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# A Pharmacokinetic Study of Diphenhydramine Transport Across the Blood-Brain Barrier in Adult Sheep: Potential Involvement of a Carrier-Mediated Mechanism

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D) List of Abbreviations:

$AUC_{0\rightarrow\infty}$ – Total area under the plasma concentration versus time curve

$AUC_{CSF\ 0\rightarrow\infty}$ - Total area under the CSF concentration versus time curve

$AUC_{ECF\ 0\rightarrow\infty}$ - Total Area under the ECF concentration versus time curve

$AUC_{CSF\ 0\rightarrow4}$ - Area under the CSF concentration versus time curve from 0 to 4 hours

$AUC_{ECF\ 0\rightarrow4}$ - Area under the ECF concentration versus time curve from 0 to 4 hours

$AUC_{4\rightarrow8}$ – Area under the plasma concentration versus time curve from 4 to 8 hours

$AUC_{CSF\ 4\rightarrow8}$ - Area under the CSF concentration versus time curve from 4 to 8 hours

$AUC_{ECF\ 4\rightarrow8}$ - Area under the ECF concentration versus time curve from 4 to 8 hours

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$\beta$ - Elimination rate constant

$C_{Pt}$ – Total plasma DPHM concentration

$C_{Ptss}$ – Total plasma DPHM concentration at steady state

$C_{Pu}$ – Free plasma DPHM concentration

$[DPHM]_{dialysate}$ – Diphenhydramine concentration in the output dialysate

$f_{CSF}$ – the ratio of $AUC_{CSF\ 0\rightarrow\infty}/AUC_{0\rightarrow\infty}$

$f_{CSF}$ – the ratio of $AUC_{CSF\ 0\rightarrow4}/AUC_{0\rightarrow4}$

$f_{CSF}$ – the ratio of $AUC_{CSF\ 4\rightarrow8}/AUC_{4\rightarrow8}$

$f_{ECF}$ – the ratio of $AUC_{ECF\ 0\rightarrow\infty}/AUC_{0\rightarrow\infty}$

$f_{ECF}$ – the ratio of $AUC_{ECF\ 0\rightarrow4}/AUC_{0\rightarrow4}$

$f_{ECF}$ – the ratio of $AUC_{ECF\ 4\rightarrow8}/AUC_{4\rightarrow8}$

$[^2H_{10}]$-DPHM – Deuterium-labeled diphenhydramine

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**Abstract**

The purpose of this study was to examine the disposition of diphenydramine (DPHM) across the ovine blood-brain barrier (BBB). In 6 adult sheep we characterized the CNS pharmacokinetics of DPHM in brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) using microdialysis in 2 experiments. In the first experiment, DPHM was administered via a 5-step i.v infusion (1.5, 5.5, 9.5, 13.5, and 17.5 μg/kg/min; 7 h per step). Average steady-state CNS/total plasma concentration ratios (i.e. [CNS]/[total plasma]) for steps 1-5 ranged from 0.4-0.5. However, average steady-state [CNS]/[free plasma] ratios ranged from 2-3, suggesting active transport of DPHM into the CNS. Plasma protein binding averaged 86.1±2.3% (mean±SD) and was not altered with increasing drug dose. Plasma, CSF, and ECF demonstrated biexponential pharmacokinetics with terminal elimination half lives ($t_{1/2\beta}$) of 10.8 ± 5.4, 3.6 ± 1.0, and 5.3 ± 4.2 h respectively. The bulk flow of CSF and transport-mediated efflux of DPHM may explain the observed higher CNS clearances. In the second experiment, DPHM was co-administered with propranolol (PRN) to examine its effect on blood-brain CSF and blood-brain ECF DPHM relationships. Plasma total DPHM concentration decreased by 12.8±6.3% during PRN, whereas ECF and CSF concentrations increased (88.1±45.4 and 91.6±34.3%, respectively). This increase may be due to the inhibitory effect of PRN on a transporter-mediated efflux mechanism for DPHM brain elimination.

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The blood-brain barrier (BBB) is composed of several specialized elements which act together to regulate the internal milieu of the brain (Smith, 1989; Farrell and Risau, 1994; Davson and Segal, 1996), by controlling the exchange of compounds between two barrier structures – one located between the blood and brain extracellular fluid (ECF), termed the blood/brain barrier; and the second between the blood and cerebrospinal fluid (CSF), known as blood/CSF barrier. Diphenhydramine, [2-(diphenylmethoxy)-N,N-dimethylethylamine (DPHM)], is a potent histamine $H_1$-receptor antagonist (Douglas, 1980) widely used for its antiallergic properties, as well as for its anti-emetic, sedative, local anaesthetic and hypnotic effects (Runge et al., 1992; Ernst et al., 1993; Pontasch et al., 1993). Like other "first-generation" antihistamines, DPHM occupies central $H_1$-receptors to result in drowsiness, sedation, incoordination and with higher doses, convulsions and death (Douglas, 1980; Nicholson, 1983; Koppel et al., 1987; Gengo et al., 1989). However, there are limited data on the CNS levels of the drug or on the mechanisms of transfer involved. Results from previous studies in rats, guinea pigs, and rabbits suggest that DPHM enters the brain tissue and CSF extremely rapidly to achieve CNS concentrations exceeding those in plasma (Glazko and Dill, 1949; Glazko and Dill, 1949; Takasato et al., 1984; Goldberg et al., 1987). Since only ~2.5% of DPHM (pKa ~ 9.0) is unionized at the physiological pH, the above results cannot be explained by the passive diffusion of this unionized form through the blood-brain and blood-CSF barriers. Moreover, there is evidence for saturable BBB transporter mechanisms for lipophilic, amine drugs (Pardridge et al., 1973; Pardridge et al., 1984; Spector, 1988; Yamazaki et al., 1994; Yamazaki et al., 1994; Yamazaki et al., 1994). This includes mepyramine, a histamine $H_1$-antagonist. The available data suggest that these compounds cross the blood-brain and blood-CSF barriers by both simple diffusion of the

