Blood-to-Brain Transfer of Various Oxicams: Effects of Plasma Binding on Their Brain Delivery

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Received December 24, 1996; accepted February 18, 1997

Purpose. The objective of this work was to assess the influence of binding to plasma proteins and to serum on the brain extraction of four antiinflammatory oxicams.

Methods. The brain extraction of isoxicam, tenoxicam, meloxicam and piroxicam was investigated in rats using the carotid injection technique. Blood protein binding parameters were determined by equilibrium dialysis using human serum, human serum albumin (HSA) and alpha-1-acid glycoprotein (AAG) solutions at various concentrations.

Results. All oxicams had low values of brain extraction, between 19% and 39% when dissolved in serum, i.e. under physiological conditions. Brain efflux rate constants calculated from the wash-out curves were the same in the absence or presence of serum. Brain efflux was inversely related to the polarity of the oxicams, such that the higher their H-bonding capacity, the lower their brain efflux. The free dialyzable drug fraction was inversely related to protein concentration. However, rat brain extraction was always higher than expected from in vitro measurements of the dialyzable fraction.

Conclusions. Except for piroxicam whose brain extraction was partially decreased in the presence of proteins, the serum unbound and initially bound fractions of oxicams both seem available for transfer into the brain. Modest affinities for AAG rule out any related effect. More surprising is the apparent lack of effect on brain transfer of the high-affinity binding to HSA and serum. The enhanced brain uptake of meloxicam in the presence of AAG could be a result of interactions between this globular protein and the endothelial wall.

KEY WORDS: blood-brain barrier; drug brain transfer; in vivo brain extraction; plasma protein binding; oxicams.

INTRODUCTION

Drugs are generally bound in plasma to a significant extent by several transport proteins, both with high and low affinity. It has long been thought that only the free drug concentration was available for diffusion into tissues (1). Indeed, in most situations, plasma binding may impair the distribution of drugs to tissues, with drug distribution then mainly restricted to the distribution compartment of the drug-binding protein. However, several studies (2,3) have shown that a fraction of the total bound drug can dissociate in some capillaries and thus becomes available for transfer. In such cases, the plasma drug-binding is permissive and does not limit drug distribution to tissues. Thus, a given transport protein may have either a permissive or a restrictive effect on drug distribution, depending on the tissue. For example, HSA exerts a permissive effect on propranolol uptake by the liver, but a restrictive effect on brain transfer (3).

Oxicams are non-steroidal antiinflammatory drugs (NSAIDs) which exhibit a specific pharmacokinetic behavior, namely a high percentage of protein binding, a low apparent volume of distribution (always lower than the volume of body-exchangeable water, 0.6 L kg⁻¹), and a long plasma half-life (>20 h). Piroxicam and tenoxicam are zwitterions in the pH range 2-5 and anions at pH 6 and above. Meloxicam is a zwitterion in the pH range 1-4 and an anion above pH 5. In contrast, isoxicam is mainly neutral below pH 4 and mainly anionic above. Therefore, all oxicams will be mainly anionic at physiological pH and as such have a modest capacity to partition into octanol (log D₂₅°C = −0.3 to 0.1) (4,5). Such properties are expected to markedly influence distribution and tissue penetration. NSAIDs seem to cause headaches and dizziness in a relatively small number of recipients. These reactions are commonly observed with indomethacin, whereas few central nervous reactions have been reported for oxicams.

The aim of our study was to compare the brain extraction of various oxicams and to assess the influence of physicochemical factors and plasma protein binding on their brain extraction.

MATERIALS AND METHODS

Radiolabeled Compounds

[³H]Piroxicam (specific activity = 374 GBq M⁻¹) was a gift from Pfizer (France), [¹⁴C]isoxicam (specific activity = 282 GBq M⁻¹) from Warner Lambert (USA), [¹⁴C]meloxicam (specific activity = 178 GBq M⁻¹) from Thomas (Germany) and [¹⁴C]tenoxicam (specific activity = 499.5 GBq M⁻¹) from Roche (Switzerland). Their chemical structure is shown in Figure 1.

