Local anesthetic activity of mixtures of cis- and trans-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates

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1. Introduction

Over the past century, local anesthetics have been used to prevent acute pain and to ameliorate chronic pain associated with various conditions. Despite their remarkable efficacy, the risk of toxicity associated with local anesthetics remains a recurring issue (Dillane and Finucane 2002; Borgeat and Aguirre 2010). Therefore, it is important to develop new local anesthetic compounds as well as to improve existing ones. The interaction between two anesthetic drugs can be: (i) additive, (ii) synergistic, or (iii) antagonistic (infra-additive) according to whether their combined effect is equal to, greater than, or less than the sum of their individual effects. The identification of synergistic drug combinations is essential because these combinations allow lower doses of each drug to be used, therefore potentially reducing the side-effects caused by the individual drugs and making them particularly attractive for clinical use (Hendrickx et al. 2008; Shafer et al. 2008). In our previous studies, we focused on the effect of stereoisomerism on the local anesthetic activity of selected carbamate compounds (Gregan et al. 2011). The stereochemistry of local anesthetics can affect their potency by altering various characteristics of the drugs, such as their binding to target molecules, rate of metabolism and lipophilicity (Gregan et al. 1995; Blesova et al. 1985; Whiteside 2011). The stereochemistry of local anesthetics can affect their potency by altering various characteristics of the drugs, such as their binding to target molecules, rate of metabolism and lipophilicity (Gregan et al. 1995; Blesova et al. 1985; Whiteside 2011). Therefore, it is important to study individual cis- and trans-isomers as well as the two isomers in combination.

2. Investigations, results and discussion

In our previous study, we synthesized two homologous series of racemic stereoisomeric cis- and trans-(2-dimethylaminomethylcycloheptyl)-2-alkoxyphenylcarbamates with alkyl chain lengths ranging from C1 to C8 and analyzed their local anesthetic activity. Here, we show that the local anesthetic activities of cis-1 and trans-1 stereoisomers are higher than the sum of activities calculated for the individual stereoisomers at all molar fractions. We conclude that an appropriate ratio of cis- and trans-stereoisomers is necessary to achieve the maximum anesthetic activity of the studied stereoisomeric carbamates.

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**3. Experimental**

### 3.1. Surface Local Anesthetic Activity (SLAA)

Equation (1) was used to calculate the Surface Local Anesthetic Activity, \( P \), for the individual cis-1 (trans-1) stereoisomers in the mixture (see Fig. 2 and Table 1).

\[
P = \frac{\text{SLAA} \times M}{100}
\]

where \( P \) is the calculated SLAA value of the individual stereoisomers in the mixture of cis-1 and trans-1 stereoisomers, SLAA is the Surface Local Anesthetic Activity of the individual isomers, and \( M \) is the molar fraction of the individual stereoisomers in the mixture.

### 3.2. Chemistry

The starting compounds for the synthesis of the studied racemic cis- and trans-2-dimethylaminoalcohol (Gregan et al. 2011, 1995; Cizmarik et al 1976; Forro et al 1998; Forro and Fulop 1999). The starting cis- and trans-aminoketones were purified by column chromatography using silica gel with an eluent of ethylacetate:methanol (1:1) and distilled. The purity of all compounds was verified by TLC on Merck Silica Gel 60 F254 plates using ethylacetaet:methanol (1:1) as the mobile phase (detection under UV light at 254 nm).

**Table 2**: Regression coefficients for the linear relationships between the sums of the calculated SLAA values and the molar fractions (\( x \)) of cis-1 and trans-1 carbamates using the equation: SLAA = \( ax + b \)

<table>
<thead>
<tr>
<th>( P )</th>
<th>( a )</th>
<th>( b )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3</td>
<td>-0.5603</td>
<td>89.058</td>
<td>1.000</td>
</tr>
<tr>
<td>P6</td>
<td>-0.9603</td>
<td>289.06</td>
<td>1.000</td>
</tr>
<tr>
<td>P8</td>
<td>-0.7361</td>
<td>178.03</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*\( P \) = R-propyl, P6 = R-hexyl, P8 = R-octyl.
*\( r^2 \) = Correlation coefficient.

