Basic characteristics and kinetics of degradation in aqueous buffer of selected diclofenac prodrugs intended for joint injection
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Basic characteristics and kinetics of degradation in aqueous buffer of selected diclofenac prodrugs intended for joint injection
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Introduction
Recently, a novel ester prodrug approach for the accomplishment of local and sustained diclofenac action after injection into joints was reported (1). It is to be expected that both onset and duration of diclofenac action can be modified by variation of inherent ester prodrug properties including their pH-dependent solubility and charge as well as their susceptibility to undergo esterase facilitated hydrolysis. In the present study, three diclofenac ester prodrugs differing with respect to the spacer carbon chain length (Fig. 1) were synthesized and evaluated in vitro. Thus, the objectives of the present study were: (i) to determine the effect of the spacer chain length on the pKₐ value and aqueous pH-dependent solubility of the prodrugs, (ii) to investigate the kinetics and mechanism of degradation of the three prodrugs in aqueous solution in the pH-range 1-10 as well as in 80 % (v/v) human synovial fluid (SF) and 80 % (v/v) plasma at 37°C, (iii) to characterize in vitro release of diclofenac from prodrug suspensions using the rotating dialysis cell model.

Prodrug concept
The prodrug approach involves the employment of pro-moieties that besides having an OH group for diclofenac ester bond formation comprises a weak base functionality with a pKₐ value of about 6-8 (2). Hence, this prodrug type experiences a complex relationship between solubility, dissolution and prodrug cleavage rate.

Mechanism of degradation
The stability of the diclofenac ester prodrugs after incubation in human 80 % (v/v) SF and 80 % (v/v) plasma was studied at 37°C. Compared to the stability in 67 mM phosphate buffer solution at pH 7.4, the prodrugs underwent much faster degradation in the biological media indicating the involvement of enzyme-mediated prodrug conversion (Table 1) to yield the active diclofenac.

Stability in human plasma and synovial fluid
The stability of the diclofenac ester prodrugs after incubation in human 80 % (v/v) SF and 80 % (v/v) plasma was studied at 37°C. Compared to the stability in 67 mM phosphate buffer solution at pH 7.4, the prodrugs underwent much faster degradation in the biological media indicating the involvement of enzyme-mediated prodrug conversion (Table 1) to yield the active diclofenac.

pH-rate profile
The degradation rate of the prodrugs was studied over the pH range 1-10 in aqueous solutions at 37°C. Based on the shape of the pH-rate profiles (Fig. 3) an expression for the overall apparent first-order rate constant for the degradation of the individual diclofenac ester prodrug in the pH-range 1-10 was proposed

$$k = k_0 \cdot e^{-\frac{n \cdot pK_a - pH}{10}}$$

where k₀ refers to the pseudo-first-order rate constant for prodrug degradation in buffer free solution (pH 7-10) and in dilute HCl, pH 1-1.5 (n = 10). The full lines in Fig. 3 which are obtained by fitting the data to Eq. 1 using a nonlinear least-squares regression reveal significantly different release behaviour among the prodrugs and indicate a complex relationship between solubility, dissolution and prodrug cleavage rate.

Conclusion
The rate of degradation of the prodrugs was about 6-fold faster in 80 % (v/v) human plasma than in 80 % (v/v) human SF, which might reflect the higher protein content of human plasma relative to SF or the presence of different esterases in the biological media. In vitro release studies performed on pro-formed suspensions of the prodrugs revealed significantly different release behaviour among the prodrugs and indicate a complex relationship between solubility, dissolution and prodrug cleavage rate. Overall the results from the study indicated that design of prodrugs suited for local pain management through joint injection may be feasible by tuning the physicochemical properties.

Table 1: pKₐ, half-lives and solubility (mean ± S.D., n=3) of prodrugs in aqueous solution at pH 7.4, 80 % (v/v) SF and 80 % (v/v) plasma at 37°C.

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>pH 7.4 (µg/mL)</th>
<th>pH 7.4 (min)</th>
<th>pH 37°C (µg/mL)</th>
<th>pH 37°C (min)</th>
<th>SF (µg/mL)</th>
<th>SF (min)</th>
<th>Plasma (µg/mL)</th>
<th>Plasma (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP-1</td>
<td>1.297 \cdot 10^{-3}</td>
<td>1.52 \cdot 10^{-4}</td>
<td>4.97 \cdot 10^{-3}</td>
<td>1.32 \cdot 10^{-4}</td>
<td>1.7 \cdot 10^{-3}</td>
<td>1.5 \cdot 10^{-4}</td>
<td>1.8 \cdot 10^{-3}</td>
<td>2.0 \cdot 10^{-4}</td>
</tr>
</tbody>
</table>

References: