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Anti-tussive activity of benproperine enantiomers on citric-acid-induced cough in conscious guinea-pigs

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Abstract

The anti-tussive effect of the *R*-(+)- and *S*-(–)-enantiomers of benproperine was evaluated and compared with that of the racemate on cough induced by 7.5% citric acid in conscious guinea-pigs. All the three compounds, intraperitoneally administered 1.5 h before the test, significantly inhibited citric-acid-induced cough. The ID50 values (effective doses for 50% inhibition) (with 95% confidence intervals) were 16.1 (9.1–28.4), 23.3 (11.2–48.6), 25.4 (11.7–55.1) mg kg^{-1} for the number of coughs in the 3 min of challenge, and 11.9 (5.3–26.6), 13.5 (5.6–32.4), 19.2 (12.8–28.9) mg kg^{-1} for the number of coughs in the 5 min immediately after the challenge, for (±)-benproperine, *R*-(+)-benproperine and *S*-(–)-benproperine, respectively. These findings suggest that the use of either enantiomer does not show any advantage over the racemate with regard to their anti-tussive effect.

Introduction

Benproperine (1-[1-methyl-2-[2-(phenylmethyl)phenoxy]ethyl] piperidine, CAS 2156-27-6) is a central and peripheral non-narcotic anti-tussive agent and was first synthesized by the Pharmacia Research Laboratory (Sweden). It has been found that the anti-tussive activity of benproperine is approximately equal to that of codeine in dogs and cats (Yamatsu et al 1967). In fact, benproperine is a racemate composed of an equimolar mixture of *R*-(+)- and *S*-(−)-enantiomers (Figure 1). Benproperine showed significant enantioselective pharmacokinetics in man after an oral dose of the racemate (Du et al 2000). However, the pharmacological effects of benproperine enantiomers have not been reported so far.

Many drugs, including some β-adrenoceptor antagonists, calcium-channel blockers and class I anti-arrhythmics, are in clinical use as racemates (Turgeon et al 1990). If enantiomers differ in their pharmacological actions, then administration of racemates can be viewed as administration of a fixed combination of two different agents. Moreover, if enantiomers interact with each other, then the effects of the racemate may differ from those predicted by the actions of the individual enantiomers. For example, in-vitro and in-vivo experiments have indicated that metabolism of (*S*)-propafenone is inhibited by the (*R*)-enantiomer (Kroemer et al 1994). Since, in some cases, the use of only one enantiomer instead of a racemate can increase the efficacy or the tolerability of a compound, we evaluated the anti-tussive effects of benproperine enantiomers.

In experimental animal models, coughing has been elicited by mechanical and chemical irritation or by electrical stimulation of tracheal mucosa or by nerve stimulation. Of these methods, the chemical stimuli carried in aerosols are preferred, because they are rapid to use, can be applied with simple equipment and the stimulus can be precisely quantified (Salem & Aviado 1970). Because the guinea-pig has a high sensitivity to many kinds of stimuli, it is the most widely used animal in this kind of study. The aim of our present work was to evaluate the anti-tussive effect of benproperine phosphate enantiomers and compare it with that of racemic benproperine on citric-acid-induced cough in conscious guinea-pigs.

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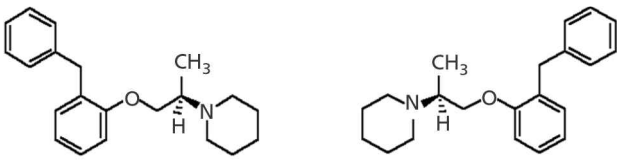

Figure 1 Structure of benproperine enantiomers..

Materials and Methods

Materials

(±)-Benproperine phosphate was provided by Aosen pharmaceutical Ltd Co. (Dalian, China) and was recrystallized by ourselves, mp 152–154 °C. Its chemical purity was up to 98.5% by HPLC. *R*-(+)- and *S*-(−)-enantiomers of benproperine phosphate were chirally synthesized by ourselves, mp 162–163 °C for *R*-(+)-benproperine and mp 159–161 °C for *S*-(−)-benproperine. The chemical purity of two enantiomers was 99% by HPLC. The optical purity was 98.5 and 97% by chiral HPLC for *R*-(+)- and *S*-(−)-benproperine, respectively. Citric acid was purchased from Shenyang Dongxing Reagent Factory (Shenyang, China). The test compounds and citric acid were all dissolved in physiological saline.

