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Influence of perfusate composition on drug disposition in the in-situ perfused rat lung

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ABSTRACT

This study compared the impact of two perfusates (A: 4.5% BSA-MOPS buffer and B: 4% dextran and 0.5% BSA-MOPS buffer) on the pharmacokinetics of the physiological markers [^{3}H]-water, [^{14}C]-sucrose, [^{14}C]-antipyrine and Evans Blue-labelled albumin; and the drugs atenolol and propranolol using an in-situ single pass perfusion model in the rat lung. The multiple indicator dilution approach was used to define disposition. Similar perfusion pressures (17.6 ± 6.71 vs 17.7 ± 8.87 cmH_2O), lung wet/dry ratio (6.14 ± 1.16 vs 5.16 ± 0.87), physiological spaces, and permeability-surface area products were found for the two perfusates. However, the recovery of propranolol using perfusate A ($49.3 \pm 10.1\%$) was significantly higher than that using perfusate B ($38.9 \pm 9.91\%$). This difference was consistent with changes in perfusate oncotic pressure associated with water and albumin distribution between the vascular, interstitial and cellular volumes of the lung.

1. Introduction

The perfused lung model has been applied widely in pharmacokinetics to study the pulmonary disposition of drugs. One of the distinguishing features between various studies in the single pass perfused lung is the range of different perfusate compositions used. Currently, the perfusates used include a range of different Kreb–Ringers or modified buffer containing various concentrations of bovine serum albumin (BSA) (ranging 2–8%) and dextran (ranging 2–6%) (Aoyama et al., 2009; Audi et al., 1996; Baker et al., 1999; Bongard et al., 1993; Cremona et al., 1995; Linehan et al., 1989; Weksler et al., 1995). Weksler et al. (1995) advocated 6% buffered hetastarch over saline, low-potassium-dextran, 5% albumin, 6% hetastarch and 5% buffered albumin in rat studies on doxorubicin (as an agent to clear experimental sarcoma lung metastases) because it gave the lowest lung wet to dry ratio. Cremona et al. (1995) examined the exhalation of NO from pig lungs perfused with 3.5% dextran and 0.5% albumin in Kreb–Henseleit buffer. A combination of dextran and BSA has also been used in other organ perfusions, including the kidneys (Jacobsen, 1978; Wang et al., 2004), pancreas (Ostenson et al., 1989) and intestine (Mokuda et al., 1989).

Perfusates have widely differing oncotic pressures, as shown in Table 1. The oncotic pressure exerted by colloid prevents leakage of fluid into the interstitial space by counteracting the intravascular pressure. Little is known about whether different perfusate buffer compositions impact on pulmonary pharmacokinetics. The work of Negrini et al. (2003) suggested that lung tissue has macromolecular sieving properties that exclude albumin from the interstitial space when a hypo-oncotic solution of normal saline is infused intravenously. They suggested that, *in vivo*, the pulmonary intravascular volume decreases during saline infusion; whereas such infusions cause an increase in skin and muscle intravascular volumes. In perfused systems, skin and muscle intravascular volumes were unaffected by hypo-oncotic solutions (Wu et al., 1993). The distribution volume depended on factors like the amount of fluid filtration into the tissue, mechanical tissue compliance and lymphatic fluid drainage, which vary between lung, skin and muscle. Pulmonary vasculature showed both a mechanical and metabolic response to progressive fluid accumulation while skin or muscle did not. Negrini et al. (2003) suggested that pulmonary interstitial albumin decreases after saline infusion, leading to pulmonary interstitial oedema. In our previous work, interstitial volumes were relatively independent of albumin concentrations at low perfusate flow rates; but at higher flow rates, low albumin perfusates raised the interstitial volume (Wu et al., 1993).

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Table 1
Perfusate composition and oncotic pressure for lung perfusion.

