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Anti-inflammatory and antipyretic effects of boldine

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Abstract. Boldine, an antioxidant alkaloid isolated from *Peumus boldus*, exhibits a dose-dependent anti-inflammatory activity in the carrageenan-induced guinea pig paw edema test with an oral ED_{50} of 34 mg/kg. Boldine also reduces bacterial pyrogen-induced hyperthermia in rabbits to an extent which varied between 51% and 98% at a dose of 60 mg/kg p.o. *In vitro* studies carried out in rat aortal rings revealed that boldine is an effective inhibitor of prostaglandin biosynthesis, promoting 53% inhibition at 75 μM. The latter *in vitro* effect may be mechanistically linked to the anti-inflammatory and antipyretic effects of boldine exerted *in vivo*.

Key words: Inflammation – Fever – Prostanoid biosynthesis – Boldine – Antioxidants

Introduction

Boldine [(*S*)-2,9-dihydroxy-1,10-dimethoxyaporphine, Fig. 1] is the most abundant and characteristic alkaloid of the leaves and bark of boldo (*Peumus boldus* Mol., Monimiaceae), a widely distributed evergreen tree native of Chile [1]. Boldine-containing galenicals and pharmaceutical preparations based on boldo leaves have been in use since the last century in Europe and North and South America, largely for the treatment of digestive and liver complaints and as mild sedatives. In addition, there are early reports that boldo extracts were indicated in the past for the treatment of headache, earache, toothache, rheumatism, and urinary tract inflammation [2].

Chemically pure boldine has been shown to be effective in stimulating canine gastric acid and bile secretion [3], and to inhibit basal and acetylcholine-induced rat ileal contraction [4]. Furthermore, we have recently shown that boldine is a potent antioxidant, thus protecting both biological and non-biological systems from free-radical-mediated oxidative damage [5, 6]. This antioxidative action of boldine seems to rest upon its excellent ability to trap hydroxyl and peroxy radicals [7]. Currently, it is recognized that oxygen reactive species are involved in the cyclooxygenase- and lipoxygenase-mediated conversion of archidonic acid into pro-inflammatory intermediates, and that these reactive species are also produced in substantial amounts during inflammatory processes by infiltrating phagocytes. On this basis, several natural and synthetic antioxidants [8] have been tested and shown to possess anti-inflammatory properties. In the present work, prompted by the recently demonstrated antioxidant activity of boldine, we have sought a possible anti-inflammatory action of this plant-derived alkaloid. We have used the carrageenan-induced guinea pig paw edema as an *in vivo* model of inflammation [9], a screening procedure in which the involvement of cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established [10]. We have employed aorta rings as an *in vitro* system to address the possible ability of boldine to interfere with prostacyclin biosynthesis. Further, since inflammation and fever can be mechanistically linked through the formation of intermediates of the arachidonic acid cascade, several of which are reactive peroxide species, we have also tested the possibility that boldine may show antipyretic activity. For this purpose we have used the bacterial pyrogen-induced hyperthermia assay in rabbits.

Materials and methods

Compounds

Boldine was crystallized to chromatographic (TLC) purity as its 1:1 chloroform adduct from the crude alkaloidal mixture extracted from boldo bark, as recently described [11]. The alkaloid was administered by means of an intragastric catheter as a saline solution in acacia gum (5%) prepared after dissolving the boldine in a small volume of 0.5 mol/l HCl. Sodium naproxen (donated by Laboratorios Saval, Chile) was used as a positive control, dissolved in the

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same vehicle. Lambda-carrageenan was purchased from Sigma. *E. coli* endotoxin was obtained from Instituto de Salud Pública, Chile.

Animals

Pirbright guinea pigs (200–300 g) of both sexes and adult female New Zealand rabbits were used for the anti-inflammatory and antipyretic studies, respectively. The animals were kept under standard housing conditions, and fasted overnight before the day of the experiments.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated in guinea pigs in groups of variable sizes (described in the text) for each dose, using the carrageenan-induced paw edema method as described by Winter *et al.* [9]. Paw volume was measured with an Ugo Basile plethysmometer (model 7150) immediately, and 3 h after injecting 0.1 ml of sterile saline lambda-carrageenan (1%). Either boldine-chloroform (27–50 mg/kg) or sodium naproxen (1.4–6.4 mg/kg) were administered orally 1 h prior to the carrageenan injection. Anti-inflammatory activity (%A) was estimated as

$$\%A = [(\%I_c - \%I_d)/\%I_c] \times 100,$$

where $\%I_c$ is the mean inflammation reached in control guinea pigs (37.7 ± 1.3% paw volume increase for a group of 96 animals), and $\%I_d$ is the average inflammation in drug-treated animals, expressed as

$$\%I = [(V_f - V_i)/V_i] \times 100,$$

where V_f and V_i are final and initial paw volumes, respectively, averaging $\%I$ over all the animals used in each test. The significance of the boldine-induced changes was estimated using Student's t-test.

