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Influence of the injection volume on the release pattern of intramuscularly administered propranolol to rats

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Summary

The aim of this study was to investigate the relationship between the injection volume and the bioavailability of propranolol after i.m. administration. Aqueous solutions of propranolol (3 mg) were administered i.m. in 50, 100 or 200 μl and i.v. (1 mg) in 0.5 ml to rats. Intramuscular injections were given alternately into the left and right hind leg. Blood samples were taken at regular time intervals during a period of 24 h. Propranolol concentrations in plasma were determined by HPLC. Intravenous data were fitted according to either a mono- or a bi-exponential function. The amount of non-absorbed drug remaining at the i.m. injection site was calculated according to the method of deconvolution. Absorption rate constants (k_a) were calculated from the initial phases (0–90 min) of the log fraction of drug not-adsorbed vs time curves. The area under the curve (AUC_{0-t}) to 8 h post-injection was calculated by the linear trapezoidal rule. A Kendall's rank order test ($\alpha = 0.05$) was applied for correlation testing. Intramuscular data showed a biphasic decline of propranolol in the blood; at early times the curves were steep while at later times they became flatter. Mean initial absorption rate constants (±S.D) were 0.12 (±0.02), 0.20 (±0.04) and 0.27 (±0.07) h^{-1}, respectively, and mean AUC_{0-t} per g body weight (±S.D.) after i.m. injection of 50, 100 and 200 μl were 5.3 (±1.2), 7.0 (±1.1) and 9.2 (±1.8) $\mu g\ h\ l^{-1}$, respectively. Propranolol availability after intramuscular injection was incomplete. A positive correlation between either AUC_{0-t} or k_a and the injection volume was established. An increased absorption rate at large injection volumes was shown not to be in accordance with the diffusion-controlled absorption model. This, and the incomplete release after 8 h, could be attributed to the precipitation at or binding of the drug to the injection site. It is concluded that the absorption rate as well as the availability of intramuscularly injected propranolol from aqueous solutions is influenced by the injection volume.

Introduction

Intramuscular injection is an important and frequently used route of drug administration. The rate of absorption from this injection site is a critical step in the availability of a drug. Therefore, the study of the absorption kinetics deserves close attention.

Several factors are known to influence the rate and extent of absorption of intramuscularly injected compounds. Among these are muscle activity, injection site, sex, molecular size, particle size, pK_a, drug solubility, viscosity of the formulation, injection depth, blood supply at the injection site and condition of the tissue at the injection site. A number of reviews on this subject have been published (Bederka et al., 1971; Koch-

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Weser, 1976; Mundie et al., 1988; Tse and Welling, 1989; Zuidema et al., 1990).

In a previous study on the role of drug lipophilicity on the release of intra-adiposely injected β-blocking agents, it appeared that hydrophilic drugs were absorbed both quickly and completely, whereas lipophilic drugs were absorbed slowly and incompletely (Kadir et al., 1990); the adipose layer was suggested to behave like a chromatographic system. Hydrophilic drugs are transported paracellularly with the solvent flow whereas lipophilic drugs are transported mainly transcellularly. Also, after intramuscular injection, drug availability was low in the case of lipophilic compounds. The present study aimed at investigating the relation between intramuscular injection volume and drug availability in rats. The lipophilic β-blocking agent propranolol was used as a model drug. Equivalent amounts of drug were injected intramuscularly in various volumes of aqueous solutions and the effects of the rate and extent of absorption on the blood levels of the drug were studied and compared with data obtained after intravenous injection.

Materials and Methods

Animals

Six male Wistar albino rats weighing 246–278 g were used. The animals were kept in individual cages and provided with food and water ad libitum. They were allowed to move freely. At the start of the experiment the animals were shortly anaesthetized using ether.

Study design

Sterile aqueous solutions of propranolol HCl (gift from ICI-Farma, Rotterdam, The Netherlands; 60, 30 and 15 mg/ml) were prepared for injections. The pH was adjusted to 7.0 using a 0.1 M NaOH solution. The osmolality was measured using a cryoscopic osmometer (Osmomat 030, Gonotec). If necessary NaCl was added to reach iso-osmolality.

