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Antitussive Activity of Moguisteine Enantiomers in Guinea-pigs and Rats

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Abstract

The antitussive effect of the *R*-(+)- and *S*-(−)-enantiomers of moguisteine were evaluated in comparison with the racemate in cough induced by 7·5% citric acid and 30 μM capsaicin aerosol in conscious guinea-pigs.

No difference in potency was observed between moguisteine and the enantiomers. The oral ED50 values (with 95% confidence limits) for moguisteine, *R*-(+)- and *S*-(−)-enantiomers were respectively: 20·4 (12·9–26·6), 20·9 (14·9–26) and 21·6 (11·8–30·0) mg kg^{-1} in cough provoked by citric acid and 17·7 (12·5–29·8), 18·9 (14·1–30·1) and 20·5 (15·1–36·6) mg kg^{-1} in cough induced by capsaicin. The acute oral and intraperitoneal toxicities of the enantiomers and moguisteine in the rat are very similar.

These findings suggest that the use of either enantiomer does not offer any advantage over the racemate.

Moguisteine, ethyl 2-[(2-methoxyphenoxy) methyl]-β-oxo-thiazolidine-3-propanoate, is a peripheral non-narcotic antitussive drug that has proved active in experimental models of cough as induced by chemical and mechanical stimuli in guinea-pigs, and by electrical stimulation in guinea-pigs and dogs; the potency of the drug is similar to that of codeine (Gallico et al 1994; Gandolfi et al 1995). In controlled clinical trials, moguisteine has been shown to effectively reduce cough associated with respiratory disorders (Aversa et al 1993; Del Donno et al 1994; Fasciolo et al 1994).

As moguisteine's framework bears a centre of asymmetry, the compound is composed of an equimolar mixture of *R*-(+)- and *S*-(−)- enantiomers.

Enantiomers may differ only in activity, but in many cases they differ in action and may behave antagonistically; one enantiomer could be also endowed with increased side-effects over those of the other (Ariëns 1986, 1991). Moreover differences in absorption, metabolic rate, affinities for target organs and protein binding sites have been detected as distinguishing the optical antipodes (Low & Castagnoli 1978; Jamali et al 1989). Thus, in some cases the use of only one enantiomer instead of a racemate may enable improvements in the efficacy or the tolerability of a compound.

As a consequence, we were prompted to compare moguisteine's antitussive properties, and its acute and subchronic toxicities, together with the pharmacokinetic and metabolic profile, with those of its enantiomers. This paper deals with the antitussive activity and the acute toxicity.

To assess the anticough properties, we selected citric acid- and capsaicin-induced cough in guinea-pigs. These models are relevant since both citric acid and capsaicin are used to provoke cough in healthy men for the evaluation of an antitussive agent (Bickerman & Barach 1954; Fuller et al 1988).

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Materials and Methods

Animals

We used Dunkin Hartley guinea-pigs of both sexes, 350-400 g (Rodentia, Italy), and Sprague-Dawley rats of both sexes, 100-125 g (Charles River, Italy). The animals were housed in conditioned quarters (temperature 21 ± 2°C, relative humidity 55 ± 10%, 12 h on, 12 h off, light cycle) with food and water freely available for at least one week before experimentation.

Antitussive activity

Cough was induced by the exposure of guinea-pigs both to a 7·5% citric acid aerosol (Charlier et al 1961) and to a 30 μM capsaicin aerosol (Forsberg & Karlson 1986) for 5 min. The aerosol was delivered by an ultrasonic nebulizer (G.B. Elbisonic, Bielin, Milan, Italy), particle size 0·5–6 μm and mean output 0·5 mL min^{-1}. Coughs were counted during aerosol exposure by a trained observer. The guinea-pigs (8–10 per dose) were orally treated with the compounds 1 h before aerosol exposure. The experiments were performed with a blind design so that the observer was unaware of the treatment.

Acute toxicity

Rats were treated both by the oral and the intraperitoneal routes with different doses (5 males and 5 females for each dose) and observed daily for 14 days for the recording of clinical signs and mortality. Macro- and microscopic examinations of organs were performed on those animals that died during the observation period and on the survivors at the end of observation period.

