Comparison of Compartmental and Minimal PBPK models for Nonlinear Naproxen Pharmacokinetics in Arthritic Rats

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Rheumatoid Arthritis (RA)

- Chronic, systemic inflammatory autoimmune disease
- Joint inflammation, cartilage and bone destruction
- Hypoalbuminemia is a common feature associated with RA
- Higher serum albumin concentrations in women

PK-PD of highly albumin-bound NSAIDs

**NSAID-Naproxen (NPX)**

**Inflammatory symptoms control in RA**

**Non-selective COX inhibitor, blocking PG production**

**Rapid anti-inflammatory and analgesic properties**

**Nonlinear PK in RA patients (> 500mg)**

**Concentration-dependent protein binding**

**Extensive protein binding (>99.9%), especially to albumin (>96%)**

Mechanisms Causing Nonlinear PK of NPX

- **Dose-dependent Distribution**
  - $V$ increases with dose
  - *Gillette Equation*
    \[
    V = V_p + V_t \times \frac{f_{u,p}}{f_{u,t}}
    \]
    - Physical volume ($V_p$ & $V_t$)
    - Binding ($f_{u,p}$ & $f_{u,t}$)
  - Saturated binding at high concentrations/doses, $f_{u,p} \uparrow$, $V \uparrow$

- **Dose-dependent Clearance**
  - $CL$ increases with dose

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Objective: Developing PK models incorporating nonlinear binding to account for the nonlinear PK of NPX in RA and assess the potential influences of sex.

- Effect of sex differences in albumin concentrations in RA on the protein binding and disposition of NPX has not yet been well investigated.

- The NPX PK profiles in earlier studies were described using simple linear clearance models without consideration of the nonlinearity.
Outline

- Experimental system: Rheumatoid Arthritis in CIA Rats (both sexes)
- Determination of plasma albumin concentrations and protein binding of NPX in arthritic rats
- PK studies of NPX in arthritic rats
- Development and comparison of extended compartmental and minimal PBPK (mPBPK) models incorporating nonlinear binding
RA in CIA Rats

Natural Growth & Disease Progression

Females
- Control
- Natural growth

Males
- Control
- Natural growth

Day 16 (384 h)
Day 21 (504 h)
Methods

- Protein binding study
  - Sandwich ELISA
  - Ultrafiltration

- Plasma albumin determination
  - Animals: CIA rats of both sexes
  - Sampling time: time to reach peak disease status

Diagram:
- Protein binding study
  - Ultrafiltration
Plasma Protein Binding of NPX

Fraction unbound vs. total concentrations

- Concentration-dependence

Rosenthal plots

- Two classes of binding sites
Plasma Albumin Concentrations & Protein Binding Data Analysis

Two classes of binding sites

\[
C_{bp} = \frac{n_1 Pt \cdot K_{a1} \cdot C_{up}}{1 + K_{a1} \cdot C_{up}} + \frac{n_2 Pt \cdot K_{a2} \cdot C_{up}}{1 + K_{a2} \cdot C_{up}}
\]

Parameters Estimates (CV%)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CIA females</th>
<th>CIA males</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_{a1} (\mu M^{-1}))</td>
<td>0.28 (3.53)</td>
<td>0.26 (4.00)</td>
</tr>
<tr>
<td>(K_{a2} (\mu M^{-1}))</td>
<td>0.0041 (4.2)</td>
<td>0.0056 (11.75)</td>
</tr>
<tr>
<td>(n_1)</td>
<td>1 (Fixed)</td>
<td></td>
</tr>
<tr>
<td>(n_2)</td>
<td>4 (Fixed)</td>
<td></td>
</tr>
</tbody>
</table>

