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# Measurements by microdialysis of free tissue concentrations of propranolol

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ABSTRACT

To determine the free concentration of a drug (propranolol) in the interstitial space in humans *in vivo*, seven male students were investigated by microdialysis of the periumbilical subcutaneous tissue. The microdialysis catheters were calibrated *in vivo* and the propranolol concentration was determined by high-performance liquid chromatography. Ten hours after intake of 80 mg of propranolol, the total plasma and free interstitial propranolol concentrations were $80 \pm 43$ and $7 \pm 2$ n*M*, respectively. After a second dose, maximum concentration was reached after $80 \pm 10$ min and $98 \pm 12$ min, in plasma, and the concentrations in the interstitial water were $594 \pm 138$ and $27 \pm 7$ n*M*, respectively. In a second study, microdialysis was performed on the left ventricular wall in six pigs receiving an intravenous injection of 5 mg of propranolol followed by a constant propranolol infusion for 40 min (5 mg propranolol per h). The maximum concentrations of propranolol were $97 \pm 29$ and $6 \pm 2$ n*M* in plasma and in interstitial water, respectively. The data suggest that microdialysis is a useful tool for recording the free concentrations of a drug in the interstitial space.

## INTRODUCTION

In pharmacological research the effect of a drug is generally related to its concentration in blood or plasma. However, because most drugs bind to the plasma proteins, thereby constituting an inactive pool of uncertain size, a simple method for the measurement of free and active drug concentrations is badly needed. Furthermore drugs targeted for specific organs in the body may have different local tissue concentrations. Measurements of the free local tissue con-

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centrations in the intercellular space of an organ would provide relevant information of the pharmacodynamics and pharmacokinetics at the cellular level.

So far, measurements of tissue concentrations of a drug have been carried out in tissue slices and homogenates as well as in implanted absorbing materials, such as dialysing capsules or sacs. Tissue slices clearly have limitations as well as a disadvantage in the preparation procedure, which is time-consuming and traumatic and cannot easily be used in human studies. Dialysing capsules and sacs can give information about the intercellular water, but, again, their use is traumatic and does not enable precise measurements of rapid pharmacokinetic events. The microdialysis technique, originally used for neurobiological research [1], was recently proposed as a useful tool for pharmacokinetic studies [2]. However, in that study only relative changes in drug concentrations were recorded [2]. We recently described an *in vivo* calibration technique of the microdialysis probe, enabling a very precise estimate of the free concentration of any small molecular substance in the intercellular water [3]. In the present study we have evaluated the ability of this microdialysis technique to measure the actual interstitial concentrations of propranolol *in vivo* in human subcutaneous tissue, as well as in porcine heart.

## EXPERIMENTAL

### *Human study*

Seven healthy male volunteers, aged 29–43 years, were investigated. Each subject gave his informed consent, and the study was approved by the Ethical Committee of the University of Gothenburg.

A single oral dose of 80 mg of propranolol (Inderal®, ICI, Macclesfield, UK) was taken at 10 p.m. After fasting overnight the subjects were investigated in the recumbent position in a room with a standardized temperature of 25°C. An indwelling catheter was placed in the cubital vein. A microdialysis catheter (Cuprophan B4AH, Cobe, Denver, CO, USA, 3000 daltons cut-off, length 20–30 mm, 0.3 mm O.D.) was placed in the subcutaneous tissue 5 cm lateral of the umbilicus with a fine cannula and connected to a precision pump (SAGE Instruments, Boston, MA, USA). After 60 min the microdialysis catheter was perfused with isotonic saline containing different concentrations of propranolol (5–100 n*M*), at a pump-rate of 2.5 μl/min. After the microdialysis probe had been calibrated by the procedure outlined below an additional 80-mg propranolol tablet was taken and the dialysate was collected at 10-min intervals. The propranolol concentration in the interstitial space was compared with the total propranolol concentration in venous plasma measured at the indicated times.

### *Animal study*

Six male pigs weighing *ca.* 25 kg were investigated under full anaesthesia, induced with thiopental (Abbott, IL, USA) followed by a combination of ha-

lothane, nitrous oxide and oxygen (70:30). Pancuronium (Organon Teknika, Boxtel, Netherlands) was used for neuromuscular blockade. The animals were ventilated with a servoventilator (Siemens Elema, Solna, Sweden). After a thoracotomy, the pericardial sac was divided and a microdialysis catheter inserted in the left ventricular wall with a fine cannula. The catheter was perfused as described, and the probe was calibrated. After calibration, a primary dose of 0.5 mg of propranolol was given intravenously followed by a continuous infusion of 0.5 mg propranolol per h for 40 min. The free interstitial myocardial concentration of propranolol was compared with the total propranolol level from an ear-vein. The blood samples were drawn at the indicated times. After completion of the study the animals were killed by injection of a lethal dose of barbiturate.

The animal study was approved by the Ethical Committee of the University of Uppsala.

*In vivo calibration of the microdialysis catheters*

The subcutaneous microdialysis calibration technique was recently described in detail [3]. Briefly, known concentrations of the compound to be measured in the interstitial space are added to the perfusate. In this study, five different concentrations of propranolol (0–100 n*M*) were added to the perfusate, and the net change of the propranolol concentrations was recorded in two dialysate samples at each concentration. A linear relationship was established between the added concentration of propranolol in the perfusate and the concentration change in the dialysate. Hence, the concentration of propranolol in the perfusate equilibrating with the interstitial propranolol concentration could be calculated by regression analysis in each experiment [3]. The regression analyses were performed by the least-squares method, and all correlation coefficients ($r$) were greater than 0.9. This also enabled the correct calculation of the percentage recovery of the interstitial propranolol concentration in the dialysate in each experiment.

*Determination of propranolol*

A Varian Model 5020 equipped with a Valco sample valve with a 10-$\mu$l loop was used for high-performance liquid chromatography (HPLC). A Kratos 980 fluorescence detector was employed with excitation wavelength of 210 nm and emission measured using a cut-off filter at 310 nm. A Rainin $C_{18}$ column (100 mm × 4.6 mm I.D., 3 mm) was used, with a mobile phase of acetonitrile–sodium acetate buffer (40:60, v/v) with 1% acetic acid (pH 3.4). The flow-rate was 1.2 ml/min. Serum samples (100 $\mu$l) were precipitated with 20 $\mu$l of perchloric acid (35%) and 100 $\mu$l of methanol. After mixing for 15 s, the samples were centrifuged. The water phase (25 $\mu$l) from serum or untreated dialysate was injected into the HPLC column. Quantification of propranolol was made relative to an external standard (propranolol hydrochloride from Janssen Chimica, Beerse, Belgium) dissolved in the mobile phase. The recovery of propranolol was greater than 95%. The detection limit for propranolol in dialysate was *ca.* 1 n*M*. The coefficients of intra- and inter-assay variation were 3.0 and 9.2%, respectively.

TABLE I

HUMAN STUDY

Estimation of the initial ($C_0$) and maximal ($C_{max}$) concentrations of propranolol and $t_{max}$ (mean + S.E.M., $n$ = 7).

| | $C_0$ (n*M*) | $C_{max}$ (n*M*) | $t_{max}$ (min) |
|---|---|---|---|
| Interstitial free propranolol | 7 ± 2 | 27 ± 7 | 98 ± 12 |
| Total serum propranolol | 80 ± 43 | 594 ± 138 | 80 ± 10 |

## RESULTS

### *Human study*

As a measure of the reproducibility of the microdialysis technique, the recovery of propranolol in the interstitial space was 50 ± 4% (mean ± S.E.M., $n$ = 7) and 87 ± 3% (mean ± S.E.M., $n$ = 6) in human subcutaneous tissue and porcine heart, respectively. Because serum propranolol could be expected to have a half-life of *ca.* 4 h a concentration change may occur during the *in vivo* calibration procedure (*ca.* 2 h). This was not reflected in the dialysate concentrations in control experiments when repeated calibration was done, indicating that the half-life of propranolol may not be the same in both compartments. Pharmacokinetic data are given in Table I. Twelve hours after a single oral dose of 80 mg of propranolol, the interstitial subcutaneous concentration of free propranolol was 7 ± 2 n*M* (mean ± S.E.M., $n$ = 7), whereas the total plasma concentration was 80 ± 43 n*M*. After the second dose of 80 mg of propranolol, the free interstitial

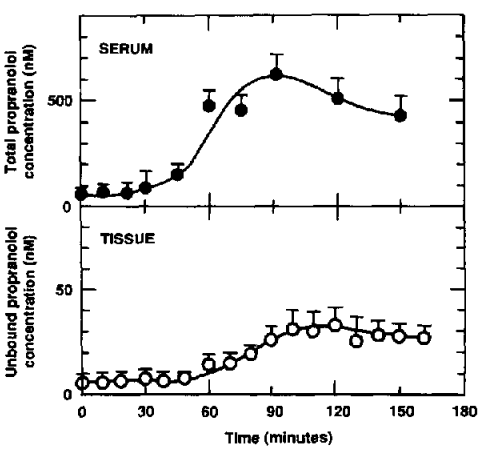


Fig. 1. Total serum (●) and free interstitial (○) concentrations of propranolol in relation to time after the oral administration of 80 mg of propranolol. Data are mean ± S.E. ($n$ = 7).