Reference	Animal	BSA (%)	Dextran (%)	Oncotic Pressure (mmHg)
Linehan et al. (1989)	Rabbit	5	–	4.10
		–	5	6.76
Sato et al. (1990)	Rat	4.5	–	3.69
Bongard et al. (1993)	Rabbit	–	5	6.76
		5	–	4.10
Cremona et al. (1995)	Pig	0.5	3.5	5.14
Weksler et al. (1995)	Rat	5	–	4.10
Audi et al. (1996)	Rabbit	4.5	–	3.69
Baker et al. (1999)	Rat	4.5	–	3.69

In the present study we sought to compare the effects of two perfusates (A: 4.5% BSA-MOPS buffer and B: 4% dextran and 0.5% BSA-MOPS buffer) on the physiological pharmacokinetics of four reference markers ([^{3}H]-water, [^{14}C]-sucrose, [^{14}C]-antipyrine- and Evans Blue labelled albumin) and two drugs (atenolol and propranolol) in the single pass perfused lung. BSA and dextran are plasma expanders which are commonly used to maintain ontotic pressure of the perfusate. Although the concentration of the solutes in both perfusates is similar (4.5% BSA vs 4% dextran and 0.5% BSA), the oncotic pressure exerted by equal concentrations of dextran and BSA is substantially different (Cross et al., 1996). The two perfusates were therefore chosen to produce highly divergent oncotic pressures.

2. Materials and methods

2.1. Materials

The chemical compounds used in this study (atenolol, D-propranolol, radio-labelled markers and Evans Blue dye) were of analytical grade and were purchased from Sigma-Aldrich (Germany) unless otherwise specified. The perfusate medium (MOPS buffer) used comprised of (in g/L) 6.9 NaCl, 0.35 KCl, 0.16 KH_2PO_4, 0.29 $MgSO_4{\cdot}7H_2O$, 0.37 $CaCl_2{\cdot}2H_2O$, 5.23 MOPS and 2 dextrose (D-(+) glucose). Two different protein regimens were then added: 4.5% bovine serum albumin (BSA) (Thermo, New Zealand) vs 4% dextran (Sigma–Aldrich, St. Louis, USA) and 0.5% BSA. The perfusates were adjusted to pH 7.4 and filtered prior to study.

2.2. In-situ perfusion of the isolated rat lung

An in-situ perfused lung model was established using male Wistar rats weighing 400–450 g. All procedures carried out in this study were approved by the Animal Ethics Committee at the University of Queensland, Australia. Two groups of experiments were performed with 4.5% BSA as perfusate (perfusate A) for one group and 4% dextran plus 0.5% BSA as perfusate (perfusate B) for the other group. The entire procedure was carried out over a heated water tank (60 °C) to maintain the temperature of the perfusion line as well as the rat; we previously found that the temperature of water tank needed to be 60 °C to maintain the temperature of the perfusion line and the rat at 37 °C. The animals were anaesthetized using an intraperitoneal injection of xylazine (10 mg/kg; Bayer, NSW, Australia) and ketamine (100 mg/kg; Parnell Laboratories, NSW, Australia). Tracheotomy was performed to cannulate the trachea and the lungs were mechanically ventilated with medical dry air (95% O_2, 5% CO_2) at 16 strokes/min and about 2 mL in total. The chest was then opened and 1000 IU/kg of heparin was administered into the right ventricle to prevent pulmonary embolism.

The lung was perfused through inflow catheter inserted into the right ventricle and outflow catheter inserted into the left ventricle. A silk ligature was then secured around the aorta to ensure an isolated system. Both insertion sites were sealed using superglue to prevent leakage. The perfusion line was attached to the inflow cannula and the oxygenated perfusates (heated to 37 °C) were delivered at approximately 10 mL/min. The preparation was monitored at regular intervals to confirm a steady flow.

The lung viability was assessed by monitoring macroscopic appearance of the lung at the start of perfusion and throughout the experiment to ensure the lung was properly perfused (no oedema present). At the end of the perfusion, the entire lung was excised from the animal's chest cavity and the wet weight determined. The lung was then placed in an oven at 70 °C for 24 h, after which the dry weight was measured. This allows the wet/dry ratio to be calculated to indicate the extent of fluid exudation in the interstitial cavity. The perfusion pressure (cmH_2O), flow rate and pH of perfusate were also closely monitored in this study. Extra tubing was connected to the inflow catheter and attached vertically against a standard height scale for measurement of the pulmonary resistance pressure.