Animals were given 3 mg propranolol HCl in 50, 100, and 200 μl of the corresponding stock solutions i.m. and 1 mg propranolol (0.5 ml) i.v. (tail vein) in a cross-over design. The i.m. injections were administered alternately into the right and left hind leg, using a small needle (Terumo, 26 gauge, 0.45 × 12 mm). A wash-out time of 2 days followed.

Blood samples were taken from the tip of the tail into heparinized capillary tubes (1.6 × 75 mm). Blood samples (2 × 100 μl) were withdrawn at regular time intervals at 0, 10, 20, 30, 40, 60, 90 min and 2, 3, 4, 6, 8, and 24 h post-injection. The blood-filled capillaries were centrifuged (3 min, 5500 × *g*, Haemofuge A, Heraeus Sepatech) and haematocrit values were monitored. Immediately after collecting the sample, the plasma was separated from the cell fraction for determination of the propranolol concentrations.

Analysis of blood samples

The concentration of propranolol in plasma was determined by HPLC. Reversed-phase chromatography with fluorimetric detection as described by Duchateau et al. (1986) was performed. The detection limit of this analytical method was 10 ng/ml.

Pharmacokinetic and statistical analysis

The concentration of propranolol in plasma at time points later than 8 h after injection was below the detection limit. Area under the curve values (AUC) up to 8 h after injection were calculated (trapezoidal rule). The bioavailability after i.m. injection was expressed as the ratio between the intramuscular and intravenous AUC.

The intravenous data were fitted with either a one- or a two-exponential equation. The method of deconvolution was used to calculate the amount of propranolol remaining non-absorbed (Vaughan and Dennis, 1978; Wagner, 1978; Tucker, 1983). This amount was expressed as a fraction of the drug administered intravenously. Absorption rate constants were calculated from the log fraction non-absorbed vs time profiles over the range 0–90 min.

Kendall's rank order test for correlation testing was applied. The level of significance chosen was $\alpha = 0.05$.

Results

Fig. 1 shows representative profiles of the concentration of propranolol in plasma vs time obtained after i.m. injection of 50, 100 and 200 μl of

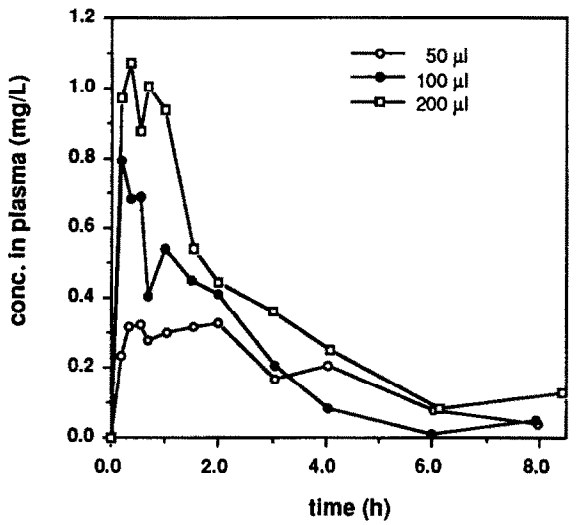

Fig. 1. Representative example of the propranolol concentration in plasma vs time profiles after intramuscular injection of 3 mg propranolol HCl in an aqueous solution in the same rat: 50 μl (○), 100 μl (●) and 200 μl (□).

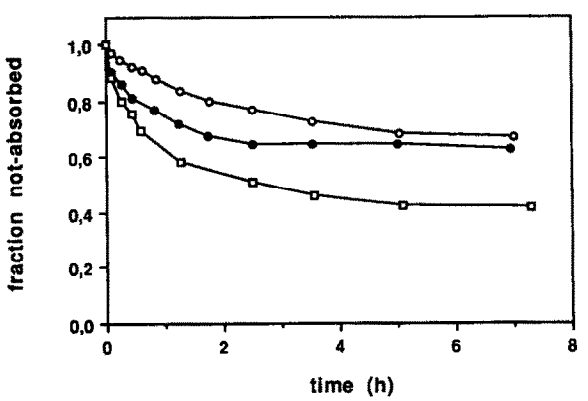

Fig. 2. A representative example of the fraction of non-absorbed drug (calculated according to the method of deconvolution) vs time (the midpoint of two consecutive sampling times) curves after intramuscular injection of 3 mg (propranolol HCl) in 50, 100 and 200 μl aqueous solution in the same rat (same symbols as in Fig. 1).

