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A new method for assessment of drug absorption from muscle: application of a local perfusion system

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Abstract—Drug absorption from muscle has been examined using the rabbit hind leg, isolated by cannulating the femoral artery and vein, and perfused with or without bovine serum albumin (BSA) using a single-pass technique. A model compound, [^{14}C]sucrose, was injected into the musculus gastrocnemius, and its appearance in the venous outflow was monitored and kinetically analysed by statistical moment theory. The fraction absorbed and mean arrival time in the venous outflow were calculated to be 103% and 50·5 min, respectively, in the BSA-containing system. The results of perfusion experiments without BSA were not significantly different, suggesting possible use of the simpler BSA-free perfusion system to obtain information on the muscular absorption of drugs.

In studies to evaluate the absorption process following intramuscular (i.m.) injection, the extent and rate of absorption of a drug is generally analysed using plasma concentration-time curves or from the residual amount at the injection site. However, such methods give only indirect and approximate estimates of drug absorption. More accurate information about absorption behaviour is required to assist the precise design of products for i.m. administration, especially those containing drugs such as peptides which have pharmaceutical disadvantages (e.g. instability) as well as high biological activity. We now describe an experimental system in which a rabbit hind leg is perfused using a single-pass technique and direct absorption behaviour of the drug is determined based on statistical moment theory. [^{14}C]Sucrose as a water-soluble model substance, was used to characterize and evaluate this system.

Materials and methods

[^{14}C(U)]Sucrose was purchased from Daiichi Radioisotopes, Tokyo, Japan. All other chemicals were purchased from Nacalai Tesque Inc., Kyoto, Japan.

Fig. 1 illustrates the experimental system. The rabbit hind leg was perfused as reported previously with slight modifications (Kakutani et al 1985, 1988). Briefly, a male domestic rabbit, 1·8–2·1 kg, fed on a commercial diet, was anaesthetized with urethane (1·5–1·8 g kg^{-1}), and the hind leg isolated from the rest of the circulation by ligating all vessels connected to the leg except for the femoral artery and vein. Heparin sodium (300 units kg^{-1}) was injected into the auricular vein and the femoral artery and vein were rapidly cannulated with vinyl tubing (i.d. 0·8 mm, o.d. 1·2 mm, Dural Plastics, Australia); infusion of perfusate, oxygenated with 95% O_2–5% CO_2 maintained at 37°C, pH 7·4, was begun immediately and maintained with a peristaltic roller pump (SJ-1215, ATTO Co., Tokyo, Japan) at 1·7 mL min^{-1}. After the perfusion was begun the animal received a lethal intravenous injection of aqueous pentobarbitone. Tyrode's solution (mM: NaCl 137, KCl 2·68, $CaCl_2$ 1·80, $NaHCO_3$ 11·9, $NaHPO_4$ 0·362, $MgCl_2$ 0·492, and D-glucose 5·55) with or without bovine serum albumin (BSA) (Fraction V, Nacalai Tesque Inc., Kyoto, Japan; 5% (w/v)) was used as the perfusate. In both systems, good recovery of the perfusate in the venous outflow (more than 93%) was obtained and mixing of blood from other parts of the body was less than 1%. After a stabilization period of 30 min, the absorption experiment was performed.

Phosphate buffer solution (100 μL, pH 7·4) containing [^{14}C]sucrose (3·7 kBq) was injected over 40 s into the centre of the musculus gastrocnemius at a depth of 8 mm using a thin needle (0·4 × 19 mm, NN-2719S, Terumo, Japan). The midpoint time of the injection was recorded as the starting point of sampling. The venous effluent was collected into previously weighed tubes for 120 min, at 30 s intervals initially and at longer intervals in the later stages. The sample volumes were calculated from the sample weights assuming the specific gravity of the perfusate to be 1·0. The sampling time was taken as the midpoint time during the sampling period. The injection needle was withdrawn slowly from muscle 10 s after the injection and the injection site covered with surgical adhesive to avoid fluid leakage. At the end of the absorption experiment, the perfused muscle was excised and the residual amount of [^{14}C]sucrose was determined (Sund & Schou 1964).