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unionized lipid-soluble form, and by carrier mediated transport of the ionized form (Pardridge et al., 1984; Goldberg et al., 1987; Yamazaki et al., 1994). In addition, there is evidence that various substances can inhibit the actions of this transport process. In vivo (rat carotid injection technique), brain uptake of mepyramine is inhibited by DPHM (Yamazaki et al., 1994). Furthermore, both in vivo and in vitro (bovine brain capillary endothelial cells) studies demonstrate that, propranolol (PRN) inhibits mepyramine uptake (Yamazaki et al., 1994; Yamazaki et al., 1994). Together these data led us to hypothesize that PRN could inhibit brain (and perhaps CSF) DPHM uptake. The purpose of our studies then, was to use in vivo microdialysis (MD) in chronically-instrumented adult ewes to investigate the transport processes of DPHM across the adult ovine blood-brain-barrier employing two different experiments. The first involved stepped infusions of DPHM at five different dosing rates to assess blood-brain CSF and blood-brain ECF drug concentration relationships in relation to variations in drug dose and hence plasma drug levels. The second involved co-administration of DPHM and PRN to examine if PRN alters blood-brain CSF and blood-brain ECF DPHM relationships.

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## Materials and Methods

### Animals and Surgical Preparation

A total of six non-pregnant Dorset Suffolk cross-bred ewes were used in these studies. All studies were approved by the University of British Columbia Animal Care Committee, and the procedures performed on sheep conformed to the guidelines of the Canadian Council on Animal Care. The sheep were between 2-4 years old with a body weight of 74.6 ± 22.0 kg (mean ± S.D.). Surgery was performed aseptically under isoflurane (1-2%) and nitrous oxide (60%) anesthesia (balance $O_2$), after induction with i.v. sodium pentothal (1 g) and intubation of the ewe. Polyvinyl or silicone rubber catheters (Dow Corning Corp., Midland, MI) were implanted in both the carotid artery and jugular vein. In addition, flexible MD probes (CMA 20, Stockholm, Sweden) were implanted in the lateral ventricle and ipsilateral parietal cortex for collection of CSF and ECF respectively. The tubing of the MD probes was tunneled subcutaneously and exteriorized via a small incision on the back of the neck of the ewe. The antibiotics, Trivetrin® (Schering Canada Inc., Pointe Claire, QC, trimethoprim, 180 mg, sulfadoxine, 900 mg) and ampicillin (500 mg), were administered to the ewe on the day of the surgery and for 3 days postoperatively. After surgery, the animals were kept in holding pens with other sheep and were allowed free access to food and water. The ewes were allowed to recover for 3 days before experimentation.

### Experimental Protocols

This was a single-site, non-randomized, open-label, two-period, single sequence study with the stepped infusion experiment followed by the DPHM-PRN co-administration study.

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There was a washout period of at least two days between the study sessions. The details of the two study periods are described below.

**(1) DPHM step-infusions**

The protocol involved bolus i.v. loading doses of DPHM (to hasten the achievement of steady-state), followed by i.v. infusion of the drug using an infusion pump (Model #600-000, Harvard Apparatus Inc., Dover, MA) at 5 different rates, with each infusion rate lasting 7 h. The DPHM loading dose was 0.15 mg/kg and the infusion rates were 1.5, 5.5, 9.5, 13.5 and 17.5 μg/kg/min. During the infusions arterial blood samples (3 ml) were collected hourly. Microdialysis (MD) sampling began at the onset of the infusion. The microdialysis pump (Model PHD2000, Harvard Apparatus Inc., Holliston, MA) infusion rate was 2 μl/min and 60-min cumulative samples of CSF and ECF were collected throughout the duration of the experiment. Both blood and MD samples were collected up to 18 h after the end of the last infusion step.

**(2) Co-administration of DPHM and propranolol (PRN)**

DPHM was infused at rate 4 (i.e. 13.5 μg/kg/min) as described in (1) above for 8 h. After 4 h, PRN was co-infused (1.5 mg/kg loading dose, 20.0 μg/kg/min; (Jones and Ritchie, 1978; Mihaly et al., 1982; Czuba et al., 1988)) for the remaining 4 h of the DPHM infusion. Blood and MD samples were collected as described above during the infusion and for up to 12 h post-infusion.

**Retrodialysis**

MD probe recovery was determined using the retrodialysis technique (De Lange et al., 1998). The MD dialysate (degassed, sterile lactated ringer solution) contained a

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calibrator ([$^{2}H_{10}$]-DPHM) at a concentration of 400.0 ng/mL. The probe recovery rate was determined by comparing the input and output concentrations of the calibrator as follows:

$$\text{Recovery} = \frac{[\text{Calibrator}_{\text{input}}] - [\text{Calibrator}_{\text{output}}]}{[\text{Calibrator}_{\text{input}}]} \qquad \text{(Equation 1)}$$

Free fraction drug concentration ($C_{CSF}$ or $C_{ECF}$) at the MD sampling site = $[DPHM]_{dialysate}$/Recovery Rate.

**Physiological Recording**

Arterial pressure was measured using strain-gauge manometers (Ohmeda Inc, Madison, WI) and heart rates from a cardiotachometer (Astro-Med, West Warwick, RI). All variables were recorded on a Grass K2G polygraph (Astro-Med, West Warwick, RI) and on a computerised data acquisition system (Chart® v4.2, ADInstruments, Grand Junction, CO).

**Plasma Protein Binding of DPHM**

Determination of plasma protein binding/unbound fraction ($C_{Pu}$) of DPHM was achieved using the equilibrium dialysis procedure described by Yoo et al. (Yoo et al., 1990) in the 7 h steady-state plasma sample from each infusion step of the 5-step infusion studies. In the case of the DPHM-PRN co-administration study, a sample collected at 8 h of the infusion was used for measurement.

**Drug Analysis**

The concentrations of DPHM ($C_{Pt}$, $C_{Pu}$, $C_{CSF}$ and $C_{ECF}$) in all samples were measured using a gas chromatographic-mass spectrometric assay capable of simultaneously measuring DPHM and [$^{2}H_{10}$]-DPHM with a limit of quantitation of 2.0 ng/mL (Tonn et al., 1993).

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**Statistical and Data Analysis**

All pharmacokinetics modelling was performed using WinNonlin®, version 1.1 (Scientific Consulting Inc., Apex, NC).

**5 Step Infusion Study**

Volume of distribution (Vd) and total body clearance ($Cl_T$) were calculated using the following respective equations (Gibaldi and Perrier, 1982):

$Vd = \text{Rate of DPHM infusion}/(C_{Ptss} \bullet \beta)$ (Equation 2)

$Cl_T = \text{Rate of DPHM infusion}/C_{Ptss}$ (Equation 3)

Where $Cp_{tss}$ = plasma total steady state DPHM concentration, and $\beta$ = terminal elimination constant.

Data were plotted using Microsoft Excel 2000® (Microsoft Corporation, Mountain View, CA). All data are reported as mean ± S.D. Statistical analyses were performed using JMP IN, version 3.2.1 (SAS, Cary, NC). The significant level was $p<0.05$ in all cases.

**Calculation of $f_{CSF}$ and $f_{ECF}$**

The extent of DPHM transfer into the brain in this study was calculated by relating the CSF and ECF $AUC_{0\rightarrow\infty}$ values to the plasma $AUC_{0\rightarrow\infty}$ value to yield the $f_{CSF}$ and $f_{ECF}$ ratios. Specifically, using $f_{CSF}$ as an example:

$$f_{CSF} = AUC_{CSF\ 0\rightarrow\infty}/AUC_{0\rightarrow\infty} \quad \text{(Equation 4)}$$

The $f_{ECF}$ value was calculated in the same manner using $AUC_{ECF\ 0\rightarrow\infty}$. This method of characterizing drug transfer across the BBB has been used in numerous other MD studies for many different drugs including acetaminophen, atenolol, gabapentin, zidovudine, morphine-6-glucuronide, lamotrigine, phenobaribital, and felbamate (Wang et al., 1993;

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Wong et al., 1993; de Lange et al., 1994; Luer et al., 1999; Bouw et al., 2001; Potschka et al., 2002).

**DPHM-PRN Co-administration**

Vd, $Cl_T$, $f_{CSF}$ and $f_{ECF}$ values in these experiments were estimated using the equations listed above. However, for comparisons of the DPHM alone and DPHM-PRN co-administration periods, DPHM concentrations and AUC values for the periods 0-4 h and 4-8 h, respectively, were used. Thus for example to assess the effect of PRN on the $f_{CSF}$ value, $AUC_{CSF\ 0\rightarrow4}/AUC_{0\rightarrow4}$ was compared with $AUC_{CSF\ 4\rightarrow8}/AUC_{4\rightarrow8}$.

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## Results

### 5-Step Infusion Study

Probe recovery rates ranged from 40-50% in all six animals and the average values for the DPHM (46.1±3.3%) and DPHM-PRN (44.3±3.7%) experiments are not significantly different (Table 1). Also, no significant differences in recovery rates were observed among the five infusion steps for both probes (ECF = 46.3±3.3%; CSF = 45.9±3.3%). The steady-state concentrations of DPHM in plasma, CSF, and ECF increased correspondingly with each infusion step (Table 2). There was no significant difference between the steady-state CSF and ECF concentrations across the 5 steps. This similarity in the two CNS concentrations is especially evident upon examination of Figure 1. Due to the high level of plasma protein binding (86.1 ± 2.3%), the plasma free DPHM concentrations were lower than that in the CSF and ECF. This relationship is demonstrated clearly in Table 3. The steady-state $C_{CSF}/C_{Pt}$ and $C_{ECF}/C_{Pt}$ ratios ranged from 0.4-0.5 whereas the $C_{CSF}/C_{Pu}$ and $C_{ECF}/C_{Pu}$ ratios ranged from 2-3. There was no significant difference between the $C_{CSF}/C_{Pt}$ and $C_{ECF}/C_{Pt}$ ratios and between the $C_{CSF}/C_{Pu}$ and $C_{ECF}/C_{Pu}$ ratios across the 5 steps. Figure 1 depicts the concentration-time relationships for the plasma, CSF and ECF compartments for the 5-step infusion study. All 3 concentrations increased in a linear manner corresponding to the increases in infusion rates. DPHM was present in the CSF and ECF within 15 minutes after starting the infusion, reaching 80-90% of the step 1 steady-state concentration.

The apparent distribution and elimination $t_{1/2}$'s of DPHM were obtained from a two compartment model fitting of the post-infusion data using WinNonlin®. Selection between

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a one or two compartment pharmacokinetic model was based upon the generation of lower AIC (Akaike Information Criterion) values for a two compartment fit of the data. All model fitting was carried out using a weighting factor of 1/predicted $y^2$ since it provides more accurate estimates at lower DPHM concentrations. The drug was extensively distributed in the animals, as shown by the high Vd value (27.9 ± 17.4 L/kg) (Table 6). Two-compartment pharmacokinetics were observed in the DPHM elimination profiles of all 3 fluids, with the CNS compartments declining at the highest rates ($t_{1/2\beta plasma}$ = 10.8 ± 5.4 h; $t_{1/2\beta CSF}$ = 3.6 ±1.0 h; $t_{1/2\beta ECF}$ = 5.3 ± 4.2 h).

**DPHM-Propranolol (PRN) Co-administration Study**

Figure 2 depicts the concentration-time relationships for the plasma, CSF and ECF compartments from the DPHM-PRN co-administration study. Consistent with results from the 5-step infusion study, elimination in all three fluids followed two-compartment pharmacokinetics (Table 6).

Tables 4 and 5 compare the DPHM concentrations in plasma, CSF, and ECF and $C_{CSF}$ and $C_{ECF}$ to $C_{Pu}$ ratios before and after propranolol co-administration. Following PRN administration, $C_{Pt}$ tended to be lower, although this did not achieve statistical significance. However, the percentage decrease in (12.8±6.3%) was significantly different from 0. In contrast $C_{ECF}$ and $C_{CSF}$ concentrations increased during PRN administration and for $C_{CSF}$ the increase was statistically significant (Table 4). For both $C_{ECF}$ and $C_{CSF,}$ the percentage increases (88.1±45.4%and 91.6±34.3%, respectively) were statistically significant. Similar to the 5-step infusion study, the CNS concentrations were higher than the $C_{Pu}$. As shown in Table 5, the $C_{CSF}/C_{pu}$ and $C_{ECF}/C_{pu}$ ratios tended to increase during

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PRN infusion, but because of inter-animal variability the changes were not significant. However, the percentage increases in the ratios were significantly different. The protein binding value with propranolol co-administration was 84.4 ± 10.5%, which was not significantly different from the value obtained in the 5-step study (86.1 ± 2.3%).