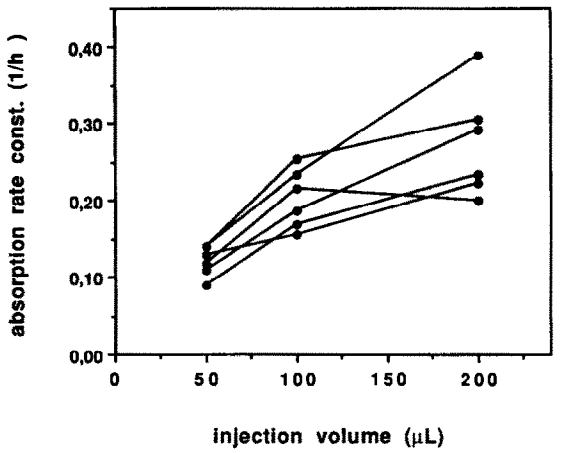

Fig. 3. Individual absorption rate constants after intramuscular injection of 3 mg propranolol HCl in 50, 100 and 200 μl aqueous solution. Lines connect individual values for the same rat.

a propranolol solution in the same rat. Other animals had similar profiles.

The fraction not absorbed after these injections is demonstrated in Fig. 2. It shows a biphasic decline of propranolol in each case: a rapid initial decline is followed by a slow depletion of the drug.

Mean absorption rate constants ($\pm$S.D) of 0.12 ($\pm$0.02), 0.20 ($\pm$0.04) and 0.27 ($\pm$0.07) h^{-1} were

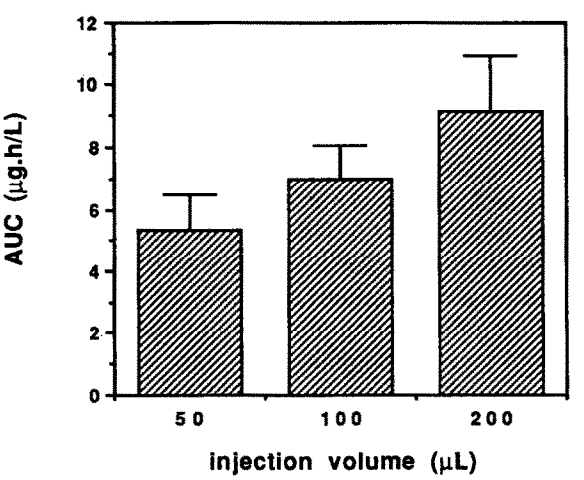

Fig. 4. Mean AUC values following intramuscular injection of 3 mg propranolol HCl in an aqueous solution: 50, 100 and 200 μl, respectively. Bars indicate standard deviation ($n = 6$).

determined for 50, 100 and 200 μl volumes, respectively. The individual absorption rate constants (k_a) at the different injection volumes, calculated from the slope over the first part of the logarithmic curve (extending up to the first 90 min), are depicted in Fig. 3. An increase in the absorption rate constant was found at higher injection volumes.

Mean AUC_{0-8h} values ($\pm$S.D.) of 5.3 ($\pm$1.2), 7.0 ($\pm$1.1) and 9.2 ($\pm$1.8) $\mu g\ h\ l^{-1}\ g^{-1}$ body weight were determined for 50, 100 and 200 μl volumes, respectively. These results are presented in Fig. 4. The calculated absorption rate constant (k_a) as well as the AUC showed a positive correlation (Kendall) with the injection volume.