Unbound NPX conc. in plasma/tissue

\[
C_{up} \text{ or } C_{ut} = (-(n_1 Pt \cdot K_{a1} + n_2 Pt \cdot K_{a2} - C_t \cdot K_{a1} + 1) + \\
\sqrt{4 \cdot (n_2 Pt \cdot K_{a2} \cdot K_{a1} + K_{a1}) \cdot C_t + (n_1 Pt \cdot K_{a1} + n_2 Pt \cdot K_{a2} - C_t \cdot K_{a1} + 1)^2})/2 \cdot (n_2 Pt \cdot K_{a2} \cdot K_{a1} + K_{a1})
\]

\[
\frac{Pt_{tissue}}{Pt_{plasma}} = 0.9
\]
NPX PK in Arthritic Rats

- **Experimental design**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Administration methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female CIA rats (n=3)</td>
<td>10, 25 and 50 mg/kg of NPX</td>
</tr>
<tr>
<td>Male CIA rats (n=3)</td>
<td></td>
</tr>
</tbody>
</table>

- $Q_{\text{hep}} \sim 1200 \text{ mL/h/kg}$
The binding affinities and numbers of binding sites on each protein molecule in ISF are the same as in plasma, with a difference in protein concentrations.

The protein binding of NPX in tissues is considered to occur primarily in the interstitial fluid (ISF).

According to the “free hormone hypothesis”, disposition processes often operate only on free drug.

The binding of NPX is concentration-dependent and only to albumin in either plasma or ISF.
Two-compartment Model (2CM) with Nonlinear Binding

Model Equations

\[ \frac{dA_a}{dt} = -k_a \cdot A_a \]

\[ \frac{dC_p}{dt} = \frac{k_a \cdot A_a + CL_{d} \cdot C_{ut}}{V_p} - \left(\frac{CL + CL_{d}}{V_p}\right) \cdot C_{up} \]

\[ \frac{dC_t}{dt} = \frac{CL_{d}}{V_t} \cdot (C_{up} - C_{ut}) \]
Table 1-3 PK parameter estimates for unbound NPX after IP administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Estimates (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (1/h)</td>
<td>Absorption rate constant</td>
<td>0.814 (7.54)</td>
</tr>
<tr>
<td>$CL$ (mL/h/kg)</td>
<td>Unbound plasma clearance</td>
<td>1370 (4.73)</td>
</tr>
<tr>
<td>$V_p$ (mL/kg)</td>
<td>Central volume of distribution</td>
<td>32.36 (Fixed)</td>
</tr>
<tr>
<td>$CL_d$ (mL/h/kg)</td>
<td>Unbound distribution clearance</td>
<td>647.2 (18.61)</td>
</tr>
<tr>
<td>$V_t$ (mL/kg)</td>
<td>Peripheral distribution volume</td>
<td>140.7 (9.27)</td>
</tr>
</tbody>
</table>

$V_{ISF} = 174$ mL/kg
From literature:
- Healthy female rats (10 mg/kg oral dose), $k_a=0.4 \, \text{1/h}$, $F=0.9$

Basic Minimal PBPK model

Whole Body PBPK Model

mPBPK Model

Lumping

Physiological restrictions:
\[ f_{d1} + f_{d2} \leq 1; \]
\[ V_1 + V_2 + V_p = BW \text{ or } ECF \]

Fick’s Laws of perfusion

- Rapid equilibrium of tissue: venous blood

Fick’s Law of Perfusion

\[
\frac{dA_T}{dt} = Q_T (C_a - C_v)
\]

- $A_T$: Amount in tissue
- $Q_T$: Blood flow
- $C_a$: Conc. arterial blood
- $C_v$: Conc. venous blood

\[
\frac{dC_T}{dt} = \frac{Q_T}{V_T} \cdot (C_a - C_{v_T})
\]

- $C_T$: Concentration in tissue
- $K_p$: Permeability coefficient

\[
C_T = K_p \cdot C_{v_T}
\]

\[
\frac{dC_T}{dt} = \frac{Q_T}{V_T} \cdot \left( C_a - \frac{C_T}{K_p} \right)
\]
Basic mPBPK Model with Nonlinear Binding