Animals

Dunkin Hartley guinea-pigs of both sexes, weighing 200–300 g (Experimental Animal Center of Shenyang Pharmaceutical University, Shenyang, China), were used in this study. All animal studies conformed to Regulations for the Administration of Affairs Concerning Experimental Animals (China, 1988) and Implementing Regulations of the Administration on Medical Experiments on Animals (China, 1989). The guinea-pigs were maintained in standard animal rooms, with food and water freely available, and on a natural light–dark cycle and they were allowed to adapt to the conditions for at least one week before use.

Statistical analysis

All results are represented as mean ± standard error of the mean (s.e.m.). Data were analysed by one-way analysis of variance. Whenever analysis of variance was significant, further comparisons between vehicle- and drug-treatment groups were performed using the Dunnett's *t*-test. A level of probability of 0.05 or less was accepted as significant. All analyses were performed using the software SPSS V11.0 for windows. Effective doses for 50% inhibition (ID50) of cough frequency were calculated by the method of Litchfield & Wilcoxon (1949). This method is based on the log transformation of the drug concentrations and the probit transformation of the percentage of inhibition.

Experimental procedure

The anti-tussive effects of (±)-benproperine and its *R*-(+)- and *S*-(−)-enantiomers were evaluated in conscious guinea-pigs against citric-acid-induced cough. The model is widely used to evaluate the activity of anti-tussive drugs (Forsberg et al 1988; Karlsson et al 1990). Guinea-pigs were placed individually in a transparent plexiglass cylinder chamber ($10 \times 10 \times 21$ cm) and exposed to a nebulized solution of 7.5% citric acid for 3 min. An ultrasonic nebulizer (402 AI; Shanghai Yuyue Medical Facilities Ltd, Shanghai, China) was used to produce an aerosol with particles having an aerodynamic mass median diameter of 1 μm, and the volume of solution aerosolized was about 0.6 mL min^{-1}. Guinea-pigs were selected for the study by the number of coughs observed 24 h before the test, and those with more than 20 or fewer than 6 coughs during the 3-min challenge were not used. The compounds and the vehicle were administered intraperitoneally (2 mL kg^{-1}) 1.5 h before the challenge. The time to the onset of the first cough, the number of coughs during the 3-min challenge and the number of coughs during the 5 min immediately after the challenge were determined. The guinea-pigs were randomly divided into 10 groups of 10 and they were used only once due to tachyphylaxis of the cough response (Lalloo et al 1995a).

The sounds of cough were recorded and amplified by use of a microphone and loudspeaker. During the experiment, the guinea-pigs were continuously watched by a trained observer who was unaware of the treatment. Sneeze and cough were differentiated by visual observation of the guinea-pigs (Laude et al 1993).

Results

Exposure to a nebulized solution of 7.5% citric acid aerosol caused coughing in control guinea-pigs within 56.8 ± 6.8 s (n = 10), and all three compounds (6–37.5 mg kg^{-1} i.p.) produced a progressive increase in the latency of the first cough (Table 1). At the highest dose (37.5 mg kg^{-1}), (±)-benproperine, *R*-(+)-benproperine and *S*-(−)-benproperine prolonged the latency of the first cough by 86.6%, 69.4% and 60.9%, respectively. Immediately after exposure to citric acid, there was a hypersensitive period for the guinea-pigs and they tended to cough continuously. For the control group, the mean number of coughs during the 3-min challenge was 13.1 ± 1.3, and the mean number of coughs during the 5 min immediately after the challenge was 27.0 ± 3.2. All three compounds reduced these two parameters in a dose-dependent manner. The ID50 values (with 95% confidence intervals) for (±)-benproperine, *R*-(+)-benproperine and *S*-(−)-benproperine were, respectively, 16.1 (9.1–28.4), 23.3 (11.2–48.6) and 25.4 (11.7–55.1) mg kg^{-1} for the number of coughs in the 3-min challenge (Table 2) and 11.9 (5.3–26.6), 13.5 (5.6–32.4) and 19.2 (12.8–28.9) mg kg^{-1} for the number of coughs in the 5 min immediately after the challenge (Table 3). In conclusion, *R*-(+)-benproperine and *S*-(−)-benproperine have significant anti-tussive activity as well as the racemate.

