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SHORT COMMUNICATION

Hypotensive Effect of O-Methylisothalicberine, A Bisbenzylisoquinoline Alkaloid Isolated from *Berberis chilensis* on Normotensive Rats

J. L. Martínez[1]*, R. Torres[2] and M. A. Morales[3]

[1] Department of Physiology and Biophysics, Faculty of Medicine, University of Chile
[2] Department of Chemical Sciences, Faculty of Chemistry and Biology, University of Santiago of Chile
[3] Department of Pharmacology, Faculty of Medicine, University of Chile

O-Methylisothalicberine (O-MI) is a bisbenzylisoquinoline alkaloid isolated from *Berberis chilensis*, structurally similar to alkaloids previously described in the literature as calcium antagonists of natural origin (berbamine, tetrandrine, antioquine, 7-O-demethylisothalicberine and others). O-MI caused a significant reduction of mean arterial pressure (MAP) in normotensive anaesthetized rats. Doses of 1.0; 2.5; 5.0; 7.5 and 10.0 mg/kg were administered via the femoral vein. MAP was reduced by 5.8%; 10.1%; 35.6%; 67.9% and 60.5% respectively. The onset of hypotensive action was 5 s after 5 mg/kg i.v. and the effect lasted for about 120 s. O-MI exhibited an LD_{50} of 5 mg/mL towards the brine shrimp (*Artemia salina*). © 1997 by John Wiley & Sons, Ltd. Phytother. Res. **11**, 246–248, 1997

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INTRODUCTION

At present, more than 400 bisbenzylisoquinoline alkaloids are known. They are found principally among the *Ranunculaceae* and *Menispermaceae* and with less frequency in the *Berbidaceae, Monimiaceae* and *Laureaceae* plant families (Schiff, 1987).

The genus *Berberis* (Fam. *Berberidaceae*) is widely distributed in Chile. *Berberis chilensis* Gillies ex Hook, (traditional names michai, richa or palo amarillo), is an endemic shrub of the Central Valley (Navas, 1973). The *Berberis* species used in Chile in traditional medicine is *Berberis empetrifolia* (traditional name: zarcilla) administered for mountain sickness (San Martín, 1983; Houghton and Manby, 1985). The chemical characterization of *B. empetrifolia* and *B. chilensis* relates to berberine only (Fajardo *et al.*, 1986). Of the other species, *B. chilensis* also contains berbamine, which was first isolated from a Chinese plant, and suggested as a calcium channel blocker of vegetable origin (Li *et al.*, 1986; Pachaly, 1990). This shows similar activity to tetrandrine (Fang and Jiang, 1986) isolated from *Stephania tetrandra*, and 7-O-demethylisothalicberine (7-O-DI) (Morales *et al.*, 1989; Martínez *et al.*, 1992; Morales *et al.*, 1993) isolated only from *B. chilensis* (Torres *et al.*, 1979a). We found that 7-O-DI, bisbenzylisoquinoline alkaloid structurally related to O-methylisothalicberine (O-MI), induced bradycardia and complete cessation of spontaneous firing of cardiac pacemaker cells, through a slow inward calcium current blockade (Morales *et al.*, 1989).

Among several bisbenzylisoquinoline alkaloids isolated from *B. chilensis* (Torres *et al.*, 1979a; 1979b; Torres, 1989), O-MI, which has also been obtained from the Uruguayan *Berberis laurina* plant (Falco *et al.*, 1968), has remained unknown from a pharmacological point of view.

The hypotensive effects of bisbenzylisoquinoline alkaloids have been generally tested with crude alkaloid fractions obtained principally from the genus *Thalictrum* (Patil and Beal, 1987; Banning *et al.*, 1982; Pachaly, 1984).

In this paper we report for the first time the hypotensive activity of a purified fraction of O-MI. Furthermore, the toxicity of this compound has also been estimated utilizing the brine shrimp (*Artemia salina*) as the test organism (Meyer *et al.*, 1982).

MATERIALS AND METHODS

Animals. Sprague-Dawley adult normotensive rats, of both sexes, weighing 200–300 g, were anaesthetized with Nembutal (50 mg/kg i.p.). The rats were cannulated through the femoral vein and femoral artery, with PE 10 and PE 50 Clay Adams cannula respectively and maintained in a thermoregulated bed. The drug was dissolved in physiological solution and injected i.v. at different doses (1.0; 2.5; 5.0; 7.5 and 10.0 mg/kg), employing a maximum volume of 300 μL. The femoral artery was connected to a pressure transducer (Gould, model p23 ID) and blood pressure was recorded in a Nihon Khoden polygraph. The results were expressed as the change in mean arterial pressure (MAP) ± standard deviation, in mmHg. The rectal temperature was monitored using a Simpson electric telethermometer (model 43 TA). Cardiac activity was monitored concurrently.

Drug preparation. O-MI was isolated from the leaves and stems of *Berberis chilensis* according to a published

* Correspondence to: J. L. Martínez, P.O. Box 70005, Santiago 7, Chile.