Discussion

Intramuscular drug absorption is a complex process. Many factors may influence the absorption of an injected compound. Some tend to enhance absorption whereas others have the opposite effect (Groothuis et al., 1980; Cockshott et al., 1982; Al-Hindawi et al., 1987). This study has demonstrated the role of the injection volume in the intramuscular absorption of propranolol, a lipophilic model compound. In a previous study, a biphasic kinetic profile was found after i.m. and intra-adipose injection of a series of β-blocking agents including propranolol (Kadir et al., 1990). Only hydrophilic atenolol was absorbed completely and rapidly. The more lipophilic compounds showed a biphasic absorption profile, i.e., an initial rapid phase followed by a slow phase. The latter was supposed to be responsible for incomplete absorption at the end of the observation period of 24 h. However, subsequent absorption of propranolol from the injection site when plasma concentrations are below detectable levels at later time points cannot be excluded.

As a rule, lipophilic drugs have a high affinity for lipid structures as encountered in cell membranes. They will distribute readily over cells and be transported transcellularly, whereas hydrophilic drugs have a low lipid solubility and, consequently, will be transported at a slow rate across cell membranes (Artursson and Karlsson, 1990). Therefore, the paracellular route is of critical importance for the transport of hydrophilic drugs. The process might be understood as a type of partition chromatography. As influences of solvent supply on the absorption rate are of minor clinical relevance in cases of rapidly and completely absorbed hydrophilic model compounds, this study is focussed on solvent supply effects on the absorption of a lipophilic compound, propranolol.

In this study the fraction of non-absorbed propranolol as a function of time after i.m. injection shows a biphasic decline in all rats, suggesting that at least two different mechanisms are involved in the release from the injection site. The absorption rate constant, k_a, was obtained from the rapid first phase. In the literature, the influence of injection volume on the absorption pattern of drugs has been the subject of investigation only in a few particular cases.

Sodium ions (Warner et al., 1953) and sugars (Sund and Schou, 1964) were found to be absorbed more rapidly when the compounds were administered as smaller injection volumes. However, such compounds are highly water-soluble. On the other hand, Kakemi et al. (1969, 1971, 1972) found that the intramuscular absorption of the hydrophilic compound, isonicotinamide, was independent of the injection volume which ranged from 5 to 20 μl. Another relationship between the injection volume and the absorption rate was found in rabbits which were injected subcutaneously with 0.5, 1.0 and 2.0 ml of a solution of a substituted benzimidazole (Kent et al., 1981). The absorption rate was found to be directly proportional to the volume injected. The authors attributed this deviation from the result predicted by diffusion-controlled absorption (as described by Fick's Law) to the precipitation of the drug at the interface of the injected solution and surrounding tissue. As demonstrated in this study, the same relationship is found in the case of intramuscular injections of propranolol. Apparently, the predicted influence of the concentration decrease is outweighed by the influence of the increased injection volume. The low drug availability 8 h after injection might have been caused by precipitation or binding of the drug at

the injection site. Unfortunately, no excisions of the injection sites were made in order to verify this point.

Several explanations can be put forward for the eliciting of an influence of the injection volume on the absorption rate reported in this study. One possibility is solvent drag during the first absorption phase which dominates the absorption process, whereas passive diffusion of the drug molecules was the driving force for transport during the second phase. Another explanation might be that drug in that portion of the injected solution which has permeated muscle tissue away from the bolus injection site encounters a larger absorbing tissue surface and is, therefore, absorbed rapidly while the drug remaining at the original injection site is absorbed more slowly from a smaller tissue surface.

The substantial solvent drag may change the relative contributions of the different transport routes for the lipophilic test drug as compared to the 'stagnant fluid' situation. As the injection volume increases, the paracellular route becomes of greater importance for the lipophilic drug. This can be attributed to the increased solvent flow between the cells in the direction of the blood circulation or lymph vessels. The portion of the lipophilic drug that will be dragged along with the flow of the vehicle, and thus will be transported paracellularly, will be higher.

To conclude, the results of the experiment performed in this study showed an effect within the volume range tested. Not only the rate of absorption but also the extent of drug absorption of the lipophilic drug propranolol during the first 8 h are affected. A good strategy for optimizing the availability of lipophilic drugs seems to be that of administration in larger volumes. This might be of importance when a rapid onset of drug action is desirable.

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