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Differences between gastric antiulcer effects of trapencaine enantiomers

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The spatial arrangement of single stereoisomers may influence pharmacodynamic, pharmacokinetic and toxicological properties of a drug. Trapencaine (I. N. N.), (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid, was developed as an antiulcer drug with gastroprotective, local anaesthetic and spasmolytic effects. Limited information is available about the potential pharmacodynamic differences of the enantiomers of trapencaine. Therefore, the enantiomers of (±)-*trans*- or *cis*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxy carbanilic acid were synthesised and tested on models of acute gastric damage induced by indomethacin and/or ethanol. A difference was found between their antiulcer effect, with the (+)-*trans*-enantiomer being the most effective and the (–)-*cis*-enantiomer the least effective in the models used.

1. Introduction

Many drugs exist as stereoisomers: optical, geometrical and conformational isomers. There are pharmacodynamic, pharmacokinetic as well as toxicological differences between the effect of stereoisomers. Very small changes in the structure of a given drug molecule can lead to radical changes in the elicited biological activity. This can be expected since the majority of receptors, enzymes and channels are stereospecific and interact differently with single stereoisomers [1]. Concerning antiulcer drugs, data on the effect of the spatial arrangement of single stereoisomers on their protective properties is still limited. Differences between the effects of stereochemical isomers have been documented in the case of proton pump inhibitors [2].

Trapencaine (I. N. N.) (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid (**1**) (Scheme) was developed originally as a local anaesthetic from the group of basic alkoxysubstituted carbanilates [3]. Trapencaine was demonstrated to exhibit also gastroprotective [4], antisecretory and spasmolytic effects [5]. It was shown to stimulate the synthesis of gastric mucus [6] and decrease the liberation of histamine from mast cells [7].

Endogenous prostaglandins and sulfhydryls play a role in the gastroprotective effect of trapencaine. The effect of free oxygen radicals, the important mediators of gastric injury, was inhibited by trapencaine pretreatment [8]. The antimicrobial activity against *Helicobacter pylori* is another important property of trapencaine [9]. Unlike the effect of most common local anaesthetics, the effect of trapencaine increases with low pH [10], an advantage with regard to the action of trapencaine in regions where pH is shifted toward acidity, e.g. in the stomach or at sites of inflammation.

The molecule of trapencaine is synthesised from 2-(pyrrolidin-1-yl)cyclohexanol (cyclohexan substituted in position 1,2 by different substituents), which exists in two geometrical stereoisomers, *cis*- and *trans*-, both geometrical stereoisomers creating a pair of (+)- and (–)-enantiomers. Trapencaine, (±)-*trans*-isomer, in a 135-times lower concentration was as effective as standard cocaine in lower anaesthesia, and in a 91-times lower concentration it was as effective as procaine in infiltration anaesthesia [11]. The (±)-*cis*-isomer [12], by comparison, was found to be less effective against ethanol induced gastric injury and failed to prevent gastric damage induced by phenylbutazone [13].

Scheme

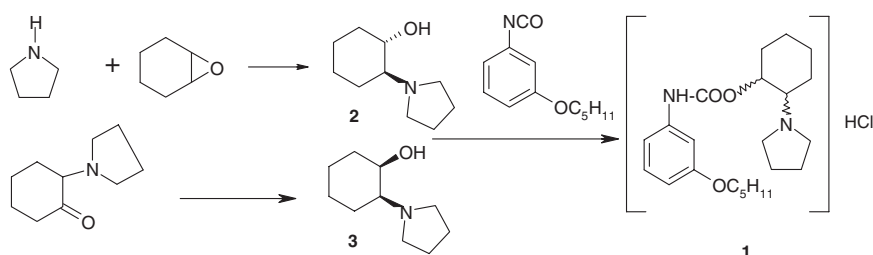


Table 1: Effect of (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid and its (+)-*trans*- and (–)-*trans*-enantiomers on length and number of gastric lesions induced by indomethacin in rats

	Mean ± SEM	% Control	% Inhibition
Lesion length (mm)			
Control	23.40 ± 3.73	100.00 ± 15.96	–
(±)- <i>trans</i>	7.78 ± 5.63*	33.24 ± 24.06	66.76
(+)- <i>trans</i>	4.61 ± 1.67*	19.75 ± 7.12	80.25
(–)- <i>trans</i>	15.35 ± 5.65	65.60 ± 24.14	34.40
Lesion number			
Control	15.45 ± 1.94	100.00 ± 12.55	–
(±)- <i>trans</i>	4.28 ± 2.46**	27.69 ± 15.93	72.31
(+)- <i>trans</i>	3.61 ± 1.07**	23.37 ± 6.95	76.63
(–)- <i>trans</i>	9.00 ± 2.97	58.25 ± 19.22	41.45

Drugs tested were administered orally in a dose of 20 mg/kg 30 min before indomethacin.