Table 6 provides a summary comparing the pharmacokinetic parameters before and after PRN administration. $Cl_T$ tended to be higher with PRN co-administration, while Vd was lower, but theses changes were not statistically significant. The elimination half-life in plasma tended to be lower during PRN and the half-life values in CSF and ECF higher, but none of these changes were statistically significant. The f ratios for both CSF and ECF were significantly increased.

**Physiological Responses**

In the 5-step infusion study, during the administration of infusion steps 4-5, 3 animals showed symptoms of agitation including restlessness, tremor, excessive bleating, and heavy breathing. These symptoms disappeared 1-1.5 h after the end of the infusion. During steps 4 and 5 which involved the highest infusion rates, the mean arterial pressure (110±2.41 mmHg for step 4 and 110±0.98 mmHg for step 5) was not significantly different from the mean baseline value of 111±0.41 mmHg. In terms of mean heart rate, no significant changes from baseline were observed during all 5 infusion steps.

In the DPHM-PRN study, no significant difference was observed in mean arterial pressure after PRN co-infusion (101±1.47 mmHg) compared to DPHM administration alone (102±0.86 mmHg). However, a significant drop in mean heart rate was observed

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with PRN co-administration (77.7±3.47 bpm) compared to DPHM alone (95.5±2.37 bpm) and this fall in heart rate persisted for the full 20 h duration of the experiment (Figure 3).

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**Discussion**

For both the blood/brain and blood/CSF barriers, the most important element is the tight junctions in the brain capillary endothelial cells and in the epithelial cells of the choroid plexus respectively (Saunders et al., 1999). The tight junctions primarily restrict the entry of proteins and other large, hydrophilic molecules into the CNS (Davson and Segal, 1996; Habgood et al., 2000). For lipophilic compounds, not significantly bound to plasma proteins, there is a good correlation between the BBB permeability coefficient and the octanol/water partition coefficient, provided the molecular weight is <400-600 Da (Levin, 1980). However, numerous transporters are present in brain endothelial cells, which transfer substances into the CNS at rates higher than could occur *via* simple diffusion. There is also evidence for saturable transporter mechanisms in the BBB for a number of lipophilic amine drugs including PRN and the histamine $H_1$-antagonist, mepyramine (Pardridge et al., 1984; Yamazaki et al., 1994). There are two possible paths for a substance from the CNS to return to the systemic circulation. One is efflux (transporter-mediated or not) *via* brain or choroidal blood. The second route involves efflux *via* bulk flow of CSF draining into either the lymphatic system or venous blood. (Bradbury et al., 1972). The latter phenomenon is termed the sink effect, whereby the brain concentrations of different compounds under steady-state conditions are different from each other and lower than the unbound concentration in blood (Davson and Segal, 1996), as a consequence of the continuous removal of the substances *via* the CSF.

In this study we applied the microdialysis technique to investigate the blood-brain CSF and blood-brain ECF DPHM relationships in 2 different experiments. In the first experiment, the results from the 5-step infusion showed that brain concentrations increased

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correspondingly to increases in dose, suggesting that the transfer of DPHM into the CNS was a concentration-dependent process. However, considering the high degree of plasma protein binding (86.1±2.3%), the CNS DPHM concentrations were actually higher than the free DPHM concentration in plasma (Tables 2-5). This suggested that the entry of DPHM into the CNS was most likely due to an active transport process since if passive diffusion was the only driving force, then free plasma DPHM concentrations should be comparable to the CNS levels. In fact, our finding indicated that DPHM concentrations were at least two times higher than free plasma concentrations (Table 3). As mentioned earlier, there are data suggesting that lipophilic, amine compounds such as DPHM can cross the blood-brain and blood-CSF barriers by both simple diffusion of the unionized lipid-soluble form and by carrier mediated transport of the ionized form (Pardridge et al., 1984; Goldberg et al., 1987; Yamazaki et al., 1994).

The postulation of an active transport process is supported by the $C_{CSF}/C_{Pu}$ and $C_{ECF}/C_{Pu}$ ratios, both ranging from 2-3 (Table 3). Transfer of DPHM into the CNS was rapid after administration, this is not a surprising observation considering the highly lipophilic nature of this compound (octanol/water partition coefficient 1862; (Douglas, 1980). Other reports also suggested rapid distribution of DPHM into tissues, with the maximum tissue uptake occurring at 1-3 min after i.v. injection (Drach et al., 1970). The close similarities between the two CNS drug concentrations (Table 2, Figure 1) suggest that drug clearances in the choroid plexus and the cerebral cortex are comparable to each other. This observation can further be assessed by comparing the $f_{CSF}$ and $f_{ECF}$ values (0.4±0.2 and 0.4±0.2, respectively), which were not significantly different from each other. Two-compartment pharmacokinetics were observed in the DPHM elimination profiles of

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all three fluids in the 5-step infusion studies, with the CNS compartments declining at the highest rates (Table 6). This was reflected in the half-life ($t_{1/2\beta plasma}$ = 10.8±5.4 *vs* $t_{1/2\beta CSF}$ = 3.6±1.0; $t_{1/2\beta ECF}$ = 5.3±4.2 h) values. The more rapid elimination of DPHM from the brain compared plasma is associated with higher concentrations of the drug in the CNS compared to the unbound plasma concentration (Table 2). This is likely due to active transport of the ionized DPHM into the brain, as discussed previously, so that more of the total circulating concentration of the drug is available to the brain compared to other organs and tissues.