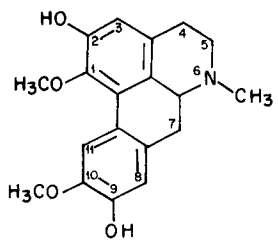

Fig. 1. Chemical structure of boldine.

Antipyretic activity

Antipyretic activity was determined in rabbits using three animals for each dose (modified from USP XXII, 1990) and repeating each experiment three times at intervals not less than two weeks. Pyrexia was induced by i.v. injection of *E. coli* endotoxin (prepared in sterile saline) at a dose of 13 mg/kg. Rectal temperatures were recorded, continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after pyrogen injection. The areas under temperature vs. time curves obtained for each pyrogen-treated animal, with or without previous oral administration of 60 mg/kg boldine or 25 mg/kg naproxen, were compared and the antipyretic effect was calculated according to the following equation:

$$\%\ \text{effect} = [1 - \text{area}_{pyr+drug}/\text{area}_{pyr}] \times 100,$$

where $\text{area}_{pyr+drug}$ represents the area under the curve obtained after plotting temperature in °C vs. time in minutes for drug-treated rabbits, and area_{pyr} is the corresponding area for animals treated only with pyrogen. These areas were calculated for the two time intervals: from 0–90 min to 90–180 min, using a computer program developed in our laboratory for this purpose, and the significance of the effect was estimated using the ANOVA test.

Inhibition of prostacyclin synthesis

Prostacyclin production was studied in aortal rings obtained from Wistar rats. The assay tissue was incubated in Krebs–Ringer solution (pH 7.4) at 37 °C during 30 min in the absence or presence of boldine (25–150 μmol/l) previously dissolved in a small volume of 0.5 mol/l HCl. Under the experimental conditions used, the rate of prostacyclin synthesis remained linear for up to 45 min. Aliquots taken from the incubation medium were immediately frozen (− 20 °C), and prostacyclin levels were quantified within 8 weeks by radioimmunoassay [12] of its stable non-enzymatic hydrolysis product, 6-keto-PGF. For the sake of comparison, the effects of the anti-inflammatory and antipyretic drugs naproxen (10–50 μmol/l) and aspirin (25–50 μmol/l), dissolved in the incubation medium, were also determined.

Results

Figure 2 shows the relative anti-inflammatory effects of boldine and naproxen on the carrageenan-induced edema of the guinea pig hindpaw, as a function of the oral dose. The administration of boldine reduces the increase in paw

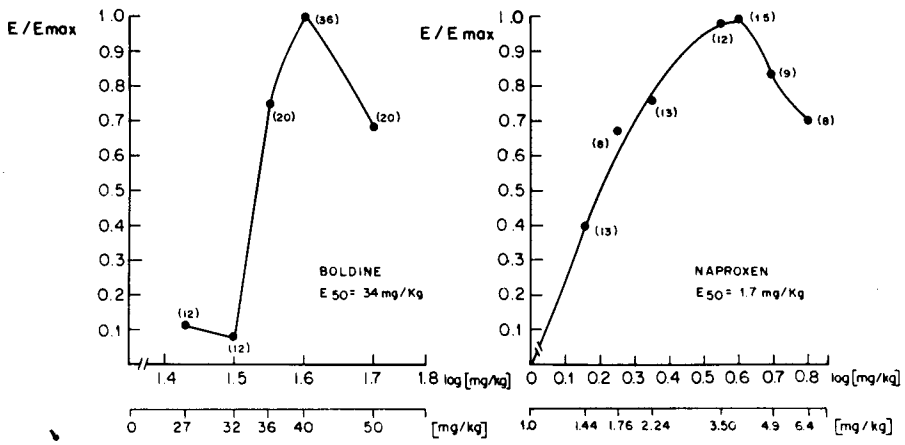

Fig. 2. Anti-inflammatory effects of boldine and sodium naproxen on the carrageenan-induced edema in the guinea pig hindpaw. E/E_{max} represents the ratio between the %inhibition of edema at each dose and the maximal %inhibition achieved by boldine and naproxen, respectively. Figures in parentheses represent the total number of animals used for each tested dose. SD were always less than 5% of the means.

volume dose-dependently, with a maximal effect ($\%A = 50\%$) at 40 mg/kg. At a higher dose, the anti-inflammatory effect of boldine appears to decrease slightly. The effect of naproxen, described by a comparable curve, reaches its highest value at a dose of 4 mg/kg.