Data were analysed in a model-independent fashion based on statistical moment theory (Yamaoka et al 1978; Kakutani et al 1985). The zero, first and second moments for venous appearance curves after i.m. injection are defined as follows:

$$F_a = \int_0^\infty J\,dt \quad (1)$$

$$\bar{t}_a = \int_0^\infty t \cdot J\,dt/F_a \quad (2)$$

$$\sigma_a^2 = \int_0^\infty (t - \bar{t}_a)^2 \cdot J\,dt/F_a \quad (3)$$

where t is the sampling time and J is the absorption rate of the drug at each sampling period as the percentage of the dose absorbed min^{-1}. F_a, $\bar{t}_a$, and σ_a^2 are the fraction absorbed, the mean arrival time to the venous sampling point, and variance of the mean arrival time, respectively. The cumulative fraction absorbed (F_t) and residual fraction (M_t) of the drug in the muscle at each sampling time are calculated as follows:

$$F_t = \int_0^t J\,dt \quad (4)$$

$$M_t = X_0 - F_t \quad (5)$$

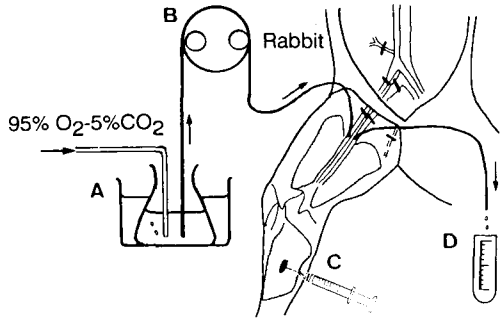

FIG. 1. Experimental system for exploring the absorption of i.m. injected drugs. A, oxygenated perfusate maintained at 37°C; B, peristaltic roller pump; C, injection syringe; D, venous outflow sampling.

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where X_0 is the initial dose (= 100). The mean residence time in the muscle ($\bar{t}_r$) is defined as:

$$\bar{t}_r = \int_0^\infty M_t \, dt / X_0 \quad (6)$$

The dispersion ratio (d_i), a measure of the mixing conditions within the transport process (Kakutani et al 1985, 1990), is calculated as:

$$d_i = \sqrt{\sigma_a^2}/\bar{t}_a \quad (7)$$

The moments are calculated by numerical integration using the trapezoidal rule and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al 1978; Kakutani et al 1985).

The results were statistically analysed by Student's *t*-test. Differences with a P value of less than 0·05 were considered significant.

Results

Fig. 2 illustrates typical venous appearance curves of [^{14}C]sucrose after i.m. injection. The absorption rate of [^{14}C]sucrose via blood capillaries reached a peak at about 10–15 min after the injection, then fell off as described by a monoexponential equation in each experimental system. In the BSA-containing perfusion system, peak time (t_{max}) and maximum absorption rate (J_{max}) of [^{14}C]sucrose in venous effluent was 15·0 min, and 1·58% of dose min^{-1}, respectively. In the BSA-free perfusion system, t_{max} and J_{max} were 12·2 min and 1·79% of dose min^{-1}, respectively. The moments calculated from equation 1–3 are summarized in Table 1, together with the slope of the terminal monoexponential phase (λ_z). The fraction absorbed (F_a) and the mean arrival time at the venous sampling point ($\bar{t}_a$) were 103·4% and 50·5 min, respectively, in the BSA-containing perfusion system and 100·5% and 46·0 min, respectively, in the BSA-free perfusion system. In the BSA-containing and BSA-free perfusion systems, d_i was 0·883 and 0·865, respectively.

Fig. 3 illustrates the calculated muscle clearance profiles of [^{14}C]sucrose and the final residual amounts observed at the end of the experiment. As shown in Table 2, mean residence time ($\bar{t}_r$) was 48·7 min in BSA-containing perfusion system. The results of the BSA-free perfusion system were not significantly different from the BSA-containing perfusion system.