Table 1 Effect of (±)-benproperine and its *R*-(+)- and *S*-(−)-enantiomers on the time to onset of first cough induced by 7.5% citric acid aerosol in guinea-pigs.

Drug	Dose (mg kg^{-1})	Time to onset of 1st cough (s)
Vehicle	—	56.8 ± 6.7
(±)-Benproperine	6	74.7 ± 12.8
	15	96.8 ± 11.4*
	37.5	106.0 ± 12.5**
R-(+)-benproperine	6	52.6 ± 5.7
	15	71.6 ± 3.9
	37.5	96.2 ± 10.5*
S-(−)-benproperine	6	56.1 ± 7.4
	15	68.3 ± 3.3
	37.5	91.4 ± 5.3*

Values represent means ± s.e.m. from 10 guinea-pigs. *$P < 0.05$, **$P < 0.01$ compared with control, Dunnett's *t*-test after analysis of variance.

Table 2 Effect of (±)-benproperine and its *R*-(+)- and *S*-(−)-enantiomers on the number of coughs during a 3-min challenge induced by 7.5% citric acid aerosol in guinea-pigs.

Drug	Dose (mg kg^{-1})	No. of coughs during the 3-min challenge	ID50 (95% CI) (mg kg^{-1})
Vehicle	—	13.1 ± 1.3	
(±)-Benproperine	6	10.6 ± 0.8	
	15	7.1 ± 1.0**	
	37.5	2.9 ± 0.7**	
			16.1 (9.1–28.4)
R-(+)-benproperine	6	11.4 ± 1.3	
	15	7.9 ± 0.7*	
	37.5	5.2 ± 0.9**	
			23.3 (11.2–48.6)
S-(−)-benproperine	6	11.2 ± 1.8	
	15	8.6 ± 1.1*	
	37.5	6.1 ± 1.0**	
			25.4 (11.7–55.1)

Values represent means ± s.e.m. from 10 guinea-pigs. *$P < 0.05$, **$P < 0.01$ compared with control, Dunnett's *t*-test after analysis of variance. The ID50 values were evaluated with 95% confidence intervals using the method of Litchfield & Wilcoxon (1949).

Table 3 Effect of (±)-benproperine and its *R*-(+)- and *S*-(−)-enantiomers on the number of coughs during the 5 min immediately after a challenge induced by 7.5% citric acid aerosol in guinea-pigs.

Drug	Dose (mg kg^{-1})	No. of coughs during the 5 min immediately after the challenge	ID50 (95% CI) (mg kg^{-1})
Vehicle	—	27.0 ± 3.2	
(±)-Benproperine	6	17.9 ± 2.6*	
	15	14.4 ± 2.1**	
	37.5	4.1 ± 1.2**	
			11.9 (5.3–26.6)
R-(+)-benproperine	6	18.2 ± 2.1*	
	15	12.2 ± 2.2**	
	37.5	8.4 ± 1.7**	
			13.5 (5.6–32.4)
S-(−)-benproperine	6	19.1 ± 2.0	
	15	15.0 ± 1.1**	
	37.5	10.9 ± 1.6**	
			19.2 (12.8–28.9)

Values represent means ± s.e.m. from 10 guinea-pigs. *$P < 0.05$, **$P < 0.01$ compared with control, Dunnett's *t*-test after analysis of variance. The ID50 values were evaluated with 95% confidence intervals using the method of Litchfield & Wilcoxon (1949).

Discussion

To assess the anti-tussive effect of (±)-benproperine and its *R*-(+)- and *S*-(−)-enantiomers, we selected the model of citric-acid-induced cough in guinea-pigs. This model is relevant since it is the one most frequently adopted and extensively studied in animals and also in man (Braga & Allegra 1989). Although guinea-pig and man showed similar responses to some stimuli (Laude et al 1993), the relationship between the cough reflex of the two species is incompletely understood. Guinea-pigs have been widely used in studies of the respiratory system, which have provided much valuable information for clinical therapeutics (Forsberg & Karlsson 1986; Lalloo et al 1995b; Girard et al 1996; Lai et al 1999). This study shows for the first time that benproperine racemate and its *R*-(+)- and *S*-(−)-enantiomers, given intraperitoneally, all have obvious anti-tussive efficacy.