Figure 3 compares the effects of boldine and naproxen as the percent decrease of the area under the rectal temperature vs. time curves for pyrogen-treated rabbits. Following administration of the pyrogen, the antipyretic effect of the drug was calculated for two intervals of the curve, the first ranging from time 0 (immediately after injection of the bacterial extract) to 90 min, and the second from 90 to 180 min. It can be seen that, in the first interval, boldine exerts a marked effect on the rectal temperature, with an area reduction of 84%. However, during the period from 90 to 180 min, the area under the curve for boldine-treated rabbits did not differ from that obtained with animals which did not receive the drug.

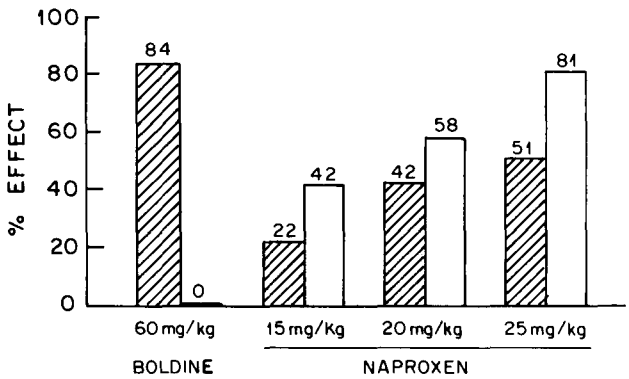

Fig. 3. Comparative antipyretic effects of boldine and sodium naproxen for pyrogen-treated rabbits. Antipyretic activity was determined using three animals for each dose and repeating each experiment three times at intervals not less than two weeks. Numbers over the bars represent the mean values of the percent decrease of the area under the rectal temperature vs. time curves in the 0–90 (shaded bars) and 90–180 (white bars) minute intervals after pyrogen administration. SD were always lower than 10% of the means. The means over the shaded and white bars are significantly different (paired data) for boldine at $p < 0.001$, and for naproxen at $p < 0.05$.

Table 1. In vitro inhibition of prostaglandin biosynthesis in rat aortic rings

Agent	Concentation (μM)	% Inhibition
Aspirin	25	51 ± 3
Aspirin	50	62 ± 6
Naproxen	10	48 ± 5
Naproxen	50	63 ± 9
Boldine	25	29 ± 2
Boldine	50	39 ± 4
Boldine	75	53 ± 5
Boldine	100	51 ± 5
Boldine	150	48 ± 3

Data represent the means + SD of three separate experiments in duplicate. The production of 6-keto-PGF was significantly ($p < 0.01$) reduced with respect to controls for each of the tested drugs and concentrations. Experimental conditions were as described in the Methods Section.

Table 1 shows the *in vitro* inhibitory effect of boldine on the biosynthesis of prostacyclin by aortal rings. This effect is approximately 30% at 25 μmol/l and, appears to attain a maximum at around 75 μmol/l, a concentration at which prostacyclin synthesis is inhibited by 53%. Approximately, 50% inhibition was reached with 25 μmol/l aspirin or 10 μmol/l naproxen.

Discussion

The association of antioxidants and inflammation stems from the recognition that free radicals are produced during the inflammatory process by phagocytosing cells and generated as by-products of the oxidative degradation of arachidonic acid [8]. The present study demonstrates that boldine, a natural antioxidant with potent *in vitro* hydroxyl- and peroxyl-radical scavenger properties [5, 7], exerts *in vivo* a significant anti-inflammatory activity. This effect was observed at oral doses ($ED_{50} = 34$ mg/kg) far below the lethal dose (1000 mg/kg) reported in the same species [3]. Previously, Lanhers *et al.* [13] had reported dose-dependent anti-inflammatory activity for a purified leaf extract of *Peumus boldus*, in an attempt to validate some of the traditional medicinal uses of this plant [2]. Nevertheless, these authors were unable to demonstrate any significant anti-inflammatory effect of boldine, administered intraperitoneally to rats (10 and 20 mg/kg), on carrageenan-induced hindpaw edema. Conceivably, Lanhers' results may have arisen from an insufficient i.p. dosing of boldine in the rat. However, our data indicate that, despite dosing orally, which will result in a lower bioavailability than the intraperitoneal route, only a slightly higher dose of boldine is required to reduce effectively hindpaw inflammation in guinea pigs.