2.3. Bolus studies

Two 50 μL injectates were prepared for each perfused lung preparation. Injectate 1 consisted of [^{14}C]-sucrose (1.3×10^6 dpm; PerkinElmer), [^{3}H]-water (1.3×10^6 dpm; PerkinElmer) and atenolol (1 mg/mL). Injectate 2 consisted of [^{14}C]-antipyrine (1.0 dpm $\times$ 10^6 dpm; PerkinElmer), Evans Blue (3 mg/mL) and D-propranolol (0.5 mg/mL).

Injectate was administered via the inflow catheter in each perfused lung. Background samples were collected prior to each bolus administration as the control. A 5 min wash out period was employed in between each bolus injection. Outlet samples were collected from the outflow catheter using a computer-operated fraction collector over 210 s.

All the samples were centrifuged and 75 μL of supernatant was then counted for [^{14}C] and [^{3}H] in a Tri-Carb 2700TR scintillation counter (Packard, Meriden, USA). The remaining supernatant was used for determination of the atenolol and propranolol concentrations by HPLC (Hung et al., 2001). The presence of Evans Blue dye was analysed using the Packard SpectraCount. A single aliquot (50 μL) of each outflow perfusate sample was transferred into a 96 well plate for spectrophotometric measurements at a wavelength of 490 nm.

2.4. Perfusion medium binding

The experiments were carried out in 4.5% BSA MOPS buffer (perfusate A) and 4% dextran plus 0.5% BSA MOPS buffer (perfusate B). A solution of each drug (1 mg/mL; atenolol and propranolol) was prepared in either perfusate A or B and incubated in a 37 °C water bath for 30 min. The unbound fraction (f_{uB}) of the drug was investigated by the ultra-filtration method (Hung et al., 2001).

2.5. Lung tissue content of propranolol

To determine potential sequestration of propranolol in the lung after perfusion, the tissue was weighed and homogenised in 5$\times$ its weight of PBS buffer (i.e., 5 mL of PBS to 1 g of lung tissue). A 100 μL aliquot of homogenate was added to 200 μL of 0.1 M $ZnSO_4$ to enable protein precipitation. The sample was then centrifuged at 13,000 rpm for 10 min, and a 50 μL aliquot of the supernatant was directly injected into the HPLC system (Shimadzu, Tokyo, Japan) to determine the concentration of propranolol in the lung tissue (Siebert et al., 2004).

2.6. Data analysis

The raw data collected from liquid scintillation counting, HPLC assays and spectrophotometric analysis were subtracted from the background signals. The recovery value (F) and the mean transit time (MTT) for a given solute were estimated using the following equations.

$$F = \frac{Q\int_0^\infty C\,dt}{\text{Dose}} = \frac{Q\text{AUC}}{\text{Dose}} \quad (1)$$

And

$$\text{MTT} = \frac{\int_0^\infty tC\,dt}{\int_0^\infty C\,dt} = \frac{\text{AUMC}}{\text{AUC}} \quad (2)$$

where AUC is the area under the curve obtained from the outflow profiles and Q is the flow rate (mL/min). In the present study, the AUC was estimated using the trapezoidal rule and appropriate correction for the time period after the last sample.

Empirical concepts of the transit time density also indicate that the MTT for a given non-extracted solute is directly related to its volume of distribution (V_D) and inversely proportional to Q (Eq. (3)). In this study, the normalised variance (CV^2) for a given marker was estimated using Eq. (4).

$$\text{MTT} = \frac{V_D}{Q} \quad \text{Hence; } V_D = \text{MTT} \times Q \quad (3)$$

And

$$CV^2 = \frac{\text{AUMC} \times \text{AUC}}{\text{AUMC}^2} - 1 \quad (4)$$

The vascular and the cellular spaces within the lung were determined from the V_D of sucrose and water. Volume of vascular space equals to $V_{sucrose}$ and volume of cellular space equals to $V_{water} - V_{sucrose}$. The V_D parameters obtained were corrected for the catheters effect.

In addition, a two phase membrane-limited vascular dispersion model (Hung et al., 2001) and non-linear regression of data with a weighting of $1/y$ (Scientist®, Salt Lake City) were used to estimate the pharmacokinetic parameters for atenolol and propranolol in the two systems.