**Table 1**: Experimental and calculated SLAA values for varying molar fractions of cis-1 and trans-1 carbamates in the mixture

<table>
<thead>
<tr>
<th>( M )</th>
<th>SLAA (exp)</th>
<th>SLAA (calc)</th>
<th>( \Delta P )</th>
<th>( % P )</th>
<th>( % M )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.0</td>
<td>89.0</td>
<td>0</td>
<td>0</td>
<td>100</td>
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<tr>
<td>0.1</td>
<td>75.7</td>
<td>80.1</td>
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<tr>
<td>0.2</td>
<td>66.8</td>
<td>81.1</td>
<td>4.3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.3</td>
<td>53.4</td>
<td>74.6</td>
<td>11.2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.4</td>
<td>44.5</td>
<td>71.3</td>
<td>16.8</td>
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</tr>
<tr>
<td>0.5</td>
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<td>34.5</td>
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</tr>
<tr>
<td>0.6</td>
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<td>62.1</td>
<td>41.5</td>
<td>0</td>
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</tr>
<tr>
<td>0.7</td>
<td>13.3</td>
<td>58.4</td>
<td>45.1</td>
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<tr>
<td>0.8</td>
<td>0</td>
<td>89.0</td>
<td>89.0</td>
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<tr>
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<td>89.0</td>
<td>89.0</td>
<td>0</td>
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</tr>
</tbody>
</table>

**Fig. 1**: Structures of the studied stereoisomeric cis- and trans-carbamates (R = alkyl chain with 3, 6 or 8 carbons).

**Fig. 2**: Dependence of the experimental SLAA values (\( \bullet \), \( \boxdot \), \( \square \)) and calculated SLAA values (\( \circ \), \( \triangle \), \( \triangleleft \)) on the molar fractions of cis-1 (trans-1) stereoisomers in the mixture of studied carbamates. P3: R = propyl, P6: R = hexyl, P8: R = octyl.
The cis- and trans-2-dimethylaminomethylcycloheptyl-2-alkoxyphenylcarbamates (Fig. 1) were prepared by the reaction of cis- and trans-2-dimethylaminomethylcycloheptanols with 2-alkoxyphenylisocyanates in anhydrous toluene under argon atmosphere, as previously described (Gregan et al. 2011). For the biological assays, hydrochlorides of these basic carba-
mates were used, and cis- and trans-2-dimethylaminomethylcycloheptanols were pre-
bred by the selective reduction of 2-dimethylaminomethylcycloheptane with lithium tris(butyldimethylsilyl)oxide in anhydrous THF at 70°C (Gregan et al. 1995; Forro et al. 1998; Forro and Fulop 1999; Per-
lia 1987). The cis-2-dimethylaminomethylcycloheptanols were prepared by the reduction of 2-dimethylaminomethylcycloheptane with sodium borohydride in water (Gregan et al. 2011; Forro et al. 1998; Forro and Fulop 1999; Perlia 1987). These reductions were previously described for analogical cis- and trans-2-dimethylaminomethylcycloheptane and cis- and trans-2-dimethylaminomethylcycloheptanol (Gregan et al. 1995). 2-Alkoxyphenylisocyanates with propyl-, hexyl- and octyl-
were prepared by the selective reduction of 2-dimethylaminomethylcycloheptane by reac-
tion with palladium in anhydrous toluene (Čižmarik et al. 1976; Gregan et al. 1997). Experimental physico-chemical data for cis- and trans-2-
dimethylaminomethylcycloheptanols are not available in the literature, with the exception of the 1H NMR spectra and optical rotations (Forro et al. 1998; Forro and Fulop 1999).

3.3. Pharmacology

The studied carbamate stereoisomers were tested in the hydrochloride form. The Surface Local Anesthetic Activity indices (SLAA) of the analyzed com-
pounds were determined on rabbit cornea according to the method of Vhta and Sekera (1959). Various concentrations of the compounds were applied to the conjunctival sac for 30 min. Afterwards, corneal sensitivity was repeat-
ly tested using a hair aesthesiometer at 3-min intervals. Full anesthesia was consid-
ered to occur if no response was elicited by 6 consecutive stimulations. Each compound was tested in at least three independent experiments. The

surface local anesthetic activity index (SLAA) was calculated as the ratio of receptive-field concentrations of the standard and the compound (dimension-
less value) (Racanska and Gregan 1999). All biological tests were performed in compliance with regulations for biological testing on animals.

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