Control rats received vehicle. Each group consisted of at least 9 rats. * $P < 0.05$, ** $P < 0.01$ versus control.

The aim of this study was to synthesise the enantiomers of (±)-*trans*- and (±)-*cis*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid and to study their effect on models of acute gastric injury in rats.

2. Investigations and results

2.1. Effect of *trans*-stereoisomers

In the model of indomethacin induced gastric lesions, oral pretreatment of rats with the drugs tested prevented the development of gastric damage. In the case of lesion length parameter, there was a significant difference between the control (vehicle treated) group and the group treated with (±)-*trans*- (trapezine) as well as between the control group versus (+)-*trans*-enantiomer (Table 1). The difference between (±)-*trans*-, (+)-*trans*- and (–)-*trans*- was not significant, however, the best protective effect, around 80% inhibition of gastric injury, was achieved after pretreatment with (+)-*trans*-enantiomer. The least effective drug was (–)-*trans*-enantiomer. Similar results were obtained for lesion number.

In ethanol induced gastric damage, the extensive haemorrhagic lesions present in the untreated controls were inhibited by administration of *trans*-isomers. Again, the most pronounced protective effect was observed after pretreatment with (+)-*trans*-enantiomer. A significant inhibition

Table 2: Effect of (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid and its (+)-*trans*- and (–)-*trans*-enantiomers on length and number of gastric lesions induced by 96% ethanol in rats

	Mean ± SEM	% Control	% Inhibition
Lesion length (mm)			
Control	128.50 ± 20.57	100.00 ± 16.01	–
(±)- <i>trans</i>	14.75 ± 4.77**	11.48 ± 3.71	88.52
(+)- <i>trans</i>	6.27 ± 3.34***	4.88 ± 2.60	95.12
(–)- <i>trans</i>	19.11 ± 12.08*	14.87 ± 9.40	85.13
Lesion number			
Control	19.00 ± 1.56	100.00 ± 8.21	–
(±)- <i>trans</i>	4.60 ± 1.11**	24.21 ± 5.84	75.79
(+)- <i>trans</i>	2.00 ± 0.67***	10.53 ± 3.53	89.47
(–)- <i>trans</i>	4.33 ± 1.89**	22.79 ± 9.95	41.45

Drugs tested were administered orally in a dose of 20 mg/kg 30 min before 96% ethanol. Each group consisted of at least 9 rats. ** $P < 0.01$, *** $P < 0.001$ versus control.

Table 3: Effect of (±)-*cis*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid and its (+)-*cis*- and (–)-*cis*-enantiomers on length and number of gastric lesions induced by indomethacin in rats

	Mean ± SEM	% Control	% Inhibition (Potentiation)
Lesion length (mm)			
Control	14.50 ± 5.23	100.00 ± 36.10	–
(±)- <i>cis</i>	7.50 ± 2.38	51.72 ± 16.43	48.28
(+)- <i>cis</i>	14.07 ± 3.91	97.03 ± 26.97	2.97
(–)- <i>cis</i>	18.04 ± 5.03	124.41 ± 34.67	+24.41
Lesion number			
Control	8.68 ± 2.49	100.00 ± 12.55	–
(±)- <i>cis</i>	5.20 ± 1.44	59.91 ± 16.55	40.09
(+)- <i>cis</i>	9.07 ± 2.09	104.49 ± 24.17	+4.49
(–)- <i>cis</i>	9.00 ± 2.16	103.69 ± 24.90	+3.69

Drugs tested were administered orally in a dose of 20 mg/kg 30 min before indomethacin. Each group consisted of at least 6 rats. + values mean more than 100% (control).

was found after pretreatment with (±)-*trans*-, (+)-*trans*-, and also with (–)-*trans*- enantiomer. The lesion length and lesion number were influenced in a similar way (Table 2).