2.7. Statistic analysis

All data are expressed as mean ± standard deviation, unless stated otherwise. Statistical analysis was performed using the unpaired t-test with a p value of <0.05 taken as being significant.

3. Results

Table 2 shows the lung viability measures obtained from continuous monitoring of perfusions conducted using perfusate A and B. No significant differences were found in the macroscopic appearance of lung, oxygen consumption, pH of perfusate, perfusion flow rate, perfusion pressure and the lung wet/dry ratio.

Fig. 1 shows the typical outflow profiles for all the substrates in perfusate A and perfusate B. There is no major difference between the profiles for reference markers (sucrose, albumin, water, antipyrine) or atenolol. However, the profile for propranolol in perfusate B showed delayed elution compared to that in perfusate A.

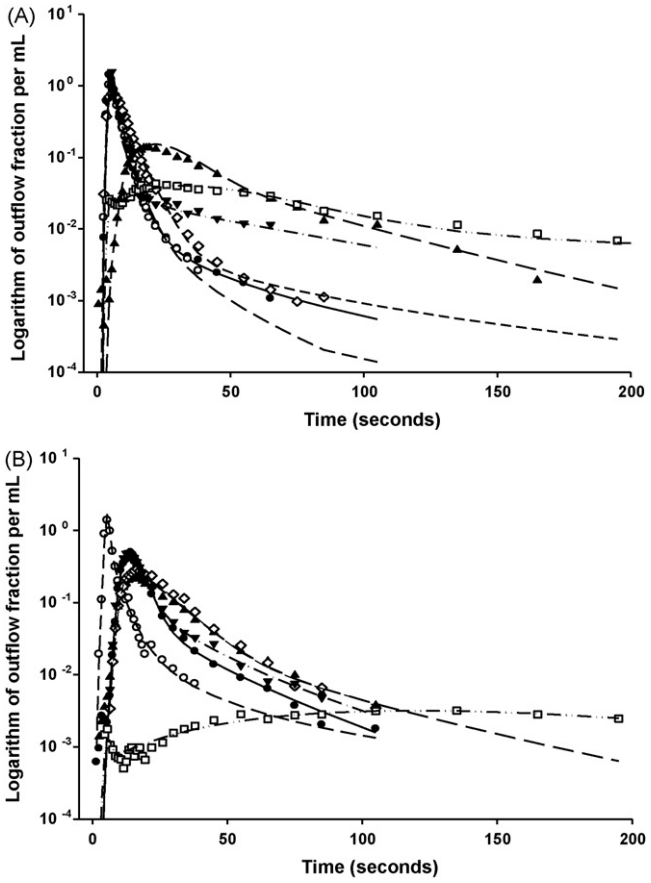

Fig. 1. Typical outflow profiles for reference markers, atenolol and propranolol in (A) 4.5% BSA perfusate and (B) 4% dextran and 0.5% BSA perfusate. ●, [^{14}C]-sucrose; ○, EB-albumin; ▲, [^{3}H]-water; ◊, [^{14}C]-antipyrine; ▼, atenolol; □, propranolol. Lines stand for the fits of the outflow profiles.

Table 3 shows the pharmacokinetic parameters estimated for each of the reference markers and drugs (derived using statistical moments). The vascular spaces, as defined by the marker sucrose, were slightly lower in the perfusate A relative to perfusate B (Table 3), however the difference was not statistically significant. In addition, the relative albumin space ($V_{EB}/V_{sucrose}$) increased by about 10% (from 0.95 for the perfusate B to 1.06 for the perfusate); however, the increase was not statistically significant. The lung tissue distribution volume of both antipyrine ($V_{antipyrine} - V_{sucrose}$) and water ($V_{water} - V_{sucrose}$) was about 40% higher for the perfusate A compared to that for the perfusate B.

There was no difference in the moments data for atenolol and propranolol, except for the recovery of propranolol, which was significantly ($p < 0.05$) lower with perfusate B (38.9 ± 9.91%) as compared with perfusate A (49.3 ± 10.1%). For both perfusates, the

Table 2
Experimental parameters during isolated lung perfusions using 4.5% BSA (BSA) or 4% dextran and 0.5% BSA (Dextran) as the perfusate medium (mean ± SD, $n = 6$).