There are two sorts of cough: the useful and the useless. Cough is useful when it effectively expels secretions, exudates, transudates or extraneous material from the respiratory tract (i.e. when it is productive). When citric acid is employed in some expectorant preparations, it is held to encourage productive cough by increasing the volume of bronchial secretion (Laurence et al 1999). On the other hand, citric acid may induce cough in the guinea-pig by acting on capsaicin-sensitive sensory neurons (Forsberg & Karlsson 1986; Forsberg et al 1988) or by disturbing the pH of the airway surface liquid (Lalloo et al 1995b; Wang et al 1999). More recent studies have identified that some neuropeptides, such as the tachykinins substance P and neurokinin A, are also involved in the complex process (Ricciardolo 2001).

Although (±)-benproperine has been widely used, the definite mechanism of its anti-tussive action has yet to be determined. It has been generally assumed that there are many types of afferent receptor in the airways. Karlsson et al (1988) reported that within the airways, certain sites are particularly sensitive to stimulation of cough and rapidly adapting stretch receptors (RARs) play a role as the cough receptors. RARs in the airway mucosa are found from the

nasopharynx to the bronchi (Widdicombe 2003). It has been found that RARs show very varied sensitivity to different stimuli, including mechanical and chemical irritant stimuli and many inflammatory and immunological mediators (Sant'Ambrogio & Widdicombe 2001). Furthermore, RAR-like fibres have been demonstrated to be involved in the activation of guinea-pig airway afferents by low pH (Kollarik & Undem 2002). Consequently, it is reasonable that a stereospecific interaction with RARs is not required for the anti-tussive action of benproperine and its enantiomers. This possibility is supported by the findings reported for the anti-tussive agents moguisteine and its enantiomers. In several experimental cough models in guinea-pigs, they have all shown equivalent anti-tussive potency (Gallico et al 1996). According to the complicacy of pharmacokinetics and pharmacodynamics of chiral drugs, another hypothesis we considered in our attempt to explain our results is their in-vivo enantiomeric inversion. However, in a previous study, we have found that the mean AUC_{0-t} and C_{max} values for *S*-(−)-benproperine were 2.12 and 2.18 times higher than those of *R*-(+)-benproperine, when the racemate was administered orally in man (Du et al 2000), which seems discrepant with this report. The most likely explanation is that there are intrinsic differences in the pharmacokinetics of benproperine between guinea-pig and man. Even in a given species, there is no evidence that the C_{max} value and pharmacological effect of a drug would be absolutely relevant. Consequently, a further study of the pharmacokinetics of the *R*-(+)- and *S*-(−)-enantiomers would be of great value to establish the possibility with greater certainty and would provide data of the duration of the effect of the drugs, which has direct value for their clinical application. Further studies with different chemical or electrical stimuli, to get more information about the anti-tussive effect and mechanism of benproperine phosphate enantiomers, are in progress.

Conclusion

This study demonstrates that in the citric-acid-induced cough test in unrestrained guinea-pigs, the *R*-(+)- and *S*-(−)-enantiomers of benproperine, a racemate used as an anti-tussive drug, both have significant anti-tussive activity never reported before. All the drugs studied reduced the number of coughs during the challenge, lengthened the time of onset and reduced the number of coughs after cessation of the challenge. The data imply that the use of either enantiomer may not show any advantage over the racemate with regard to their anti-tussive effect.