Statistical analysis

The ED50 values with 95% confidence limits were determined according to Hubert et al (1988); the LD50 values were evaluated with 95% confidence limits using the method of probit analysis.

Drugs

Moguisteine and its enantiomers were synthesized by

Table 1. Antitussive activity of moguisteine, *R*-(+)- and *S*-(−)-enantiomers assessed as inhibition of cough induced by citric acid (7·5%) aerosol (5 min) in guinea-pigs.

	Dose	No. of coughs	ED50 (95% CL)
Controls	–	16·8 ± 0·7	
Moguisteine	15	9·8 ± 0·9	
	30	6·5 ± 0·9	
	60	3·5 ± 0·5	
			20·4 (12·9–26·6)
Controls	–	17·3 ± 0·9	
R-(+)-Enantiomer	15	10·5 ± 0·6	
	30	7·2 ± 0·6	
	60	3·6 ± 0·6	
			20·9 (14·9–26·0)
Controls	–	17.1 ± 0·8	
S-(−)-Enantiomer	15	10·1 ± 1·0	
	30	7·2 ± 1·0	
	60	4.5 ± 1·2	
			21·6 (11·8–30·0)

The results are expressed as mean ± s.e.m.; the guinea-pigs (8 per dose) were treated with the compounds 1 h before aerosol exposure.

Boehringer Mannheim Italia. Optical purity of the enantiomers was confirmed by chiral HPLC, and their absolute configuration, *R*-(+)- and *S*-(−)-, was elucidated by means of several spectroscopic analytical methods (NMR, X-ray diffraction, circular dichroism). The compounds were given suspended in 0.5% methylcellulose (Formenti, Italy). Citric acid (Carlo Erba, Italy) was dissolved in distilled water, capsaicin (Fluka, Switzerland) was dissolved in 10% ethanol (Carlo Erba, Italy) and 10% Tween 80 (Fluka, Switzerland) and diluted with distilled water.

Results

Antitussive activity

At oral doses of 15, 30 and 60 mg kg^{-1}, moguisteine reduced the coughs induced by the 5 min of 7·5% citric acid aerosol in a dose-related way with an ED50 (95% confidence limits) of 20·4 (12·9–26·6) mg kg^{-1} (Table 1). Both enantiomers induced a similar dose-dependent inhibition of cough with the same potency as that of the racemate, with respective ED50 values of 20·9 (14·9–26·0) and 21·6 (11·8–30·0) mg kg^{-1} for the *R*-(+)- and *S*-(−)- enantiomers (Table 1).

In cough elicited by the 5 min 30 μM capsaicin aerosol, moguisteine, *R*-(+)- and *S*-(−)- enantiomers, orally administered at doses of 7·5, 15 and 30 mg kg^{-1}, induced a dose-dependent reduction in cough and displayed an antitussive activity of similar potency, as shown by the respective ED50 values of 17·7 (12·5–29·8), 18·9 (14·1–30·1) and 20·5 (15·1–36·6) mg kg^{-1} for the racemate, and the *R*-(+)- and *S*-(−)- enantiomers (Table 2).

Acute toxicity

The acute toxicities of moguisteine and its enantiomers were very low. No general toxic signs or particular target organs were discovered on the basis of clinical symptoms, body weight changes, or by macro- and microscopical examination of organs. Moreover, the LD50 values were very similar for the two enantiomers and moguisteine (Table 3).

Discussion

The antitussive activity of the *R*-(+)- and *S*-(−)-enantiomers overlaps that of moguisteine, since the three preparations display the same antitussive potency in citric acid- and capsaicin-induced cough. These findings suggest that the *S*-(+)- and *R*-(−)-enantiomers of moguisteine contribute equally to the effectiveness of the racemate. The *R*-(+)-

Table 2. Antitussive activity of moguisteine, *R*-(+) and *S*-(−)-enantiomers assessed as inhibition of cough induced by capsaicin (30 μM) aerosol (5 min) in guinea-pigs.