Model Equations

\[
\frac{dA_a}{dt} = -k_a \cdot A_a
\]

\[
V_p \cdot \frac{dC_p}{dt} = k_a \cdot A_a + f_d \cdot Q_{co} \cdot (C_{ut} - C_{up}) - CL_p \cdot C_{up}
\]

\[
V_t \cdot \frac{dC_t}{dt} = f_d \cdot Q_{co} \cdot (C_{up} - C_{ut})
\]

\[K_p = \frac{f_{u,p}}{f_{u,t}}\]

Model Structure

\[f_d \leq 1, \quad V_p + V_t = ECF \ (206.29 \text{ ml/kg})\]
Possible Tissue Distribution of NPX

- NPX is a weak acid (pKa=4.5)
- Henderson-Hasselbalch equation for acids:
  \[ \text{pH} = \text{pKa} + \log \left( \frac{[\text{ionized}]}{[\text{unionized}]} \right) \]
- The unionized fraction of NPX in ISF/plasma (pH 7.4) is much less than that in cell water (pH 7.0).
- pH Partition Hypothesis: only the unbound unionized drug is able to permeate cell membranes in vivo.
- Therefore, it is very likely that NPX distributes mainly into the extracellular space with minimal cell distribution.

- \( V_{\text{NPX}} \) (Human) \( \sim 0.14 \) L/kg
- \( V_{\text{ECF}} \) (Human) \( \sim 0.26 \) L/kg

Basic mPBPK Model with Nonlinear Binding

Model Fittings

PK Parameter Estimates

Table 1-4 PK parameter estimates for NPX in CIA rats after IP administration

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Definition</th>
<th>Estimates (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (1/h)</td>
<td>Absorption rate constant</td>
<td>0.98 (8.3)</td>
</tr>
<tr>
<td>$f_d$</td>
<td>Fraction of cardiac plasma flow</td>
<td>0.15 (12.9)</td>
</tr>
<tr>
<td>CL (mL/h/kg)</td>
<td>Unbound plasma clearance</td>
<td>1438 (3.2)</td>
</tr>
<tr>
<td>$V_p$ (mL/kg)</td>
<td>Plasma volume</td>
<td>32.36 a</td>
</tr>
<tr>
<td>$V_t$ (mL/kg)</td>
<td>ISF volume</td>
<td>173.93 a</td>
</tr>
<tr>
<td>$Q_{co}$ (mL/h/kg)</td>
<td>Cardiac plasma flow</td>
<td>7650 a</td>
</tr>
</tbody>
</table>

CIA Females

CIA Males

NPX concentrations (µg/mL)

Time (h)
Model Simulations Overlaid with Published Human Data

Basic mPBPK Model with Nonlinear Binding

## Model Comparison

<table>
<thead>
<tr>
<th></th>
<th>Compartmental model</th>
<th>mPBPK model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model structure</strong></td>
<td>Limited physiologic or anatomic reality</td>
<td>Physiological and anatomical properties</td>
</tr>
<tr>
<td><strong>Model fittings</strong></td>
<td>Similar fitting results for the plasma PK data of NPX</td>
<td></td>
</tr>
<tr>
<td><strong>PK parameters</strong></td>
<td>Depend highly on the quality of the PK data; Unclear physiologic relevance</td>
<td>Physiological-relevant PK parameters; Separates system- and drug- specific parameters</td>
</tr>
<tr>
<td><strong>Assessment of tissue distribution</strong></td>
<td>Drug concentrations outside of plasma, especially when protein binding is nonlinear, cannot be reasonably predicted</td>
<td>Allows reasonable calculation of total and unbound NPX concentrations in ISF</td>
</tr>
</tbody>
</table>
Summary

- Concentration–dependent protein binding was incorporated into a classic type of 2CM and the basic mPBPK model to account for the nonlinearity of NPX PK in arthritic rats.

- Both models operated distribution and elimination processes using unbound drug and allowed a global analysis of all PK data over a range of doses simultaneously.