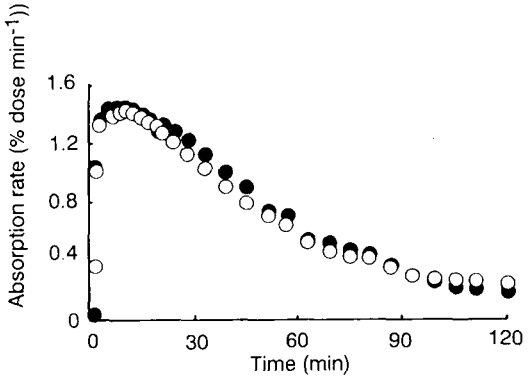

FIG. 2. Typical venous appearance curves of [^{14}C]sucrose in the BSA-containing (●) and in the BSA-free (○) perfusion system after i.m. injection. Each curve was obtained from a single animal.

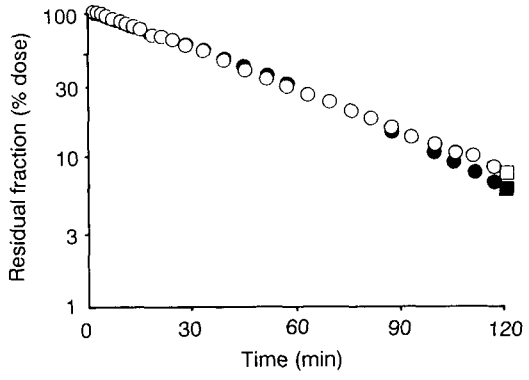

FIG. 3. Semilogarithmic plots of typical muscle clearance curves of [^{14}C]sucrose in the BSA-containing perfusion system (●) and in the BSA-free perfusion system (○) after i.m. injection. Residual fractions of [^{14}C]sucrose observed at the end of the 120 min experiments are also shown both in the BSA-containing perfusion system (■) and in the BSA-free perfusion system (□). Each curve was obtained from a single animal.

Table 2. Mean residence time in the muscle and the slope of the terminal phase for [^{14}C]sucrose derived from muscular clearance curves after i.m. injection.

Perfusion system	$\bar{t}_r$ (min)	λ_z ($\times 10^3$ min^{-1})
With BSA (3)	48·7 ± 2·8	25·3 ± 2·1
Without BSA (5)	50·4 ± 8·8	22·5 ± 6·0

Values given are mean ± s.d. Numbers in parentheses represent the number of experiments for each perfusion system.

Discussion

The aim of this work was to evaluate an experimental system to assess drug absorption from muscle. Conventional absorption studies of i.m. injected drugs through determination of residual drug at the injection site yields only a single datum point for each animal (Schou 1971) and therefore a large number of experiments and statistical evaluation are needed (Schou 1961). In addition, since disappearance from the injection site is deter-

Table 1. Moments and the slope of the terminal phase for [^{14}C]sucrose derived from venous appearance curves after i.m. injection.

Perfusion system	Moments: F_a (% dose)	Moments: $\bar{t}_a$ (min)	Moments: σ_a^2 (min^2)	Slope of the terminal phase λ_2 ($\times 10^3$ min^{-1})
With BSA (3)	103·4 ± 6·5	50·5 ± 4·0	1983 ± 399	23·0 ± 2·0
Without BSA (5)	100·5 ± 13·3	46·0 ± 4·5	1608 ± 455	25·1 ± 4·0

Values given are mean ± s.d. Numbers in parenthesis represent the number of experiments for each perfusion system.

mined as a whole, removal of injected substances via blood flow and lymphatic drainage and further metabolic degradation cannot be separately evaluated.

Drug absorption rate may also be derived from drug blood concentration after i.m. injection by deconvolution analysis of the complex profile of systemic disposition of the drug which includes distribution, metabolism, and excretion; precise separation of the absorption process from other disposition processes is difficult.