TABLE II

ANIMAL STUDY

Estimation of the maximal concentration of propranolol ($C_{max}$) and $t_{max}$ (mean + S.E.M., $n = 6$).

| | $C_{max}$ (n$M$) | $t_{max}$ (min) |
|---|---|---|
| Interstitial unbound propranolol | 6 ± 2 | 18 ± 5 |
| Total serum propranolol | 97 ± 29 | 10[a] |

[a] First sample time.

concentration was 27 ± 7 n$M$ and the total plasma level 594 ± 138 n$M$. No significant difference was seen between the two compartments in $t_{max}$ (Table I).

Data from these experiments are depicted in Fig. 1. The propranolol concentrations were not followed for a sufficiently long time period to study the elimination phase.

*Animal study*

Table II shows pharmacokinetic data from the study in porcine myocardium. The highest plasma propranolol concentration was seen in the first sample, drawn 10 min after the injection of the primary dose. No significant delay of $C_{max}$ was seen in the interstitial space. The free propranolol concentration in the tissue averaged 6% of the total concentration in plasma. Fig. 2 shows the variation of the propranolol concentration in both compartments over 120 min.

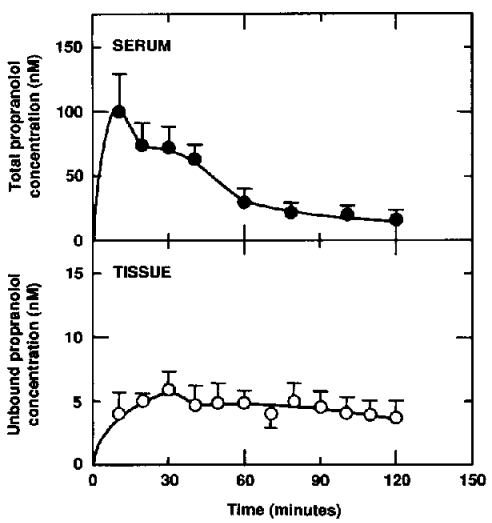


Fig. 2. Mean total serum (●) and myocardial interstitial unbound (○) concentration of propranolol in relation to time after intravenous administration of propranolol. Data are mean ± S.E. ($n = 6$).

## DISCUSSION

The present study clearly shows that the microdialysis technique can be used for recording the free drug concentration in the interstitial space in target organs. Furthermore, the method allows pharmacokinetic variables to be studied concomitantly. The procedure used has recently been validated in a number of studies in humans [3–6]. These studies convincingly show that the technique enables measurements in the interstitial fluid, since the tissue can be drained of glucose with the microdialysis catheter [3]. Also, no major tissue trauma is induced by the catheter because the adenosine concentration in the dialysate is low [5,6]. Furthermore, the rapid concentration change of a low-molecular-mass compound detected in the dialysate following a change in the ambient concentration excludes interference by local concentration changes and oedema [4,6]. Histological examination of tissue microdialysed for 12 h has also shown that the tissue is intact without any majore inflammatory reaction or bleeding adjacent to the microdialysis membrane [7].

The levels of free propranolol in the interstitial water space were 5–9% of the total plasma propranolol concentration in both studies. This is in agreement with previous estimates of the relative binding of propranolol to proteins in serum [8,9]. Since the microdialysis procedure requires fluid-sampling for several minutes (10 min in the present study), a finite delay occurs before an ongoing concentration change is detected. Furthermore, a dialysis sample reflects the average tissue concentration over the sample collection time. Hence, a 12-min delay (collection time plus dead space perfusion period) between changes in the interstitial concentration and that of the dialysate should be expected. The present data thus indicate that the unbound propranolol in plasma equilibrates rapidly with the interstitial fluid. Exact measurements of this equilibration time were not obtained in this study, however, since plasma–protein binding was not determined and only total propranolol concentrations were measured in the plasma.

In the second study, the concentration of propranolol was measured for a sufficiently long period to follow the elimination phase of propranolol in the plasma. It appeared that the initial decrease in the propranolol concentration was delayed in the interstitial space. This may be further investigated in experiments allowing microdialysis measurements during the entire elimination phase in both compartments.

In summary, microdialysis studies in two organs in different species have shown that concentrations of unbound propranolol can be recorded at a target cellular level.

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