42.47x + 112.53$; gadolinium chloride (● --- ), $r = 0.91$, $y = -35.94x + 104.25$; ytterbium chloride (△ --- ), $r = 0.91$, $y = -8.64x + 104.82$; phenylbutazone sodium (▲ --- ), $r = 0.98$, $y = -31.53x + 89.52$. a) Significantly different ($p < 0.05$) from lanthanides chlorides regarding to their intercepts.

orally, produced no inhibitory effect when compared to control group treated with saline (Table I).

**Nystatin-Induced Oedema**

The oedema induced by nystatin suspension increased progressively until the 10 h later, when the control group reached about 47% increase (Fig. 2). The oedema was significantly reduced 8 h after nystatin. This effect persisted for more than a 48- h period. The gadolinium chloride was the most effective among the tested salts, showing a pronounced decrease of oedema mainly at 24 and 48 h after gadolinium administration.

**Vascular Permeability**

Intracutaneous injections of histamine and serotonin caused a pronounced dye exudation. Serotonin produced more severe exudative effect than histamine in the same doses. This permeability response to both histamine and serotonin was significantly reduced by praseodymium, gadolinium and ytterbium chloride. The mean inhibition of histamine-induced permeability was 58.5%, whereas that of serotonin-induced permeability was 45.5%. There were no consistent differences in exuded dye content among lanthanide chlorides treated groups (Table II).

**Arthritis**

Fig. 3 shows the effects of lanthanide chlorides

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**TABLE I. Effect of Lanthanide Chlorides on Carrageenin-Induced Paw Oedema**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>% increase of paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p.o.</td>
</tr>
<tr>
<td>Saline (control)</td>
<td></td>
<td>69.56±12.66</td>
</tr>
<tr>
<td>Phenylbutazone, sodium</td>
<td>50</td>
<td>48.16±3.62 b)</td>
</tr>
<tr>
<td>Praseodymium chloride</td>
<td>50</td>
<td>69.24±10.13</td>
</tr>
<tr>
<td>Gadolinium chloride</td>
<td>50</td>
<td>64.66±9.53</td>
</tr>
<tr>
<td>Ytterbium chloride</td>
<td>50</td>
<td>66.18±6.74</td>
</tr>
</tbody>
</table>

p.o. = oral administration.

i.p. = intraperitoneal administration.

Each value represents the mean± SD of 6 animals, and was obtained at 3rd h after carrageenin injection.

a) Significantly different from the control ($p < 0.05$, Tukey).

b) Significantly different from the control and lanthanide chlorides groups ($p < 0.05$, Tukey).
TABLE II. Effect of Lanthanide Chlorides on the Permeability Increasing Response to Histamine and Serotonin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>Histamine (µg)</th>
<th>Serotonin (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>0.821±0.139</td>
<td>0.993±0.101</td>
<td>1.197±0.123</td>
</tr>
<tr>
<td>Praseodymium chloride</td>
<td>0.365±0.047&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.463±0.027&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.733±0.095&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(55.5%)</td>
<td>(53.4%)</td>
<td>(38.8%)</td>
<td>(39.4%)</td>
</tr>
<tr>
<td>Gadolinium chloride</td>
<td>0.313±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.395±0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.636±0.052&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(61.9%)</td>
<td>(60.2%)</td>
<td>(46.9%)</td>
<td>(47.4%)</td>
</tr>
<tr>
<td>Ytterbium chloride</td>
<td>0.343±0.073&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.406±0.044&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.589±0.065&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(58.2%)</td>
<td>(59.1%)</td>
<td>(50.8%)</td>
<td>(48.4%)</td>
</tr>
</tbody>
</table>

Each value represents the mean absorbance value ± SD obtained from 6 animals. The values in parenthesis represent % inhibition.

<sup>a</sup> Significantly different from the control (p < 0.05, Tukey).

![Graph](image)

FIG. 2. Effect of Lanthanide Chlorides on Nystatin-induced Oedema

Animals were treated at 6th h after nystatin with saline or 50 mg/kg, i.p., of lanthanide chlorides. Each point is the mean of 6 animals, and the bars represent standard deviations of mean values.

Legends: saline (△), praseodymium chloride (○), gadolinium chloride (●) and ytterbium chloride (△);

<sup>a</sup> Significantly different from the control (p < 0.05, Turkey) ○ b) Significantly different from the praseodymium and ytterbium groups (p < 0.05, Turkey).

and phenylbutazone sodium on right hind paw arthritis produced by the injection of mycobacterial adjuvant. In the control group, the paw volume increased gradually over a 20-d period. On day 13, the last day of drug administration, the percent increase of oedema was significantly reduced in the groups treated with lanthanide salts and with phenylbutazone sodium when compared to the control groups. This inhibitory effect persisted further for 3 d, and then the paw volume increased, reaching maximal value on days 20–27 with a 60% increase seen in animals treated with praseodymium chloride, 68% with ytterbium chloride, 50% with gadolinium chloride and 40% with phenylbutazone sodium. The peak oedema increase in the control group was about 80% at 20th and 27th days.

Granuloma

Table III shows the inhibitory effect of lanthanide chlorides on granuloma tissue formation by implantation of cotton pellets. In the control group, the mean weight of dry granuloma was 458.9 mg. Among the tested salts, ytterbium chloride exhibited the greatest effect with 43.3% inhibition. The positive control drug, phenylbutazone sodium, elicited 68.7% inhibition.

DISCUSSION

Anti-inflammatory effects are elicited by a variety of chemical agents, having no remarkable correlation between their pharmacological activity and chemical structure. This fact, associ-
ated with the complexity of inflammatory process, makes the use of different experimental models necessary in their pharmacological trials.