The rapid efflux of DPHM from CNS could be due to the bulk flow of CSF (sink effect) and also involvement of a transporter-mediated efflux mechanism for the drug. The validity of the above assumption can be assessed by examining the results from the DPHM-PRN experiment. The CSF DPHM concentration increased significantly during PRN co-administration and for both CSF and ECF the percentage increase in drug levels was statistically significant (Table 4). In addition, both the $f_{CSF}$ and $f_{ECF}$ increased after the co-administration of propranolol (from 0.40±0.20 and 0.40±0.20 to 0.69±0.03 and 0.95±0.05, respectively), with $f_{ECF}$ being significantly increased. The trend for an increase in the CSF and ECF DPHM elimination half-life following PRN is also consistent with reduced clearance of the drug from the brain. However, these changes were not statistically significant. Although the PRN infusion was not continued into the elimination phase, the persistence of the PRN-elicited bradycardia for the entire duration of the experiment (Figure 3) indicates that there was sufficient PRN still present during the elimination period for this action and thus perhaps also for an effect on DPHM transfer

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across the BBB. Overall the findings suggest lower DPHM clearances from the CNS after PRN co-administration.

As mentioned earlier, besides the bulk flow of CSF, efflux of substances (transporter-mediated or not) can occur via brain or choroidal blood back to the systemic circulation. To date, there is no information showing that PRN lowers CSF formation or its secretion rates, and that it has any interference with the passive diffusion process of substances back to the cerebral circulation. Also, besides a slight decrease in heart rate, PRN causes no systemic or cerebral physiologic changes in sheep (O'Brien et al., 1999). In another study, PRN infusion did not significantly change choroid plexus blood flow in sheep (Townsend et al., 1984). Results from these studies are consistent to our current findings in that only heart rate, but not arterial pressure, was affected by PRN. Therefore, the lowered brain clearances are likely due to lowered rates of efflux of the drug. Propranolol can be involved in this process in one or both of the following manners – by directly inhibiting the efflux mechanism and/or by competing with DPHM for the efflux process. Both of these actions could be responsible for the observed lowered rates of CNS clearances; however, the exact mechanism cannot be elucidated by our current experiments.

In contrast to the situation with DPHM concentrations in CSF and ECF, plasma total DPHM level fell by 12.8% during PRN co-administration. This may have been due to an increase in systemic clearance of the drug, given the trend for an increase in this variable during PRN and for a decrease in $t_{1/2\beta}$ (Table 6). DPHM has a high hepatic extraction in sheep, and thus its hepatic clearance is largely dependent on hepatic blood flow (Kumar et al., 1999). However, PRN has been reported to decrease hepatic blood flow

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in humans (Zoller et al., 1993; Orszulak, 1995); thus the mechanisms involved in the decreased plasma DPHM concentration are unclear.

In summary, using in vivo microdialysis in chronically-instrumented adult ewes, we have demonstrated that DPHM enters the brain rapidly after administration by passive diffusion and an active transfer process. Drug concentrations are markedly higher in the brain relative to unbound plasma levels and this may, in part, explain the significant CNS effects of the drug. DPHM clearances from brain ECF and CSF were similar and faster than plasma clearance, as indicated by the relatively short half-lives in the brain. The rapid efflux of DPHM from the CNS could be due to the bulk flow of CSF (sink effect) and also a transport-mediated efflux mechanism for the drug. The DPHM-PRN co-administration study suggests that PRN inhibits an efflux rather than influx mechanism. This latter finding was rather unexpected and warrants further investigation.

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**Footnotes**

A) Financial support for this work was provided by the Canadian Institutes of Health Research (CIHR). Sam CS Au-Yeung is a recipient of a CIHR Doctoral Fellowship. The current project is a part of Sam CS Au-Yeung's Ph.D thesis at the University of British Columbia, Vancouver, BC, Canada. Part of this work was previously presented as a poster in the 2002 ISSX meeting held in Orlando, FL. Below is the citation of the abstract: Sam CS Au Yeung, Nancy Gruber, K Wayne Riggs, and Dan Rurak (2002). Investigation of the CNS pharmacokinetics of diphenhydramine (DPHM) in sheep using microdialysis. *Drug Metab Rev* 34(1):134.

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**Figure Legends**

**Figure 1.** Plasma, CSF, and ECF DPHM concentrations achieved with the 5-step infusion (loading doses 0.15 mg/kg and the infusion rates 1.5, 5.5, 9.5, 13.5 and 17.5 μg/kg/min). The duration of each infusion step was 7 h. Steady-state was achieved in all fluids by 4 h. Error bars are omitted for clarity.

**Figure 2.** Plasma, CSF, and ECF DPHM concentrations achieved with the DPHM-PRN co-administration study. DPHM was infused for 8 h at 13.5 μg/kg/min and propranolol was co-infused from 4-8 h at 20 μg/kg/min. The ECF curve was based on results from 3 animals due to failure of ECF probes in 3 other animals. Both the plasma and CSF curves represent the results from 6 animals. Error bars are omitted for clarity.

**Figure 3.** Heart rate *vs* time in the DPHM-PRN co-administration study (n=6). The arrow denotes the start of the PRN infusion. *denotes a significant decrease from Pre-PRN heart rate ($p<0.05$). Error bars are omitted for clarity.

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**Table 1.** Summary of microdialysis probe recovery rate in the 5-step infusion and DPHM-PRN co-administration experiments.

| | Step 1 | Step 2 | Step 3 | Step 4 | Step 5 | DPHM-PRN |
|---|---|---|---|---|---|---|
| **CSF[a] Probe (%)** | 46.3±4.0 | 48.0±2.6 | 46.1±3.8 | 45.8±2.7 | 43.3±3.5 | 44.5±4.0 |
| **ECF[a] Probe (%)** | 47.9±4.0 | 47.4±2.7 | 46.2±2.3 | 44.8±3.6 | 45.4±3.9 | 44.0±3.4[b] |

Data are shown as mean ± S.D.

[a] Probe recovery rates were determined using Equation 1.

[b] N=3 due to failure of ECF probes in 3 of the animals. Otherwise, n=6.

