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The anti-inflammatory effects of D-myo-inositol-1.2.6-trisphosphate (PP56) on animal models of inflammation

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Introduction

D-myo-inositol-1.2.6-trisphosphate (PP56, Perstorp Pharma, Sweden) is an isomer of inositol trisphosphate (IP_3), and is produced by hydrolysis of phytic acid by the phytase enzyme of yeast (M J Sirén 1984, US Pat No. 4735936).

Another isomer of IP_3, D-myo-inositol-1.4.5-trisphosphate, is an intrinsic part of membrane phospholipids and is released intracellularly following a wide variety of stimuli. The 1.4.5-isomer acts as a second messenger and releases calcium from intracellular stores. Unlike 1.4.5-IP_3, PP56 does not release intracellular calcium.

PP56 is a metal chelator and may therefore influence free radical generation. Transition metals have been implicated in the pathogenesis of inflammatory synovitis by virtue of their ability to reduce oxygen via univalent electron transfer pathways to produce highly damaging reactive oxygen species, which initiate lipid peroxidation of cellular membranes [1, 2].

We have assessed the efficacy of PP56 as an anti-inflammatory agent in animal models of acute (foot pad oedema) and chronic (adjuvant arthritis) inflammation. In addition, we have studied the effects of PP56 on metal-induced lipid peroxidation *in vitro*.

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Materials and methods

Irritancy Tests

Both the calcium and sodium salts of PP56 were tested using the standard Evans Blue capillary leakage technique following intradermal injection in rats. The doses tested were 50 and 200 mg/kg and the degree of blueing in comparison to vehicle control (Kreb's ringer) one hour post injection were noted.

Paw Oedema

Male Wistar rats (200–250 g) in groups of 5 were given either a sodium-salt of PP56 (Na-PP56, intraperitoneal route) or a calcium-salt of PP56 (Ca-PP56, oral route) in doses ranging from 20–1500 mg/kg, administered as a single bolus 1 hr prior to initiation of inflammation. Two controls were used: Saline vehicle and inositol (400 mg/kg). Acute inflammation was initiated in one hind-paw using a single subplantar injection of 0.1 ml 1% w/v carrageenan (Viscarin 402, Marine Colloids Inc) in sterile normal saline. The inflammation/oedema was assessed hourly for 5 hrs and finally at 24 hrs by measurement of paw circumference (mm) immediately proximal to the two hindmost spurs of the foot.

Adjuvant Arthritis

Adjuvant disease was induced in male Wistar rats (200–250 g) in groups of 10. Rats received a single

intradermal injection of 0.1 ml of a 10 mg/ml suspension of Mycobacterium butyricum (Gibco) in paraffin oil, in the base of the tail. Both Na- and Ca-PP56 and two controls were administered as described for paw oedema, dosing being on a daily basis (5 days per week) from induction of disease or from onset of clinical disease (day 12). The Na-salt doses were 60 and 260 mg/kg and day and the Ca-salt doses were 50, 200 and 400 mg/kg and day. Animals were assessed daily for 27 days, chronic inflammation being quantified by both joint score [2] and hindpaw circumference measurement.

In vitro studies of lipid peroxidation

Lipid peroxidation was induced in rat liver microsomes or bovine brain liposomes by 0.1 m*M* Fe^{2+} or by 0.1 m*M* Fe^{3+} and 0.1 m*M* ascorbate, or in human erythrocytes by H_2O_2 [1]. The lipid peroxidation was measured with the TBA-method [1].

Results

Neither of the PP56 salts possessed any appreciable irritant properties in the Evans Blue capillary leakage test (data not shown). The paw oedema studies showed that both intraperitoneal Na-PP56 and oral Ca-salt, at doses from 200 mg/kg, significantly reduced paw swelling compared to the controls. The suppression of oedema was dose-dependent and lasted at least 5 hours with the Na-salt (Fig. 1 A) and 4 hours with the Ca-salt (data not shown). In the chronic inflammation produced by adjuvant disease, daily dosing with 400 mg/kg Ca-PP56 from the time of disease initiation showed marked suppression in inflammation from days 12–24 in comparison to both saline and inositol controls (Fig. 1 B). Dosing from day 12 resulted in suppression from day 14 and onwards, but the effect was less marked (Fig. 1 B). The two lower Ca-PP56 doses gave no statistically significant effects. Both doses of Na-PP56 resulted in a marked suppression of inflammation only when given from the time of initiation of adjuvant disease (data not shown).