2.2. Effect of *cis*-stereoisomers

Contrary to the above mentioned results, the effect of *cis*-isomers was not significant in the indomethacin model of gastric injury. Pretreatment with (–)-*cis*-enantiomer even worsened the damage: both the lesion length and lesion number were greater as compared to control values (Table 3). The differences between the enantiomers tested were also nonsignificant.

3. Discussion

The gastric antiulcer effect of trapezine was investigated as to its stereoselectivity. The development of indomethacin and/or ethanol induced gastric lesions was prevented by oral administration of the racemic stereoisomers and optical enantiomers of 2-(pyrrolidin-1-yl)cyclohexylester of 3-(*n*)-pentyloxycarbanilic acid, given in a dose of 20 mg/kg. Using *trans*-isomers, the inhibition of gastric lesion length as well as lesion number was significant, while *cis*-isomers were not effective. The results presented confirm and extend the described stereospecific antiulcer effect of some carbanilates, which was found also in the model of phenylbutazone induced gastric damage [13].

Though not always significant, there was also a difference between the effects of the optical isomers. On the models used, the most pronounced antiulcer and gastroprotective effect was observed after pretreatment with the (+)-*trans*-enantiomer and the least one after (–)-*cis*-enantiomer. Compared to the controls, the effect of (+)-*trans*-enantiomer was significant in all cases studied. On the contrary, the *cis*-enantiomers were not effective and even worsened the gastric injury. Although their effect was tested only in the indomethacin model because of the lack of available substances, but based on the previous experimental data [13] similar results may be expected also for the ethanol model.

Pharmacodynamic differences have been established for many enantiomeric pairs. The ratio of pharmacodynamic activities of the more active enantiomer, the eutomer, to the less active enantiomer, the distomer, may be up to 100 or greater, e.g. for some beta blockers and nonsteroidal antiinflammatory drugs (NSAIDs). In other instances,

however, the differences are less profound, as in the case of anticoagulants, or even negligible, e.g. concerning bupivacaine. The activity of enantiomers may also differ qualitatively, where the enantiomers have opposing dose-related effects, e.g. methadone [14]. In the case of the drugs tested, there was a marked difference in the antiulcer activity against NSAIDs – but not ethanol-induced gastric damage, with the *trans*-stereoisomer being 2–3 times more effective than the *cis*-. Such a stereospecific effect did not appear in derivatives with the methylene group incorporated between the pyrrolidin and cyclohexan structure of the molecule [13]. A difference in the antiulcer activity was observed also between optical isomers of the compounds studied in the model of indomethacin induced gastric injury with (+)-*trans*-enantiomer being the most effective.

Data on the influence of spatial arrangement of single stereoisomers on the protective properties of antiulcer drugs are rather scarce. Differences between the effects of stereochemical isomers were found only in the case of proton pump inhibitors [2]. The molecule of omeprazole, a typical representative of these antisecretory drugs, is chiral due to the presence of a sulphur atom situated on the apex of the trilateral pyramid, formed by three different substituents. This arrangement is responsible for the existence of two enantiomers. Esomeprazole [15], the *S*-isomer of omeprazole, is the first proton pump inhibitor developed as a single optical isomer. It possesses a better pharmacokinetic profile and provides a greater gastric acid suppression than omeprazole. In *in vitro* studies, esomeprazole was found to undergo less metabolic transformation by the cytochrome P450 system than the racemic drug, resulting in less variation in plasma concentration between slow and rapid metabolisers.

Pharmacodynamic differences between enantiomers question the validity of using racemic preparations [16]. Advantages of enantiomerically pure drugs include more selective pharmacological profiles leading to better therapeutic indices, less complex pharmacokinetics and interactions and better estimation of plasma concentration-response relationship [17]. It is likely that new drug development will concentrate on single stereoisomers and the development of racemic mixtures will demand scientific justification. There will be therefore a continuing requirement for enantiospecific analysis [18]. The trend towards the clinical use of enantiomers will require also more research on the pharmacodynamic properties of enantiomers of racemic drugs. The additional testing of enantiomers can lead to the discovery of new indications of the original drug, improve its clinical use and increase its safety and efficacy [19].

In the work presented, the enantiomers of (±)-*trans*- or *cis*-2-(pyrrolidin-1-yl)cyclohexylester of 3-(n)-pentyloxy carbanilic acid were synthesised and tested on models of acute gastric damage induced by indomethacin and/or ethanol. A difference was found between their antiulcer effect, with the (+)-*trans*-enantiomer being the most effective and the (–)-*cis*- enantiomer the least effective in the models used.