Perfusate	O_2 consumption (μmol/min/g)	pH[a]	Flow rate (mL/min)	Perfusion pressure (cmH_2O)	Lung wet/dry ratio	Oncotic pressure[b] (mmHg)
BSA	1.27 ± 0.49	7.20 ± 0.02	10.2 ± 0.33	17.6 ± 6.71	6.14 ± 1.16	3.69
Dextran	1.37 ± 0.44	7.18 ± 0.01	10.2 ± 0.42	17.7 ± 8.87	5.16 ± 0.87	5.82

[a] Perfusate solutions were pre-calibrated to pH 7.4 prior to perfusion.
[b] Oncotic pressure (in mmHg) was calculated from the van't Hoff Equation. Therefore, no standard deviation (SD) could be derived.

Table 3
Nonparametric moments for each substrate studied in the isolated perfused lung, using either 4.5% BSA (BSA) or 4% dextran and 0.5% BSA (Dextran) as the perfusate medium (mean ± SD; $n = 6$).

Substrate	Recovery (F, %)		Mean transit time (MTT, s)		Normalized variance (CV^2)		Volume of distribution (V_D, mL)	
	BSA	Dextran	BSA	Dextran	BSA	Dextran	BSA	Dextran
[^{14}C] Sucrose	93.6 ± 8.33	96.8 ± 10.6	5.41 ± 1.17	6.18 ± 1.63	0.71 ± 0.38	0.48 ± 0.16	1.07 ± 0.45	1.16 ± 0.37
EB- Albumin	98.5 ± 12.0	97.3 ± 7.42	6.81 ± 3.58	5.80 ± 1.06	0.51 ± 0.29	0.32 ± 0.16	1.13 ± 0.46	0.99 ± 0.22
[^{3}H] Water	91.3 ± 5.79	96.8 ± 13.9	10.4 ± 2.72	9.13 ± 2.08	0.51 ± 0.15	0.28 ± 0.07	1.75 ± 0.45	1.52 ± 0.35
[^{14}C] Antipyrine	94.2 ± 8.15	92.4 ± 12.1	9.71 ± 1.24	8.29 ± 1.96	0.65 ± 0.22	0.37 ± 0.09	1.62 ± 0.54	1.49 ± 0.33
Atenolol	101 ± 3.59	98.6 ± 7.43	7.18 ± 3.25	5.71 ± 1.92	0.79 ± 0.39	0.37 ± 0.18	1.20 ± ±0.33	1.12 ± 0.56
Propranolol	49.3 ± 10.1 (96.8 ± 7.31)[b]	38.9 ± 9.91[a] (89.3 ± 7.67)[b]	106 ± 31.1	122 ± 29.3	0.35 ± 0.09	0.29 ± 0.11	17.8 ± 5.62	19.3 ± 2.05

[a] $P < 0.05$.
[b] Total recovery (recovery from perfusate plus recovery from lung).

volume of distribution of atenolol was similar with that of sucrose, which may indicate the distribution of atenolol was restricted to the vascular space. However, the V_D of propranolol was close to 20 fold higher than that of atenolol and the other physiological markers. A substantial amount of propranolol (48.5 ± 8.00% of total injection for perfusate A group; and 50.6 ± 6.73% for perfusate B group) was recovered in the lung tissues (as parent compound not metabolites) at the end of the perfusion, emphasising that lungs sequester drugs that pass through it.

As shown in Table 4, the influx (k_{in}) and efflux (k_{out}) rate constants for propranolol were much higher than those for atenolol. This indicated that the lung tissue was more permeable to propranolol compared to atenolol due to the different physicochemical properties of the two drug molecules. However, the difference in pharmacokinetic parameters for propranolol and atenolol obtained from different perfusion systems was not statistically significant.

4. Discussion

An in-situ perfused lung model was used to examine the impact of different perfusate compositions on the pulmonary distribution of atenolol and propranolol. We first assessed the viability of our lung preparations, based on oxygen consumption, pH of the outflow perfusate and the wet/dry lung weight ratio. No significant differences were seen in these parameters for the different perfusate compositions. The values obtained are consistent with those seen in other multiple indicator dilution studies, as is evident in a comparison of the wet/dry weight ratio estimated in these studies for 4.5% BSA (6.14 ± 1.16) compared to that reported by Roerig et al. (1999) (5.91 ± 4.0).