- However, there are advantages to be gained by use of the extended mPBPK model for describing the PK of NPX, particularly for describing unbound NPX in ISF and SF, adjacent to the site of action. This serves as the prelude for establishing more reasonable PK-PD relationship of NPX in RA.

- Sex differences in albumin concentrations did not produce differences in PK, suggesting that other factors (e.g., drug metabolism) are involved. However, the underlying mechanisms still need further investigations.
Effect of Disease-Related Changes in Plasma Albumin on the Pharmacokinetics of Naproxen in Male and Female Arthritic Rats

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ABSTRACT

Naproxen (NPX) is used in the treatment of rheumatoid arthritis (RA) for alleviation of pain and inflammation. In view of the extensive albumin binding of NPX, this study investigates whether chronic inflammation and sex influence the physiologic albumin concentrations, plasma protein binding, and pharmacokinetics (PK) of NPX. The PK of NPX was evaluated in a rat model of CIA (collagen-induced arthritis) in Lewis rats and in healthy controls. These PK studies included 1) NPX in female and male CIA rats that received 10, 25, or 50 mg/kg NPX i.p.; and 2) NPX in healthy female and male rats after i.p. dosing of NPX at 50 mg/kg. Plasma albumin concentrations were quantified by enzyme-linked immunosorbent assay, and protein binding was assessed using ultrafiltration. The NPX concentrations in plasma and filtrates were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasma concentration-time data of NPX were first assessed by noncompartmental analysis (NCA). Nonlinear PK was indicated by dose-dependent NCA clearances and distribution volumes was observed. A two-compartment model was fit with a first-order absorption process incorporating nonlinear protein binding in plasma and tissues. Noncompartmental analysis of the PK data of the various groups. Saturable albumin binding accounted for the nonlinearity of NPX PK in all rats as well as part of the PK differences in arthritic rats. The CIA rats exhibited reduced albumin concentrations, reduced overall protein binding, and reduced clearances of unbound NPX, consistent with expectations during inflammation. The net effect of chronic inflammation was an elevation of the Cmax and area under the plasma concentration-time curve (AUC) of unbound drug.

Compartmental model

mPBPK model

Modeling Sex Differences in Pharmacokinetics, Pharmacodynamics, and Disease Progression Effects of Naproxen in Rats with Collagen-Induced Arthritis

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ABSTRACT

Naproxen (NPX) is a frequently used nonsteroidal anti-inflammatory drug for rheumatoid arthritis (RA). Lack of quantitative information about the drug exposure-response relationship has resulted in empirical dosage regimes for use of NPX in RA. Few studies to date have included sex as a factor, although RA predominates in women. A pharmacokinetic, pharmacodynamic, and disease progression model described the anti-inflammatory effects of NPX in collagen-induced arthritis (CIA) male and female rats. Three groups of rats were included for each sex: healthy animals, CIA controls, and CIA rats given a single 50-mg/kg dose of NPX intraperitoneally. Paw volumes of healthy rats indicated natural growth, and disease status was measured by paw edema. An innovative minimal physiologically based pharmacokinetic (mPBPK) model incorporating nonlinear albumin binding of NPX in both plasma and interstitial fluid (ISF) was applied. Arthritic rats exhibited lower plasma and ISF albumin concentrations and reduced clearances of unbound drug to explain pharmacokinetic profiles. The unbound ISF NPX concentrations predicted by the mPBPK model were used as the driving force for pharmacological effects of NPX. A logistic growth function accounted for natural paw growth and an indirect response model for paw edema and drug effects (inhibition of iNOS) was applied. Female rats showed a higher incidence of CIA, earlier disease onset, and more severe symptoms. NPX had stronger effects in males, owing to higher unbound ISF NPX concentrations and lower IC50 values. The model described the pharmacokinetics, unbound NPX in ISF, time course of anti-inflammatory effects, and sex differences in CIA rats.
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