The isolated organ perfusion technique has been widely used to explore the physiological and pathophysiological aspects of the organ under study. In contrast to in-vitro methods, organ perfusion systems preserve tissue architecture, cell polarity, and tissue microcirculation, and unlike in-vivo methods, experiments can be done under well controlled conditions independent of the influence of other organs, blood constituents and neural or hormonal effects.

The perfusion system of the rabbit hind limb has been used to study drug transport into the muscle (Kakutani et al 1985, 1988; Conlon et al 1989). We have applied that method to the assessment of drug absorption from muscle since separate factors, physicochemical and physiological, contributing to the drug absorption can be examined, thereby providing information on the absorption mechanism. For drugs metabolized at the injection site, absorption profiles of the parent drugs and their metabolites can be estimated simultaneously.

In this study, [^{14}C]sucrose was chosen as a water-soluble model substance. Sucrose has no electric change and does not show any pharmacological effect, metabolic degradation or protein binding. Consequently, it has been used as an extracellular marker substance (Law 1982) and as a test substance in i.m. injection studies (Sund & Schou 1964). In the system perfused with BSA, the fraction of injected [^{14}C]sucrose recovered in the venous effluent was about 96·0% at the end of the 120 min perfusion, and the residual fraction in the injection site was about 6·0% at the same time. Table 1 shows that the calculated total recovery of [^{14}C]sucrose was almost equal to the injected dose, suggesting complete transfer of this sugar into the vein. These results confirm the validity of the experimental system perfused with BSA-containing medium.

Mean arrival time ($\bar{t}_a$) of [^{14}C]sucrose to the venous side was calculated to be about 50 min (Table 1). This period corresponds to the total time for the absorption, including diffusion in the muscle, passage through the capillary wall, and transport by the blood. The terminal slope of the venous outflow corresponding to the absorption rate constant after achieving the steady-state condition was calculated to be 0·0230 min^{-1} in the BSA-containing perfusion system. The mean weight of perfused muscle was 51·4 g in this perfusion system (Kakutani et al 1985) and perfusion rate was 1·7 mL min^{-1} in this study. However, distribution of sucrose is restricted to the extracellular space which occupies about 10–20% of the total muscle (Law 1982). The observed absorption rate is therefore slower than the calculated blood flow clearance rate constant (0·165–0·33 min^{-1}) and the contribution of the blood to absorption may be small. Heterogeneity of blood flow has been reported in the skeletal muscle (Paradise et al 1971; Iversen et al 1989) and further investigation is required to test these factors.

In this experimental system, accurate information on muscular clearance of an injected substance can be obtained if there are no local degradation and removal routes other than via the blood flow. For [^{14}C]sucrose, therefore, the mean residence time ($\bar{t}_r$) and $\bar{t}_a$, calculated from a muscle clearance profile (Fig. 3) and a venous appearance curve (Fig. 2), respectively, were in good agreement (Table 2).

In tissue perfusion experiments, BSA or dextran is often added to the perfusate to maintain colloidal osmotic pressure. In this study, rabbit hind limb muscles were perfused with or without BSA with no significant differences with respect to either rate or extent of absorption, showing that the effect of colloidal osmotic pressure is relatively small for water soluble low molecular drugs such as sucrose.

The statistical moment analysis was introduced into pharmacokinetics for systemic disposition (Cutler 1978; Yamaoka et al 1978). Moment analysis for a local perfusion system was first applied to investigate drug disposition in rabbit muscle (Kakutani et al 1985), and the wide applicability of this analysis has been shown in various organs such as the liver and in tumour tissue (Atsumi et al 1987; Nishida et al 1989; Ohkouchi et al 1990). In the present study, absorption of [^{14}C]sucrose from muscle was evaluated with respect to rate and extent based on this analysis. Though absorption of i.m. injected drugs involves a series of complex processes, the parameters obtained are free from restriction of the model. Analysis based on pharmacokinetic modeling should also be useful with the venous appearance curves and the muscular clearance curve providing useful input parameters for pharmacokinetic models.

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