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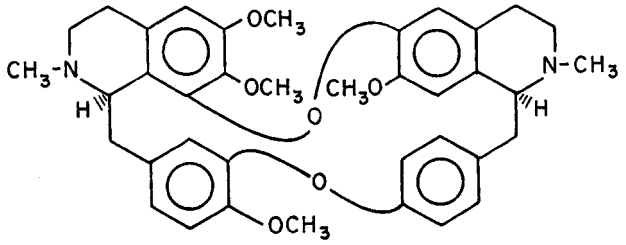

Figure 1. The chemical structure of O-MI, obtained from *Berberis chilensis,* characterized by two hydroxylated carbons in group C-7 and C-12.

procedure (Torres *et al.*, 1979a), from a phenolic extract and converted into the chlorhydrate form (Vogel, 1967).

Drug toxicity. The toxicity test was accomplished using the brine shrimp *Artemia salina*, a marine crustacean with an appropriate superficial membrane, in order to estimate the 50% lethal dose of the drug (LD_{50}). This test exhibits a great correlation with other methods which utilize cellular strains (Meyer *et al.*, 1982).

RESULTS AND DISCUSSION

O-MI (Fig. 1), a permethylated bisbenzylisoquinoline non-phenolic alkaloid has a chemical structure closely similar to 7-O-DI, another alkaloid previously reported from *Berberis chilensis* (Martínez, 1986; Morales *et al.*, 1989; Martínez *et al.*, 1992; Morales *et al.*, 1993). The structural difference between these two alkaloids is that 7-O-DI possesses two hydroxyl groups at C-7 and C-12 instead of methoxyl groups (Torres *et al.*, 1979a) and therefore would presumably be less lipophilic. O-MI was assayed in five different doses, in normotensive anaesthetized rats.

Fig 2 shows the dose-response curve of O-MI. The effect is dose-dependent up to 7.5 mg/kg with a continuous

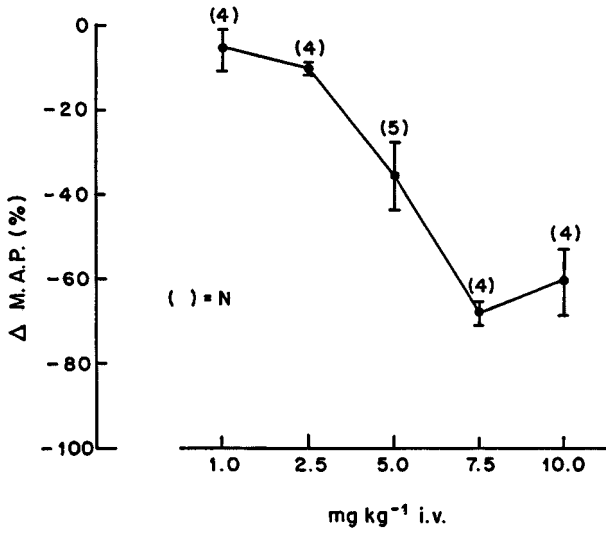

Figure 2. Dose-response curve for mean arterial pressure expressed in % of effect to five different i.v. injections of O-MI.

Figure 3. Representative polygraphic recording showing the effect of O-MI, 5 mg/kg i.v., on mean arterial pressure in normotensive anaesthetized rats.

decrease of the MAP. O-MI, 1.0 and 2.5 mg/kg i.v. injected as a single dose each time, induced a slightly, brief decrease of MAP. With a dose of 5 mg/kg i.v., a 36% decrease of control MAP was observed starting 5 s after administration and lasting for 2 min. Figure 3 shows a polygraphic recording of the hypotensive action occurring 5 s after 5 mg/kg of O-MI i.v.

Injection of 7.5 mg/kg of O-MI i.v. produced a greater effect on MAP, decreasing to almost 65%. The hypotensive effect remained for about 75 s and a slight increase in cardiac frequency of about 20 beats/min was observed throughout the duration of drug activity, maybe from sympathetic reflex origin.

With 10.0 mg/kg the MAP decrease was lower than that induced with 7.5 mg/kg. This result could be attributed to a greater sympathetic response. An inverse effect like that suggested for high concentrations of another alkaloid antioquine (Ivorra *et al.*, 1993), was not observed.

O-MI exhibited an LD_{50} of 5 mg/mL toward the brine shrimp (*Artemia salina*). The Meyer's test for toxicity is used as a correlation between LD_{50} and biological activity for natural products and other compounds (Zani *et al.*, 1995). According to this criterion the effect observed with O-MI shows low toxicity and the reversibility of its hypotensive effect seems to confirm this.

The results of the present study show that the O-MI alkaloid isolated from *B. chilensis* induces an arterial blood pressure decrease in a dose-dependent manner (Fig. 2). These results agree with previously reported observations on similar bisbenzylisoquinoline alkaloids isolated from *Thalictrum minus* (Liao *et al.*, 1978).

7-O-DI, another alkaloid previously reported by us, exhibits a long lasting hypotensive effect in anaesthetized rats (Martínez, 1986), greater than that of O-MI. This difference could be related to their chemical structure affecting the affinity to receptors. The greater solubility of O-MI could be another important factor, accelerating metabolism and renal excretion. It is probable that the O-MI-induced hypotensive activity is related to that shown by other similar bisbenzylisoquinoline alkaloids: tetrandrine, (Fang and Jiang, 1986; King *et al.*, 1988; Liu *et al.*, 1995; Wang and Lemos, 1995); antioquine, from the Colombian *Pseudoxandra sclerocarpa* (D'Ocon *et al.*, 1989) and 7-O-DI from *Berberis chilensis* (Morales *et al.*, 1989) which are all calcium channel modulators.

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