	Dose (mg kg^{-1})	No. of coughs	ED50 (95% CL)
Controls	–	13·4 ± 1·0	
Moguisteine	7·5	9·6 ± 0·9	
	15	7·7 ± 1·3	
	30	4·6 ± 0·9	
			17·7 (12·5–29·8)
R-(+)-Enantiomer	7·5	10·5 ± 1·0	
	15	7·5 ± 1·2	
	30	4·9 ± 0·9	
			18·9 (14·1–30·1)
S-(−)-Enantiomer	7·5	10·5 ± 1·1	
	15	7·5 ± 0·9	
	30	5·5 ± 1·0	
			20·5 (15·1–36·6)

The results are expressed as mean ± s.e.m.; the guinea-pigs (10 per dose) were treated with the compounds 1 h before aerosol exposure.

Table 3. Acute toxicity of moguisteine, *R*-(+) and *S*-(−)-enantiomers in rats (5 males and 5 females per dose) after oral and intraperitoneal administration.

	LD50 ($g\,kg^{-1}$, p.o.)	LD50 (95% CL) ($g\,kg^{-1}$, i.p.)
Moguisteine	>4	2·6 (2·5–2·8)
R-(+)-Enantiomer	>4	2·3 (2·1–2·6)
S-(−)-Enantiomer	>4	2·7 (2·4–2·9)

and S-(−)-enantiomers of moguisteine, both after the oral and the parenteral route in rats, displayed a low acute toxicity which is superimposable on that observed for the racemate. Electrophysiological studies support the hypothesis that the rapidly adapting irritant receptors (RARs), which are associated with the airway myelinated fibres, play a role as the cough receptors, since many recognized tussigenic stimuli can activate them (Karlsson et al 1988). Given that cough reflex can be provoked by a variety of stimuli such as bronchial excessive mucus production, inhalation of chemical irritants and of particulate matter and inflammatory mediators (Karlsson et al 1988), it is reasonable that a stereospecific interaction with RARs is not required for their modulation. Consequently, the lack of interaction with a stereospecific receptor involved in cough control could likely explain our results. This possibility is supported by the findings reported for the peripheral antitussive agents levodropropizine and racemate dropropizine (Malandrino et al 1988). In several current experimental models of cough in guinea-pigs, both compounds have shown the same antitussive potency. Another hypothesis we considered in our attempt to explain the equiactivity of the two enantiomers as well as their toxic similarity, was their in-vivo enantiomeric inversion as described for other chemical entities (Mehvar & Jamali 1988). The chemical structure of the compound, a 2-substituted -3 acylthiazolidine, and the optical stability of the enantiomers either in solution or as solid material, force us to consider metabolic inversion as highly improbable. Moreover this hypothesis seems to be ruled out on the basis of the pharmacokinetic and in-vitro and in-vivo metabolic studies with individual moguisteine enantiomers, which led to the conclusion that the interconversion phenomenon is to be excluded (unpublished data). When moguisteine is administered to animals or healthy subjects, it undergoes a prompt and complete presystemic hydrolysis to form its principal metabolite, the free carboxylic acid (M1), which consists of a mixture of two enantiomers (Bernareggi et al 1993). In guinea-pigs, M1 displays poor oral bioavailability (22% vs moguisteine), as expected for a carboxylic acid (unpublished data).

No substantial differences were observed between the pharmacokinetic profile of the M1 enantiomers in man after 200 mg moguisteine given orally (unpublished data). This suggests that moguisteine enantiomers are absorbed to the same extent, that they are completely biotransformed to the M1 enantiomers at the same rate, and that M1 enantiomers have similar disposition properties.

Time-course studies in cough models with moguisteine enantiomers were not performed as they have an overlapping pharmacokinetic profile and consequently differences in duration of action between them are unlikely.

In conclusion, since moguisteine enantiomers have a similar pharmacological and toxicological profile, and since the pharmacokinetic profiles of M1 enantiomers in man are quite similar, the clinical use of racemic moguisteine is justified, and the use of either enantiomer does not offer any advantage over the racemate.

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