References

Braga, P. C., Allegra, L. (1989) In: Braga, P. C., Allegra, L. (eds) *Cough*. Raven Press, New York, pp 73–93

Du, Z. M., Zhong, D. F., Kang, Y., Chen, X. Y. (2000) Enantioselective pharmacokinetics of benproperine in healthy volunteers. *Acta Pharm. Sin.* **35**: 909–912

Forsberg, K., Karlsson, J. A. (1986) Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea pigs. *Acta Physiol. Scand.* **128**: 319–320

Forsberg, K., Karlsson, J. A., Theodorsson, E., Lundberg, J. M., Persson, C. G. (1988) Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea pig. *Pulm. Pharmacol.* **1**: 33–39

Gallico, L., Borghi, A., Cavalletti, E., Ceserani, R., Tognella, S. (1996) Antitussive activity of moguisteine enantiomers in guinea-pigs and rats. *J. Pharm. Pharmacol.* **48**: 112–114

Girard, V., Yavo, J. C., Emonds-Alt, X., Advenier, C. (1996) The tachykinin NK2 receptor antagonist SR 48968 inhibits citric acid-induced airway hyperresponsiveness in guinea pigs. *Am. J. Respir. Crit. Care Med.* **153**: 1496–1502

Karlsson, J. A., Sant'Ambrogio, G., Widdicombe, J. (1988) Afferent neural pathways in cough and reflex bronchoconstriction. *J. Appl. Physiol.* **65**: 1007–1023

Karlsson, J. A., Lanner, A. S., Persson, C. G. (1990) Airway opioid receptors mediate inhibition of cough and reflex bronchoconstriction in guinea pigs. *J. Pharmacol. Exp. Ther.* **252**: 863–868

Kollarik, M., Undem, B. J. (2002) Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J. Physiol.* **543**: 591–600

Kroemer, H. K., Fromm, M. F., Buhl, K., Terefe, H., Blaschke, G., Eichelbaum, M. (1994) An enantiomer-enantiomer interaction of (S)- and (R)-propafenone modifies the effect of racemic drug therapy. *Circulation* **89**: 2396–2400

Lai, Y. L., Chiou, W. Y., Lu, F. J., Chiang, L. Y. (1999) Roles of oxygen radicals and elastase in citric acid-induced airway constriction of guinea-pigs. *Br. J. Pharmacol.* **126**: 778–784

Lalloo, U. G., Barnes, P. J., Chung, K. F. (1995a) Captopril inhibits tachyphylaxis to citric acid-induced cough in guinea pigs. *Am. J. Respir. Crit. Care Med.* **151**: A110 (Abstract)

Lalloo, U. G., Fox, A. J., Belvisi, M. G., Chung, K. F., Barnes, P. J. (1995b) Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. *J. Appl. Physiol.* **79**: 1082–1087

Laude, E. A., Higgins, K. S., Morice, A. H. (1993) A comparative study of the effects of citric acid, capsaicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulm. Pharmacol.* **6**: 171–175

Laurence, D. R., Bennett, P. N., Brown, M. J. (1999) In: Laurence, D. R., Bennett, P. N., Brown, M. J. (eds) *Clinical pharmacology*. 8th edn, Science Press & Harcourt Asia, Peking, pp 500–502

Litchfield, J. T., Wilcoxon, F. (1949) A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**: 99–113

Ricciardolo, F. L. (2001) Mechanisms of citric acid-induced bronchoconstriction. *Am. J. Med.* **111** (8A): 18S–24S

Salem, H., Aviado, D. M. (1970) Antitussive agents. In: Salem, H. Aviado, D. M. (eds) *International encyclopedia of pharmacology and therapeutics*. Vol. 1, Pergamon Press, New York, pp 205–231

Sant'Ambrogio, G., Widdicombe, J. (2001) Reflexes from airway rapidly adapting receptors. *Respir. Physiol.* **125**: 33–45

Turgeon, J., Murray, K. T., Roden, D. M. (1990) Effects of drug metabolism, metabolites, and stereoselectivity on antiarrhythmic drug action. *J. Cardiovasc Electrophysiol.* **1**: 238–260

Wang, C. H., Matai, R., Morice, A. H. (1999) Cough induced by low pH. *Respir. Med.* **93**: 58–61

Widdicombe, J. G. (2003) Overview of neural pathways in allergy and asthma. *Pulm. Pharmacol. Ther.* **16**: 23–30

Yamatsu, K., Ohtsu, K., Kase, Y., Miyata, T., Yuizono, T. (1967) On the site of antitussive action of 1-(2-benzylphenoxy)-2-piperidinopropane phosphate (pirexyl). *Jpn. J. Pharmacol.* **17**: 538–549