The perfusion flow rate used in this study (10 mL/min) is within the range 5–15 mL/min reported in other studies; it is the most frequently used rate in the isolated perfused lung (Baker et al., 1999; Kuhlmann et al., 2003; Lahnstein et al., 2008; Li et al., 2009; Martinez Martinez et al., 2005; Pang et al., 2005; Reinoso et al., 1998; Reinoso et al., 1999; Roerig et al., 1999; Saldias et al., 1998; Sato et al., 1990; Tronde et al., 2003; Waters et al., 1999; Weksler et al., 1995). Although the flow rate may not directly affect the viability of the preparation, studies have shown that higher flows are associated with an increased risk of interstitial oedema (Weksler et al., 1995).

We found a difference in the distribution kinetics of propranolol in the 4.5% BSA perfusate (perfusate A) compared with the 4% dextran-0.5% BSA perfusate (perfusate B). This result is consistent with the observation that the higher oncotic buffer (perfusate B) led to reduced wet/dry weight ratio in the perfused lung (by 16%; Table 2) and decreased distribution volumes of water and antipyrine (by 40% and 53% respectively; Table 3); relative to perfusate A.

A higher oncotic buffer (perfusate B) would be expected to result in a lower hydration of the lung and a reduction in the physiological water space. Foth et al. (1995) suggested that water molecules are osmotically 'absorbed' into the tissue to achieve equilibrium when a reduced oncotic pressure exists. Consistent with this idea, Weksler et al. (1995) have noted that interstitial oedema occurs when reduced BSA concentrations are used. It is also evident from Table 1 that the two perfusates we used fall within the range of oncotic pressures used in other studies.

The relative albumin space was about 10% more in perfusate A than in perfusate B. Such a finding is consistent with the accumulation of albumin in the interstitial spaces of lungs during perfusion, via transvascular albumin flux (Gerbino and Glenny, 2002). Negrini et al. (2003) has shown that saline infusion corresponding to 20% body weight *in vivo* could lead to an increase in the lung interstitial fluid volume and extravascular albumin distribution volume by 38.5% and 240.2%, respectively. They concluded that, unlike the skin and muscle, the pulmonary interstitium behaves like a restrictive porous sieve with respect to large molecular weight solutes such as albumin.

As perfusate A has a lower oncotic pressure and higher albumin concentration than perfusate B, we predicted that it would have a higher albumin driving force into the lung interstitium and that a higher albumin concentration would be found in the interstitium. However, perfusate flow might have been a confounding parameter that masked the effects of the albumin driving force. Previous work with the perfused rat hind limb suggests that whereas interstitial volumes are relatively independent of perfusate albumin concentrations at low perfusate flows, significant differences are observed at higher flows (Wu et al., 1993). Hence, a key determinant in the lung macromolecular sieving effect described by Negrini et al. (2003) is likely to be the perfusion pressure generated at higher perfusate flow rates.

The higher apparent recovery of propranolol in the presence of BSA (relative to dextran) in our experiments is consistent with propranolol being more highly bound in the BSA perfusate ($f_{uB} = 0.32$;

Table 4
Kinetic parameters derived from the two-phase barrier limited model fitting for atenolol and propranolol in two perfusates [4.5% BSA (BSA) and 4% dextran plus 0.5% BSA (Dextran)] (mean ± SD; $n = 6$, except for $n = 3$ for f_{uB}).