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**Table 2**. Steady-State DPHM total plasma ($C_{Pt}$), unbound plasma ($C_{Pu}$), CSF, and ECF concentrations for the 5 Infusion Steps.

| Infusion Step | $C_{Pt}$ (ng/mL)[a] | $C_{Pu}$ (ng/mL)[b] | $C_{CSF}$ (ng/mL)[a] | $C_{ECF}$ (ng/mL)[a] |
|---|---|---|---|---|
| **1** | 62.9±18.8 | 8.7±3.4 | 20.0±4.2* | 20.2±5.0* |
| **2** | 169.3±61.4 | 18.9±7.0 | 54.4±15.9* | 56.7±27.2* |
| **3** | 306.6±108.7 | 40.5±18.0 | 112.8±42.7* | 119.6±68.8* |
| **4** | 453.8±203.0 | 61.8±36.4 | 158.0±58.0* | 165.6±78.8* |
| **5** | 599.3±276.9 | 104.7±45.3 | 221.9±89.0* | 214.0±109.0* |

Data are shown as mean ± S.D. n=7 for $C_{Pt}$ and $C_{Pu}$; n=6 for $C_{CSF}$ and $C_{ECF}$

[a] Average concentration for each step was derived from the last sample of each infusion step. Steady-state is reached at 4 h of each infusion step.

[b] Free plasma concentrations were determined by equilibrium dialysis of the last sample of each infusion step.

*, significantly different from their corresponding $C_{Pu}$ values (one-way ANOVA)

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**Table 3.** Steady-State $C_{CSF}/C_{Plasma}$ and $C_{ECF}/C_{Plasma}$ Ratios for Each Infusion Step.

| Infusion Step | $C_{CSF}/C_{Pt}$ [a] | $C_{CSF}/C_{Pu}$ [b] | $C_{ECF}/C_{Pt}$ [a] | $C_{ECF}/C_{Pu}$ [b] |
|---|---|---|---|---|
| **1** | 0.4±0.2* | 2.3±1.2* | 0.4±0.2* | 2.3±1.5* |
| **2** | 0.4±0.2* | 2.9±2.3* | 0.4±0.2* | 3.0±3.9 |
| **3** | 0.5±0.2* | 2.8±2.4* | 0.4±0.2* | 3.0±3.8 |
| **4** | 0.5±0.3* | 2.6±1.6* | 0.4±0.2* | 2.7±2.2* |
| **5** | 0.5±0.3* | 2.1±2.0* | 0.5±0.2* | 2.0±2.4* |

Data are shown as mean ± S.D. n=6.

[a] The $C_{CSF}/C_{Pt}$ ratios were calculated by dividing the concentrations of the last sample of CSF with the last sample of plasma in each step. The same procedure was applied to the calculation of $C_{ECF}/C_{Pt}$ ratios.

[b] The $C_{CSF}/C_{Pu}$ ratios were calculated by dividing the concentration of the last sample of CSF with the free plasma concentration (determined by equilibrium dialysis) of the last plasma sample in each step. The same procedure was applied to the calculation of $C_{ECF}/C_{Pt}$ ratios.

*, Significantly different from the value of 1 (paired *t* test).

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**Table 4**. Average steady-state DPHM concentrations before and after the co-administration of PRN.

| | $C_{Pt}$ (ng/mL) | $C_{Pu}$ (ng/mL) | $C_{CSF}$ (ng/mL) | $C_{ECF}$ (ng/mL)[d] |
|---|---|---|---|---|
| **DPHM (Pre-PRN)[a]** | 398.0±199.2 | 54.2±16.0[c] | 144.9±62.2 | 198.1±91.7 |
| **DPHM+PRN[b]** | 347.2±150.7 | 54.2±15.8[c] | 277.7±88.6[*] | 372.7±211.9 |
| **Percent Change[e]** | -12.8±6.3%[#] | 0.10±0.03%[#] | 91.6±34.3%[#] | 88.1±45.4%[#] |

Data are shown as mean ± S.D.

[a] Pre-PRN concentrations were determined from samples taken at 4 h (immediately before PRN administration) of the DPHM infusion.

[b] DPHM+PRN concentrations were determined from samples taken at 8 h of the co-administration study (i.e. 4 h after PRN was co-infused).

[c] Free fraction plasma concentrations were determined by performing equilibrium dialysis on the corresponding plasma samples.

[d] n=3 for $C_{ECF}$ due to failure of ECF probes in 3 of the animals. Otherwise, n=6 for $C_{Pt}$, $C_{Pu}$, and $C_{CSF}$.

[e] Percent change in DPHM concentrations after PRN co-administration, with respect to that from DPHM administration alone.

*, significantly different from the Pre-PRN value ( paired *t* test).

#, significantly different from 0 (paired *t* test).

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**Table 5.** Steady-State $C_{CSF}/C_{Pu}$ and $C_{ECF}/C_{Pu}$ ratios before and after the co-administration of propranolol.

| | $C_{CSF}/C_{Pu}$ | $C_{ECF}/C_{Pu}$ [a] |
|---|---|---|
| **DPHM (Pre-PRN)** | 3.6±2.1 | 3.3±1.6 |
| **DPHM+PRN** | 8.0±6.5 | 5.9±3.1 |
| **Percent Change** [b] | 122.2±85.3%* | 78.8±39.8%* |

Data are shown as mean ± S.D.

[a] n=3 for $C_{ECF}/C_{Pu}$ due to failure of ECF probes in 3 of the animals; n=6 for $C_{CSF}/C_{Pu}$.

[b] Percent change in DPHM concentration ratios after PRN co-administration, with respect to that from DPHM administration alone.

*, Significantly different from 0 (paired t-test).

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**Table 6**. Pharmacokinetic parameters for DPHM in plasma CSF, and ECF (n=6 in all cases, except for the ECF concentrations in the DPHM-PRN study, where n=3).

| | **DPHM (0 – 4 h)** | | | **DPHM-PRN (4 – 8 h)** | | |
|---|---|---|---|---|---|---|
| | **Plasma** | **CSF** | **ECF** | **Plasma** | **CSF** | **ECF** |
| **$Cl_T$** (mL/min/kg) | 29.9±9.6 | N/A | N/A | 36.7±10.5 | N/A | N/A |
| **$Vd_{ss}$** (L/kg) | 27.9±17.4 | N/A | N/A | 22.5±13.5 | N/A | N/A |
| **PB** (%)[a] | 83.0±2.2 | N/A | N/A | 84.4±10.5 | N/A | N/A |
| **f ratio** | N/A | 0.40±0.20 | 0.40±0.20 | N/A | 0.69±0.03* | 0.95±0.05* |
| **$t_{1/2\beta}$** (h)[b] | 10.8±5.4 | 3.6±1.0 | 5.3±4.2 | 7.2±3.5 | 4.4±3.4 | 8.3±4.8 |
| **$t_{1/2\alpha}$** (h)[b] | 0.5±0.2 | 0.6±0.2 | 0.5±0.3 | 0.3±0.2 | 0.6±0.3 | 0.9±0.2# |

Data are shown as mean ± S.D.