Our study also shows that in the carrageenan-induced inflammation model, boldine given p.o. is approximately one order of magnitude less potent than the clinically used anti-inflammatory drug naproxen. While the higher dose requirement of boldine may reflect an inherently weaker anti-inflammatory activity of this substance, it may also be explained on the basis of large differences in the drug concentrations attained at the target tissue. Consistent with the interpretation of a poorer bioavailability of boldine, a substantially smaller difference is observed when the *in vitro* potencies of these two agents are compared with regard to their ability to inhibit prostaglandin biosynthesis.

On the other hand, the oral administration of boldine (60 mg/kg) very significantly (albeit fleetingly) prevents the increase in rectal temperature induced in rabbits treated with bacterial pyrogen. Interestingly, the dose found to elicit this antipyretic effect is in the same range as the anti-inflammatory oral ED_{50}. However, the fact that boldine only showed antipyretic activity during the first 90 min of the assay, while in the case of naproxen this effect was greater in the second 90 min interval, may reflect substantial pharmacokinetic differences between these drugs. On the other hand, the persistence of the anti-inflammatory effect of boldine in guinea pigs up to at least 180 min after its administration may be explained on the basis of pharmacokinetic and/or pharmacodynamic

species differences. A possibility that can be envisioned is that large differences exist in the timing of the biochemical events underlying inflammation and pyresis in the experimental models used, at least those with which boldine is likely to be interfering.

Known medicinal uses of boldo and boldine preparations do not seem to include the treatment of fever [2]. Nevertheless, considering that both inflammation and fever may be mediated in part by prostaglandins [14, 15], our finding that boldine displays antipyretic activity in addition to its anti-inflammatory properties is not totally surprising. Existing evidence seems to indicate that, in the endotoxin-induced fever model, endogenous pyrogen released in the brain induces PGE_2 synthesis [15, 16], and that injection of PGE_2 itself into the preoptic area of the anterior hypothalamus evokes fever in experimental animals [16]. Consequently, the *in vitro* inhibitory effect of boldine on prostaglandin synthesis, as demonstrated for the first time here, may explain both the antipyretic and antiinflammatory effects of boldine. The ability of boldine to inhibit the biosynthesis of prostanoids may stem from its reactivity toward free radicals [5, 7], some of which are known to be intermediates in the arachidonic acid cascade. Our results, showing a reduction in 6-keto-PGF levels, imply a decreased biosynthesis of PGI_2 which may be due either to a direct inhibitory effect of boldine on prostacyclin synthetase, or to a decreased availability of PGH_2 secondary to a reaction between an intermediate peroxy radical or endoperoxide and the antioxidant. If the reduction of PGI_2 biosynthesis were the major biochemical action of boldine in the cascade, the alkaloid would be expected to prevent both PGI_2-induced vasodilation as well as the ability of this autacoid to inhibit platelet aggregation. On the other hand, since PGH_2 is also a common precursor for the synthesis of prostaglandins (PGE_2, PGD_2, and PGF_{2a}) and of thromboxanes (TXA_2), an effect of boldine on PGH_2 levels would be expected to affect the synthesis of all these substances, to varying extents, depending on the tissues involved. Our studies do not allow us thus far to state at what point boldine would be affecting the biosynthesis of PGI_2.

Non-steroidal anti-inflammatory and antipyretic agents can be very effective therapeutically, but their use is often accompanied by gastric mucosal irritation or the appearance of duodenal ulcers. Our results indicate that, relative to the NSAID naproxen, higher doses of boldine are required to control effectively experimental inflammation and fever. Notwithstanding, in view of the fact that available reports on the use of boldine-containing galenicals, usually taken as an aid to digestion, do not cite any untoward gastrointestinal effects [2], further research on the antiphlogistic and antipyretic properties of boldine and chemically related natural antioxidants appears warranted.

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