4. Experimental

4.1. Chemistry

4.1.1. Chemicals and equipment

The reagents used were from Aldrich, Fluka AG and Faculty of Pharmacy Brno. Melting points were determined with a Boetius apparatus. Optical rotation was measured with a polarimeter AA5 (Optical Activity Ltd.) in methanol. IR spectra were recorded with a spectrometer FTIR Nicolet Im-

Table 4: The specific rotation of trapencaine isomers

	$[\alpha]_D^{20}$ [methanol]
(+)- <i>trans</i>	+16.4°
(–)- <i>trans</i>	–17.8°
(+)- <i>cis</i>	+5.6°
(–)- <i>cis</i>	–5.2°

pact (Nicolet) in KBr. UV spectra were recorded with a spectrophotometer HP 8453 (Hewlett Packard) in methanol. ¹H and ¹³C NMR spectra were determined with a Varian Gemini 200 (200 MHz and 50 MHz, respectively) using CDCl₃ as solvent. Tetramethylsilane was used as internal standard. TLC was performed on aluminium-backed silica gel plates with fluorescent indicator Silufol UV₂₅₄ (Kavalier).

4.1.2. Synthesis of trapencaine enantiomers

The racemic (±)-*trans*-2-(pyrrolidin-1-yl)cyclohexanol (**2**) was prepared by addition of 1,2-epoxycyclohexane to pyrrolidine (5 h, 120 °C). The racemic (±)-*cis*-2-(pyrrolidin-1-yl)cyclohexanol (**3**) was prepared by reduction of 2-(pyrrolidin-1-yl)cyclohexanone with L-selectride in tetrahydrofuran. Enantiomers of (±)-*trans*- or (±)-*cis*-2-(pyrrolidin-1-yl)cyclohexanol were prepared by crystallisation of responsible diastereoisomers with (+)-O,O-dibenzoyl-D-tartaric acid. Solutions of (±)-*trans*- or (±)-*cis*-2-(pyrrolidin-1-yl)cyclohexanol in propan-2-ol and solution of (+)-O,O-dibenzoyl-D-tartaric acid in propan-2-ol were mixed and the resulting diastereoisomers were recrystallised (propan-2-ol).

3-(n)-Pentyloxyphenylisocyanate was prepared by reaction of phosgene and 3-(n)-pentyloxyaniline in toluene (water free).

Bases of enantiomers (+)-*trans*-, (–)-*trans*-, (+)-*cis*- and (–)-*cis*-2-(pyrrolidin-1-yl)cyclohexanol of appropriate optical purity were added to 3-(n)-pentyloxyphenylisocyanate in toluene (water free, 5 h under reflux). The final bases in the form of hydrochlorides were with hydrogen chloride solution in ether (water free) prepared and recrystallised from acetone. (Scheme).

The structure of the compounds studied was confirmed by IR, UV, ¹H NMR, ¹³C NMR spectroscopy and by specific rotation (Table 4).

4.2. Pharmacology

4.2.1. Indomethacin induced gastric lesions

Female Wistar rats weighing 160–180 g were randomly assigned to experimental groups. The rats were fasted 24 h prior to the experiments with free access to water. Indomethacin was applied orally to fasted rats in a dose of 30 mg/kg b.w. Trapencaine (racemic drug) and the enantiomers used were given orally in a dose of 20 mg/kg/5 ml 30 min before indomethacin administration. Control rats received an equal volume of the vehicle (distilled water). The animals were sacrificed 4 h after indomethacin intake. The doses of the compounds used were chosen on the basis of results from previous experiments [4].

4.2.2. Ethanol induced gastric lesions

Food but not water was withheld 24 h prior to experiment. Fasted rats were given orally 96% ethanol in a volume of 5 ml/kg b.w. Trapencaine and the enantiomers tested were administered in a dose of 20 mg/kg/5 ml orally 30 min before ethanol. Control rats received an equal volume of distilled water. The animals were killed 60 min after ethanol intake.

In all the antiulcer experiments the rats were killed by cervical dislocation, the stomachs were removed, opened along the greater curvature and rinsed with tap water. The lesions were examined under a dissecting microscope by an observer who was unaware of the treatment. The length of gastric lesions (mm) was measured and the number of lesions was recorded.

The results are expressed as means ± SEM. ANOVA and Student's t-test were used for statistical analysis, P < 0.05 was considered significant.

The experiments were approved by the Bioethical Committee of the Institute.

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