Disposition Kinetics of Diclofenac in the Dual Perfused Rat Liver

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ABSTRACT: This study investigates the hepatic disposition of diclofenac as a function of route of input: portal vein (PV) versus hepatic artery (HA) in the presence of its binding protein, albumin. The *in situ* dual perfused rat liver was performed using Krebs bicarbonate buffer containing human serum albumin (HSA, 0.25%–1%) at constant PV (12 mL/min) and HA (3 mL/min) flow rates. Bolus doses of [^{14}C]–diclofenac and ^{125}I-labeled HSA were injected randomly into the HA or PV and then, after an appropriate interval, into the alternate vessel. Regardless of route of input and perfusion medium protein concentration, the hepatic outflow profile of diclofenac displayed a characteristic sharp peak followed by a slower eluting tail, indicating that its radial distribution is not instantaneous. Based on the estimated effective permeability-surface area product/blood flow ratio, hepatic uptake of diclofenac is governed by both perfusion and permeability. Fractional effluent recovery (F) increased as unbound diclofenac fraction in the perfusate decreased. Although no significant difference in hepatic clearance of diclofenac as a function of route of delivery at 0.5% and 1% HSA, it was demonstrable at 0.25% HSA ($p < 0.001$), when the extraction ratio is higher. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3220–3227, 2013

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INTRODUCTION

The isolated perfused liver preparation is an ideal experimental system that has been widely used to investigate the kinetics of hepatic disposition. The perfused liver retains characteristics that are closest to the *in vivo* situation in terms of structural and functional heterogeneity and, also particularly in the single pass mode, permits easy manipulation of the experimental conditions (e.g., perfusate flow, binding, oxygen content, temperature, etc.) without the complicating effects of recirculation. Although the liver has a dual blood supply, hepatic artery (HA) and portal vein (PV), the PV has generally been the sole source of input in the isolated perfused liver preparations studied. This modality is unphysiological in the sense that it excludes the possible contribution from the arterial input.

A survey of available data on the hepatic extraction or clearance of various compounds in relation to the route of presentation to the liver is currently ambiguous. Although some authors report a difference between arterial and venous administrations,[1–8] others have observed no such difference.[9–15] Nevertheless, choice of compound and method of assessment may be important issues when attempting to demonstrate whether any difference in recovery occurs with respect to site of vascular input. The liver is a heterogeneous organ in terms of its flow distribution, comprising a specific arterial space and a common space that occupies the remaining arterial space together with the portal space.[16] Any difference in fractional hepatic recovery of compound as a function of route of administration could therefore be attributed to the specific arterial space and its enzyme content, because the extraction across the common space will be the same regardless of the route of hepatic input.

To gain further insight into the issue of the role of hepatic input on extraction, we chose diclofenac as a probe. Although influence of albumin on hepatic disposition of diclofenac has been extensively studied

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in the single PV perfused rat liver,[17–20] there are no reports concerning its hepatic disposition kinetics as a function of route of hepatic input. Therefore, we investigated the influence of altered protein binding on uptake kinetics of diclofenac in the *in situ* dual perfused rat liver preparation.

MATERIALS AND METHODS

Chemicals

Iodine-labeled albumin [^{125}I–HSA (human serum albumin), 1.02 mCi/mg] was purchased from ICN Biomedicals (Costa Mesa, California), and ^{14}C–diclofenac (372 kBq/mg) was a gift of Pfizer (UK). All other reagents were of analytical grade.

Liver Perfusion

All experiments were conducted under appropriate Project and Personal licences issued by the UK Home Office. All animals were handled in compliance with the Home Office guidelines.

The single-pass dual perfused *in situ* rat liver technique, with male Sprague–Dawley rats (300–450 g, wet liver weight: 11–16 g; $n = 10$), has been reported by us before.[21] The rat was anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg). After cannulation of the bile duct, HA, and PV, the liver perfused with freshly prepared Krebs-bicarbonate buffer (pH 7.4) containing sodium taurocholate (6 mg/L), glucose (3 g/L), and HSA (0.25, 0.5, and 1.0%) at a total flow rate of 15 mL/min (3 mL/min via the HA and 12 mL/min via the PV). The viability of the liver was assessed from bile flow measurement, arterial perfusion pressure, perfusate recovery, and macroscopic appearance. Immediately after an experiment, the liver excised and weighed.

Experimental Procedure

An initial stabilization period of the liver preparation of 15–20 min with drug- and protein-free perfusate was allowed before commencement of the unit impulse-response experiments. The experiments were carried out under three different perfusate protein concentrations, namely 0.25%, 0.5%, and 1.0% HSA. A bolus dose (50 μL) of ^{14}C–diclofenac (0.17–0.44 μCi) in the absence or presence of ^{125}I–HSA (0.06–0.27 μCi) was introduced randomly into either HA or PV, and then after an appropriate interval (15–20 min) into the alternate blood vessel. Immediately after an injection, the automatic turntable was activated for collection of hepatic outflow perfusate at 1–2 s intervals for 1–2 min. Subsequently, samples were collected into test tubes at increasing intervals up to 5–6 min. The activities of ^{14}C–diclofenac and ^{125}I–albumin in hepatic effluent samples were determined by radiochemical analysis after the addition of 4 mL of liquid scintillation fluid, with the results expressed as disintegrations per minute (dpm). The protein used throughout the study was defatted and freeze-dried.[8]

Data Analysis

The concentration of radioactivity (dpm/mL) of each tracer at the midpoint time of the collection interval, $C(t)$, was transformed to frequency output ($f(t)$, 1/s) using the following equation

$$f(t) = \frac{C(t)Q}{\text{Dose}} \tag{1}$$

where Q is the perfusate flow rate (mL/s). The mean catheter transit time (catheter and tubing; PV: 2.5 s and HA: 2.7 s) was determined and all dilution curves obtained were corrected. The maximum $f(t)$ value, f_{max}, and the time at which it occurred, t_{max}, were determined experimentally.

Moments of the frequency outflow versus time profiles were estimated using the following equations:

$$\text{AUC} = \int_0^\infty f(t)\mathrm{d}t \tag{2}$$

$$\text{MTT} = \frac{\int_0^\infty tf(t)\mathrm{d}t}{\text{AUC}} \tag{3}$$

where AUC and MTT are area under the concentration–time profile and mean transit time, respectively. Equations 2 and 3 only deal with characterization of the material associated with transit through the liver, therefore, they apply equally both to eliminated (diclofenac) and noneliminated (albumin) compounds. In contrast, such moments can only be used to calculate the volume of distribution of a fully recovered compound, such as albumin.[8]

For the calculation of distributional volume (V_H) of albumin, the liver was divided into two spaces: a specific arterial space receiving 17% of the arterial flow and a common space receiving all the portal flow and the remaining fraction (83%) of the arterial flow.[8] The volume of distribution associated with each hepatic input were calculated as follows[22]
After the venous administration:

$$V_{PV} = \left[Q_{PV} + 0.83Q_{HA}\right]\text{MTT}_{PV} \tag{4}$$

After the arterial administration:

$$V_{HA} = Q_{HA}\left[\text{MTT}_{HA} - 0.83\text{MTT}_{PV}\right] + V_{PV} \tag{5}$$