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Publication date: 2016

Document Version
Peer-review version

Citation for published version (APA):
Basic characteristics and kinetics of degradation in aqueous buffer of selected diclofenac prodrugs intended for joint injection

Nina Mertz1, Susan Weng Larsen1, Claus Selch Larsen1
1Department of Pharmacy, Faculty of Health and Medical Science, University of Copenhagen, Denmark

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 values 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.

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.

Table 1: pKa values, 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</th>
<th>pH 7.4</th>
<th>80% SF</th>
<th>80% Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP-1</td>
<td>4.6</td>
<td>7.6</td>
<td>11.5</td>
<td>16.5</td>
</tr>
<tr>
<td>DP-2</td>
<td>4.6</td>
<td>7.6</td>
<td>11.5</td>
<td>16.5</td>
</tr>
<tr>
<td>DP-3</td>
<td>4.6</td>
<td>7.6</td>
<td>11.5</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Table 2: Dose 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>
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<td>16.5</td>
</tr>
</tbody>
</table>

pH-rate profiles

The degradation rate of the prodrugs was studied over the pH range 1-10 in aqueous solution 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:

\[ k_{oa} = k'_{oa} + k''_{oa} \]

where \( k'_{oa} \) refers to the pseudo-first-order rate constant for prodrug degradation in buffer free solution (pH 2-10 (n = 3-4) and in dilute HCl, pH 1-1.5 (n = 3-10). The full lines in Fig. 3 where obtained by fitting the data to Eq. 1 using the \( k'_{oa} \) of 1.297 - 10^(-10) (37°C; I = 0.3 M) (3). As apparent from Fig. 3, good agreement between the solid curves and the experimental determined rate constants was achieved (R² = 0.997).

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 pre-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.

References:


Figure 1: Chemical structures of the diclofenac ester prodrugs.

Figure 2: Schematic representation of the in vitro formulation principle.

Figure 3: pH-rate profiles for the degradation of the diclofenac prodrugs in aqueous solution at 37°C (I = 0.3 M).

Figure 4: Reaction schemes for formation of diclofenac and ILO from degradation of the DP-2 prodrug at pH below 2 (A) and at 5.8 ≤ pH ≤ 10 (B) and 37°C.

Figure 5: Release profiles of diclofenac from prodrug suspensions of DP-1, DP-2, and DP-3 in 80 % (v/v) human synovial fluid at 37°C in the rotating dialysis model (n=2-3).

A total dose of approximately 25 µmol prodrug was added to the donor compartment. Bars represent standard deviations.

Figure 6: Time (h) versus concentration (µg/mL) profiles for the degradation of the diclofenac prodrugs in 80% human synovial fluid at 37°C.

Figure 7: Time (h) versus concentration (µg/mL) profiles for the degradation of the diclofenac prodrugs in 80% human plasma at 37°C.

Figure 8: Time (h) versus concentration (µg/mL) profiles for the degradation of the diclofenac prodrugs in 80% human plasma at 37°C.