Kinetic parameter	Propranolol		Atenolol	
	BSA	Dextran	BSA	Dextran
k_{in}[a] (s^{-1})	2.03 ± 0.52	1.77 ± 0.77	0.042 ± 0.017	0.023 ± 0.01
k_{out}[b] (s^{-1})	1.85 ± 0.71	0.86 ± 0.36	0.032 ± 0.01	0.015 ± 0.002
k_{on}[c] (s^{-1})	1.54 ± 0.61	0.29 ± 0.14	–	–
k_{off}[d] (s^{-1})	0.004 ± 0.002	0.003 ± 0.001	–	–
k_b[e] (s^{-1})	76.9 ± 28.5	23.0 ± 8.12	–	–
f_{uB}[f]	0.32 ± 0.01	0.63 ± 0.01	0.65 ± 0.01	0.89 ± 0.02

[a] Influx rate constant.
[b] Flux rate constant.
[c] Intracellular binding rate constant.
[d] Intracellular unbinding rate constant.
[e] Equilibrium amount ratio characterizing the rapidly equilibrating binding sites.
[f] Free drug ratio in perfusate.

c.f. dextran $f_{uB} = 0.63$). This would mean that a higher fraction of propanolol was carried through the lung vessels and not extracted over the time course of our study. We analysed propranolol in the lung and found most (more than 95%) of it was parent compound, not metabolite (4-hydroxy propranolol).

The distribution of propranolol in the lung has been examined in patients. Geddes et al. (1979) examined the first-pass uptake of propranolol using a double indicator dilution technique in 10 patients undergoing cardiac catheterization. They found a mean uptake of 75% in seven patients who were not previously taking the drug. We believe that our result, which showed about 50% of propranolol recovered in the lung tissue after the perfusion, is consistent with the finding in patients and indicates substantial sequestration in lung tissue. Rodgers et al. (2005) work with whole rat perfusions also indicated that distribution of lipophilic β-blocker drugs into the lungs greatly exceeded their distribution into other tissues.

The accumulation effect for basic lipophilic amines like propranolol has been described as being due to phospholipid binding via electrostatic interactions and lysosomal sequestration; the latter being well defined for the liver (Hung et al., 2001; Hung et al., 2004; Siebert et al., 2004; Van Dyke et al., 1992). Lysosomes are subcellular organelles that are highly expressed in the cells of the liver and are abundant in the alveolar macrophages of the lung (Rodgers et al., 2005). Trapping of basic metabolites within lysosomes is usually a result of protonation, which subsequently limits the movement of the metabolite via substrate-specific transport pathways in the lysosomal membrane (Ishizaki et al., 1998; Lang et al., 1994; Lloyd, 2000; MacIntyre and Cutler, 1988; Upton and Doolette, 1999). As a result, the extent of exposure is reduced and lower concentrations of solutes are measured in perfusate outflows. The present work suggests that lung albumin (intrinsic and acquired during a perfusion) is also a major binding site for propranolol and thus sequesters the drug in the lung.

The CV^2 represents the vascular dispersion for solutes retained in the vascular space and is increased when a membrane permeability barrier exists. We saw consistently lower values for the physiological markers (i.e., [^{14}C]-sucrose, [^{3}H]-water and [^{14}C]-antipyrine) and for atenolol delivered with perfusate B (compared to delivery with perfusate A). Our physiological values are similar in magnitude to those estimated from other perfused organs that are relatively homogenous, such as the liver (Hung et al., 2001). This contrasts with the high CV^2 observed in the pancreas and the head which are heterogeneous in structure (pancreas 4.21 ± 0.75; head 7.8 ± 2.6) (Fanning and Roberts, 2007; Foster et al., 2000). Shunting of the peripheral vessels as a result of a hypersensitivity reaction (Flaim et al., 1978) would be expected to result in an increased CV^2 for water in the dextran group rather than the decrease (relative to 4.5% BSA) we observed. A large CV^2 has been previously reported in rats and attributed to a possible allergic-type reaction (Flaim et al., 1978; Harris et al., 1967).

The in-situ perfusion model allows the characterization of the kinetic profile of a drug in a single animal, thus avoiding inter-individual variability. Furthermore, the outflow concentration curves obtained can be fitted to physiological or other types of mechanistic models requiring a high number of experimental data points per curve. Interpretation of the values of the model parameters could provide additional information about the intrinsic mechanisms involved in the distribution of the drugs in the lung. It is, however, possible that *in vivo* results differ from those observed in perfused systems as the latter lack local microvascular vasomotion and lymphatic flow. In conclusion, the present study, using an in-situ perfused lung model, has shown that differing perfusate compositions can affect solute and drug sequestration within the lung, as a consequence of oncotic effects on water and albumin fluxes.

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