In the *in vitro* studies, 1–5 m*M* of Na-PP56 resulted in slight, statistically not significant, reductions (by 10–39%) of iron- and H_2O_2-stimulated lipid peroxidation. Supplementation with 0.4 m*M* of either Al^{3+}, Pd^{2+} or Cd^{2+} increased the Fe^{2+}-stimulated lipid peroxidation by 45–90% ($p<0.05$ with Student's *t*-test) in liposomes, microsomes and erythrocytes. Under these conditions, 1–5 m*M* of PP56 was able to totally block the effect of the supplementary metal-ions (data not shown).

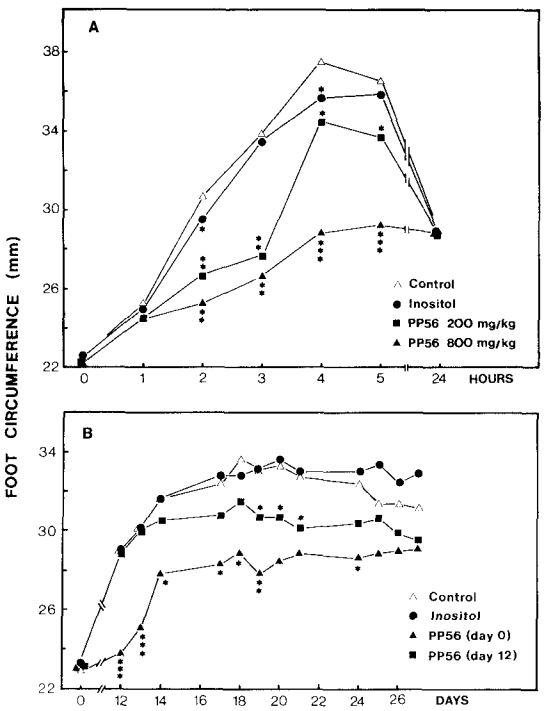

Figure 1
A) The effect of Na-PP56 (200 and 800 mg/kg), administered intraperitoneally as a single bolus dose, 1 hr before carrageenan, in rat paw oedema. B) The effect of orally aministered Ca-PP56 (400 mg/kg) in adjuvant disease in rats. Comparison of dosing from initiation of disease (day 0) and from onset of clinical disease (day 12). The inositol dose was 400 mg/kg in both experiments. Mean values, $n=5$ and 10 in A and B, respectively. Student's *t*-test was used to compare each treatment with control (vehicle): $*p<0.05$, $**p<0.01$, and $***p<0.001$.

Discussion

PP56 thus has significant anti-inflammatory effects in two widely used models of inflammation. The mechanism of action is unclear, although it may relate to the metalchelating properties of PP56, as PP56 is able to decrease metal-induced lipid peroxidation in several in vitro systems. Iron chelators, such as desferrioxamine, have been shown to suppress both acute and chronic inflam-

mation, by reducing the iron available for free radical generation [2]. In support of this, dietary iron deficiency in animals also suppresses the chronic inflammatory response [3]. Thus, although PP56 has a lower affinity for iron than desferrioxamine (log β is 14 and 32, respectively), chelation of iron and other metals may contribute to the anti-inflammatory action of PP56.

The use of iron chelators as anti-inflammatory agents in humans has highlighted toxicity problems. Desferrioxamine causes reversible ocular abnormalities which may be related to its high iron binding capacity [4]. As PP56 does not have such a high affinity for iron as does desferrioxamine, it is likely to be less toxic. This has been shown in our rat model of ocular toxicity [5] where PP56 not only was non-toxic, but also protective against desferrioxamine ocular toxicity.

References

[1] G. J. Quinlan, B. Halliwell, C. P. Moorhouse and J. M. C. Gutteridge, *Action of lead (II) and aluminium (III) ions on iron-stimulated peroxidation in liposomes, erythrocytes and rat liver microsomal fractions.* Biochim. Biophys. Acta *962,* 196–200 (1988).

[2] F. J. Andrews, C. J. Morris, G. Kondratowicz and D. R. Blake, *Effect of iron chelation on inflammatory joint disease.* Am. Rheum. Dis. *46,* 327–333 (1987).

[3] F. J. Andrews, C. J. Morris, E. J. Lewis and D. R. Blake, *Effect of nutritional iron deficiency on acute and chronic inflammation.* Am. Rheum. Dis. *46,* 859–865 (1987).

[4] D. R. Blake, P. Winyard and J. Lunec, *Cerebral and ocular toxicity induced by desferrioxamine.* Q. J. Med. *56,* 345–351 (1985).

[5] P. A. Good, A. Claxson, C. J. Morris and D. R. Blake, *Experimental models for desferrioxamine and oxygen toxicity of the retina using the Albino Rat.* In: *Free Radicals, Disease states, and Anti-Radical Intervention.* (Ed. C. Rice-Evans) Richelieu Press (in press).