The carrageenin-induced oedema is efficient for assessment of both steroidal and non-steroidal anti-inflammatory agents. In this experimental model, the dose–response relationship of small doses of antiphlogistic drugs is usually observed 3–4 h after subcutaneous injections of carrageenin. For this reason, the oedema was measured 3 h after carrageenin injection. Praseodymium, gadolinium and ytterbium chlorides produced a pronounced decrease of carrageenin-induced oedema. High correlation coefficients (r) of linear-regression from dose–response relationship of these lanthanide salts showed that their inhibitory effects are proportional to the doses.

These lanthanide chlorides revealed similar inhibitory effects among themselves, but they were less potent than phenylbutazone sodium in the same doses. Moreover, the antioedema effect of three lanthanide chlorides is seen only when they are administered intraperitoneally. Even at the dose of 50 mg/kg, by oral route, the decreasing effect in the carrageenin-induced oedema is non expressive. This fact suggests small absorption of these lanthanide chlorides by gastrointestinal tract.

The nystatin alters the lysosomal membranes causing a release of proteolytic enzymes and its local inflammatory responses have a long duration, reaching a maximal effect 6 or 8 h after

**FIG. 3. Effect of Lanthanide Chlorides on Adjuvant-Induced Oedema**

Daily dose of 20 mg/kg of lanthanide chlorides or phenylbutazone sodium was injected i.p. for 15 d. Each point is the mean of 8 animals.


- a) Significantly different from Adjuvant group (p < 0.05, Turkey)
- b) Significantly different from Adjuvant and Gadolinium group (p < 0.05, Turkey).

**TABLE III. Inhibitory Effects of Lanthanide Chlorides on Granuloma Tissue Formation by Implantation of Cotton Pellets**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>Dry granuloma weight (mean ± SD)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>4589±28.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylbutazone sodium</td>
<td>20</td>
<td>143.4±11.5&lt;b&gt;</td>
<td>68.7</td>
</tr>
<tr>
<td>Praseodymium chloride</td>
<td>20</td>
<td>329.5±16.4&lt;a&gt;</td>
<td>28.2</td>
</tr>
<tr>
<td>Gadolinium chloride</td>
<td>20</td>
<td>276.2±31.3&lt;a&gt;</td>
<td>39.8</td>
</tr>
<tr>
<td>Ytterbium chloride</td>
<td>20</td>
<td>2600.0±24.5&lt;a&gt;</td>
<td>43.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD obtained from 10 animals.

- a) Significantly different from the control (p < 0.05, Tukey).
- b) Significantly different from the control and the lanthanide chlorides groups (p < 0.05, Tukey).
injection of phlogogenic agent. After 6 h, just before the treatment with drugs, all the groups presented the same degree of oedema showing that any further variation of the paw volume are due exclusively to different treatment of animals. The lanthanide chlorides, in the dose of 50 mg/kg, i.p., reduced the nystatin-induced oedema, but the maximal inhibitory effect was slightly smaller than that observed in carrageenin-induced inflammation. The acute inflammatory responses consist of three main vascular effects, i.e., vasodilation and increased blood flow, increased permeability, and leucocytes migration into the injured tissues. Histamine and serotonin are usually responsible for eliciting the immediate responses of inflammation in rats, whereas kinins and prostaglandins mediate relatively prolonged responses obtained in delayed type effects. The main evidence for histamine and serotonin being the mediators of immediate responses in injury comes from the suppression of the responses by relatively small doses of appropriate pharmacological antagonists. Aspirin-like drugs inhibit the inflammatory process in the stage of prostaglandin release and migration of leucocytes from the blood vessels into the injured tissues. In our assays, the lanthanide chlorides inhibited strongly the enhanced vascular permeability induced by different amounts of both histamine and serotonin. However, the effect of histamine was inhibited more intensively than that of serotonin. The mean inhibitory value of the serotonin responses is similar to values obtained with carrageenin- and nystatin-induced oedema. This difference in the magnitude of their potency may be attributed to the greater inhibitory specificity of the lanthanide chlorides on the response of histamine than on the response produced by other substances, since numerous mediators take part in the inflammatory process induced by carrageenin and nystatin.

The injection of mycobacterial adjuvant into the hind paw caused inflamed lesions characterized mainly by oedema in the hind paw and, as secondary lesions, increase in the volume of the forepaws, ears and tail. The development of these lesions was similar to that described by Newbould. The most rapid increase of paw volume was observed during the first three days and between 16th and 20th day. The first stage of swelling seems to be partly due to the injection of the solvent (lanolin: mineral oil). Despite short biological half-lives of lanthanides, their inhibitory effect on adjuvant-induced arthritis persisted for the entire experimental period, even after the 13th day, when the last dose of lanthanide chlorides was injected. Among the lanthanide salts studied, gadolinium chloride showed the most potent inhibitory activity. Phenylbutazone sodium was more potent than lanthanide salts in the same doses.

The cotton pellet test, another widely used method for the assessment of anti-inflammatory agents, has particular sensitivity to steroidal compounds. It is rather insensitive to non-steroidal and requires large doses. However, some authors have obtained fair dose-response curves with non-steroidal anti-inflammatory drugs. Praseodymium, gadolinium and ytterbium chlorides significantly inhibited the granuloma tissue formation but were less potent than phenylbutazone sodium. Moreover, the mean inhibition value in the granuloma was greater than with the adjuvant-induced arthritis.

The exact mechanism of action of these lanthanide salts on inflammatory process is unknown, but our experiments show that they are effective on different kinds of experimental inflammatory models, like many other well known anti-phlogistic agents. Calcium ion antagonism may contribute, at least partially, to their inhibitory effects. Therefore, further investigation concerning the correlation between inflammatory effect and specific calcium antagonism of those metals seems to become quite important.

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REFERENCES