Radiocimolecular purities were all >97%, as assessed by TLC on silica plates using four different solvent systems: CHCl₃/CH₂COCH₃/HCOOH (70/30/4 v/v/v) for piroxicam, CHCl₃/CH₃OH/HCOOH (85/10/5 v/v/v) for isoxicam, CHCl₃/CH₃OH/H₂O (80/20/1 v/v/v) for meloxicam and CH₂COCH₃/CH₃OH/C₃H₇OH (70/20/10/2 v/v/v) for tenoxicam. The labeled compounds were stored at −80°C until use. The specific activity of the internal standards, [¹⁴C]butanol and tritiated water (NEN®, Du Pont de Nemours) were 37 GBq M⁻¹ and 370 GBq M⁻¹, respectively.

Human Plasma Proteins

Human serum albumin (A1887) (HSA) was purchased from Sigma (Saint-Quentin Fallavier, France) and alpha-1-acid glycoprotein from Behring (Marburg, Germany). Protein frac-
Effects of Plasma Binding on Oxicam Transfer Through the BBB

\[
\text{piroxicam}
\]

\[
\text{isoxicam}
\]

\[
\text{tenoxicam}
\]

\[
\text{meloxicam}
\]

Fig. 1. Chemical structure of the investigated oxicams.

\[\text{ipsilateral to the injection were dissolved in 1.5 mL of Soluene 350 (Packard Instrument Co.) at 60°C, and left overnight before double isotope liquid scintillation counting (Packard Tri-Carb 460 CD).}\]

Owing to the rapid bolus injection, the injected solution traverses the brain microcirculation as a discrete bolus, mixing only minimally with the circulating rat blood. Previous studies have shown that bolus mixing with rat blood is <5% (6).

\**Separation of the Net Brain Fraction of the Drug from the Intracapillary Fraction**

In these experiments, brain homogenates were depleted of brain microvasculature, so that net cerebral uptake could be measured, as opposed to the fraction of the drug retained by brain capillary endothelium. According to the method previously described by Triguero et al. (7), the hemisphere ipsilateral to the injection was quickly removed after decapitation (5 s after carotid injection) and homogenized with a glass homogeniser (8–10 strokes) in 5 mL of physiological glucose buffer (pH 7.4). A 28% Dextran solution (5 mL) was added and homogenized again. After an aliquot of the homogenate was taken, the remainder was centrifuged at 6000 g for 20 min at 4°C in a swinging-bucket rotor (Sorvall RC 28S). The supernatant (brain tissue) and pellet (vascular cells) were carefully separated and prepared, as described earlier, for double-isotope liquid scintillation counting. In this manner, we obtained the intra-endothelial fraction of the drug (\(E_0\)) which had remained trapped in the endothelial cells during the blood-to-brain transfer.

\**Estimation of Drug Brain Uptake**

The brain uptake index (BUI) was calculated as follows:

\[
BUI = \frac{(\text{dpm in tissue})}{(\text{dpm in injected sample})}
\]

for piroxicam and

\[
BUI = \frac{(\text{dpm in tissue})}{(\text{dpm in injected sample})}
\]

for the other oxicams.

The BUI represents the net uptake of the drug normalized by the net uptake of the reference compound. The BUI is, therefore, a direct function of the single-pass extraction of the drug (\(E_0\)):

\[
E_d = E_r \cdot BUI
\]

where \(E_r\) is the single-pass extraction of the reference compound.

The brain-to-blood transport of each drug and reference compound were simultaneously determined at 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 300 s after injection. The data were analysed using an iterative non-linear program according to the formula:

\[
E_d(t) = E_d(0) \cdot e^{-kt}
\]

\[
E_r(t) = E_r(0) \cdot e^{-kt}
\]

where \(E(t)\) represents the brain extraction at time \(t\), \(E(0)\) is the brain extraction extrapolated at time 0 and \(k\) is the efflux rate.