[a] PB = extent of protein binding.

[b] Model-fitted parameters obtained from the 2 compartment modeling of the 5 step and DPHM-PRN co-infusions.

*, Significantly different from the respective DPHM value alone (unpaired *t* test).

N/A – Data not available.

#, Significantly different from the respective 5-step infusion value (paired t-test).

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Figure 1

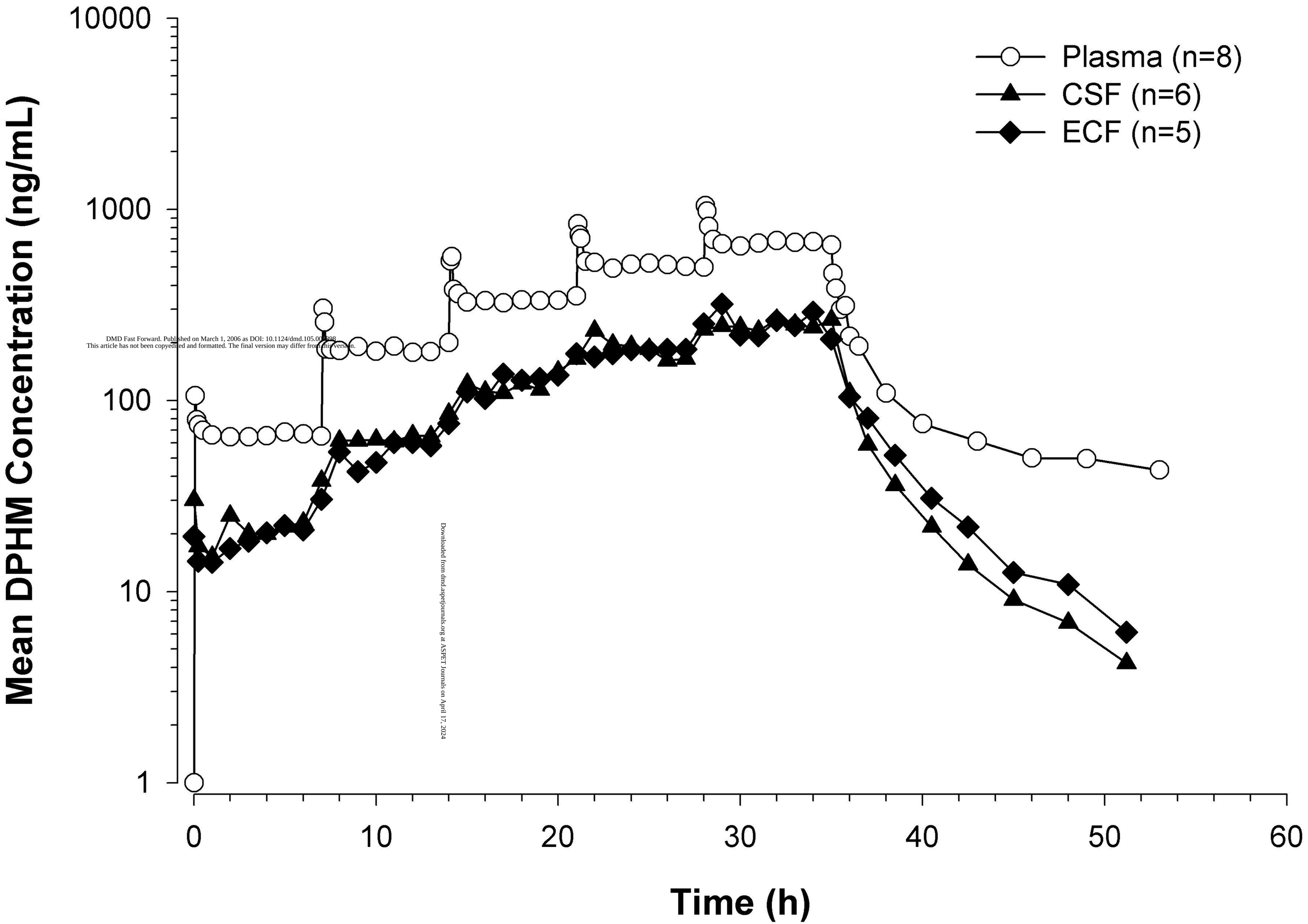


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Figure 2

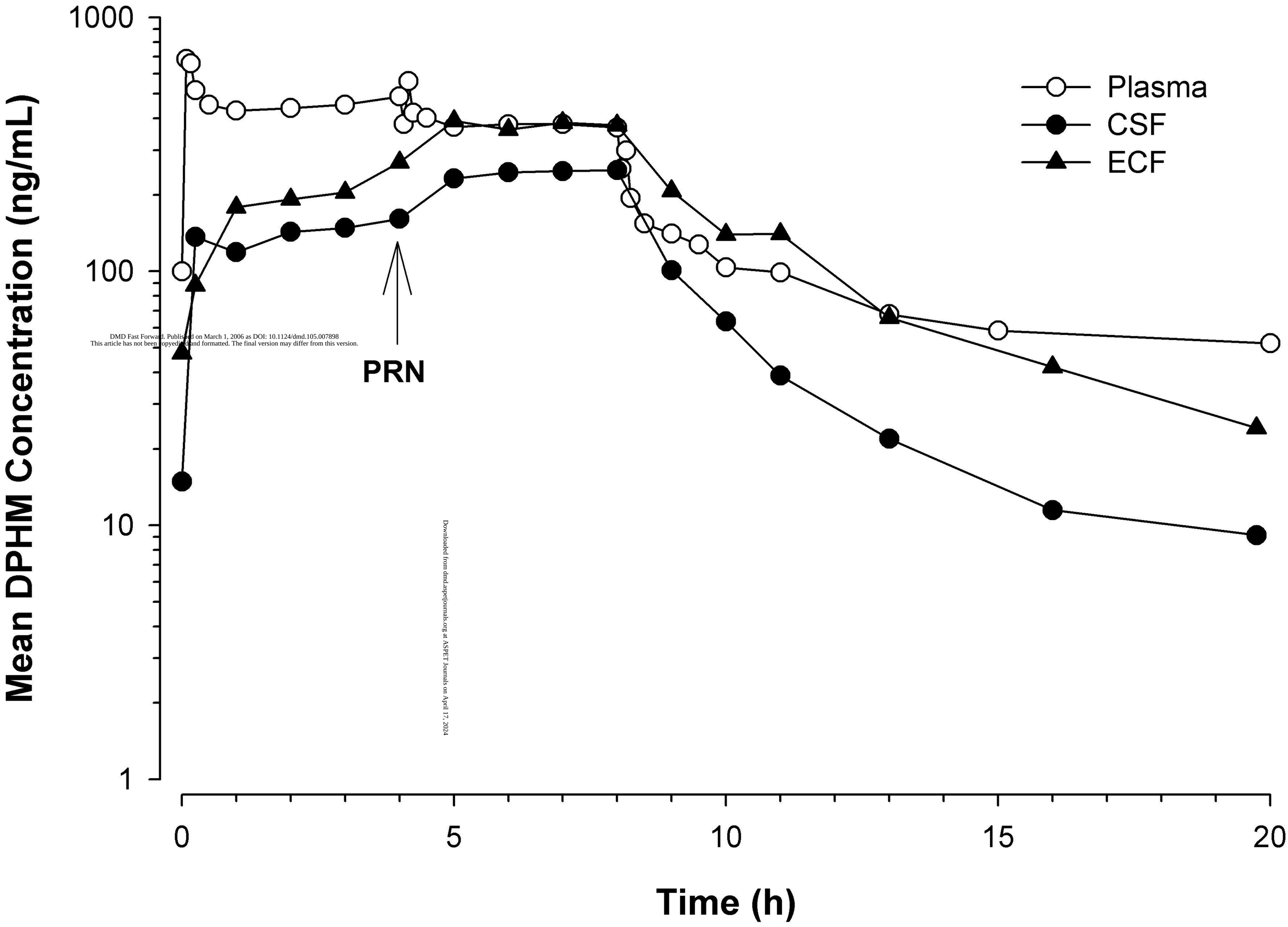


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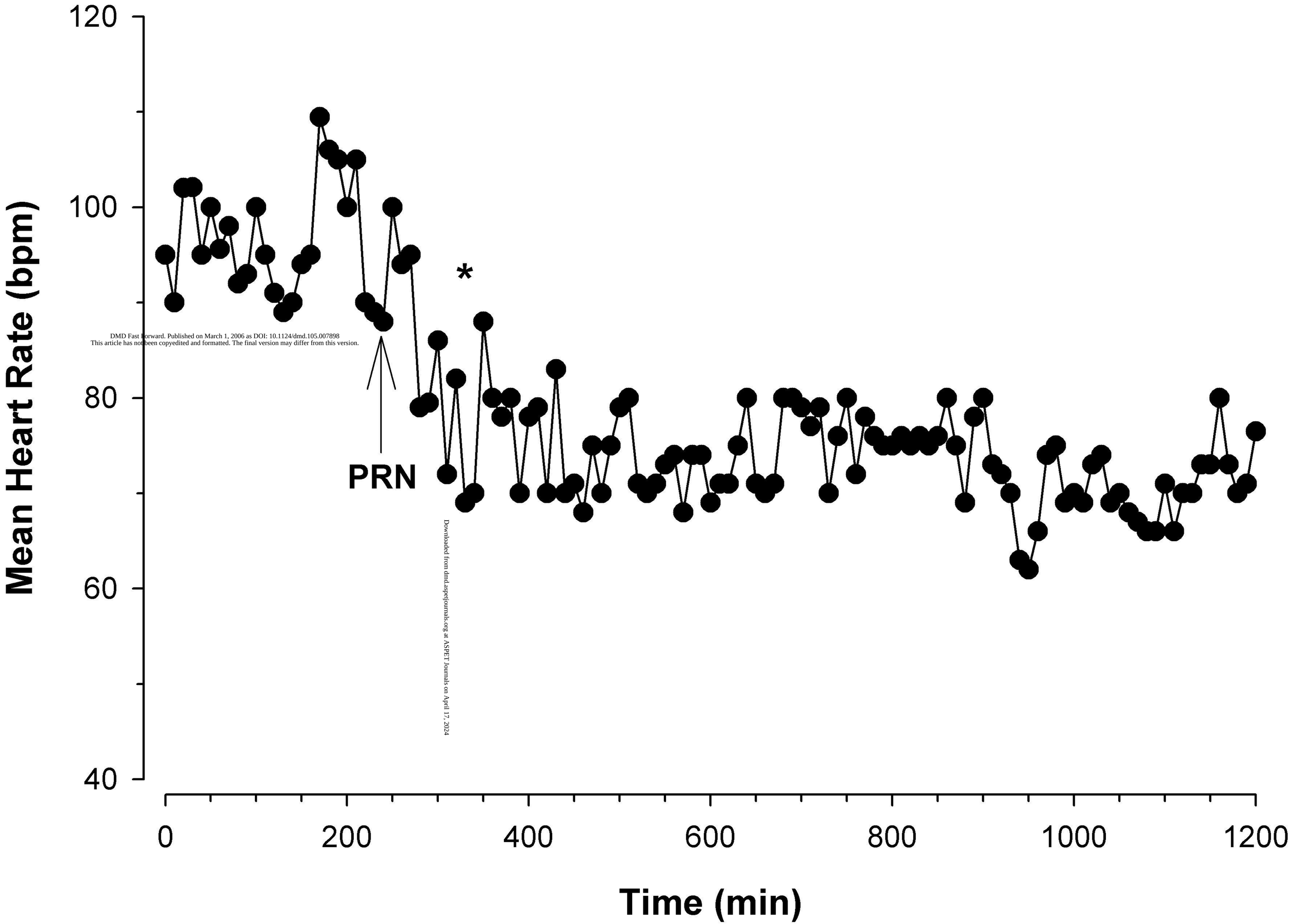
Figure 3

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