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Evaluation of intramuscular lateral distribution profile of topically administered acetaminophen in rats

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Abstract

To clarify to what extent topically administered drug molecules horizontally permeate into tissues surrounding the administration site, the intramuscular lateral concentration profile of acetaminophen was investigated *in vivo* using the microdialysis method in rats. When acetaminophen was intramuscularly administered for 6 h in a pinpoint manner at a constant rate of 3 μg/min, it was clearly detected in the muscle surrounding the administration site, being 17.5 μg/ml when measured at a 2 mm distance from the administration site. The concentration in the muscle was decreased as the distance increased, and those measured at 5 mm and 40 mm were 0.35 μg/ml and 0.09 μg/ml, respectively. In addition, it was shown that the concentration in the muscle at 40 mm reflected the compound's concentration in plasma, but not the compound's horizontal permeation from the administration site. With these observations, the intramuscular distribution profile of acetaminophen was numerically characterized according to Fick's law. As a result, it was revealed that horizontal permeation is the primary process accountable for the increased intramuscular concentration only in the area adjacent to the administration site, and the radius of the adjacent area was calculated to be 5.80 mm for acetaminophen.
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1. Introduction

Intramuscular administration is one of the common ways to administer a therapeutic compound to patients parenterally. This is partly because a large enough amount of the compound to exert its therapeutic effects can be readily deposited in muscle with little pain and local irritation, as compared with other manners such as subcutaneous injection (Pratt, 1990). In addition, as the pharmacological effects observed following intramuscular administration are usually exerted more slowly and last longer than those observed following intravenous administration, this administration manner is suitable when patients are unwilling to be frequently injected. It was indicated that the strength and duration of the pharmacological effects primarily depend on the rate and to what extent the administered compound enters the blood stream (Neubig, 1990), and various researchers have revealed that the entering process is dominated by several factors such as the compound's molecular size (Nara et al., 1992a), interstitial diffusion coefficient (Nara et al., 1992b), and muscular blood flow rate (Rowland and Tozer, 1995). However, it has been poorly elucidated whether the administered compound remains in the administration site or if it horizontally permeates from the administration site to the surrounding tissues on entering the blood stream, though such information is indispensable to optimize the design of intramuscularly-embedded drug-releasing devices to guarantee localized pharmacological effects and/or to minimize topical irritations (Maeda et al., 2003a,b).

One of the reasons for the lack of such information is due to difficulties in measuring the drug concentration in muscle *in vivo*. To date, several approaches have been proposed to overcome these difficulties, and among them, an approach using the microdialysis method seems to be the most suitable to evaluate the intramuscular drug concentration *in vivo* (Araki et al., 2002; Marchand et al., 2005). In this approach, a tiny probe

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equipped with a semi-permeable membrane is precisely embedded in muscle. When the lumen of the probe is filled with an isotonic solution, drug molecules, but not water, move through the semi-permeable membrane into the lumen according to the concentration gradient (Chaurasia, 1999). Therefore, the intramuscular drug concentration can be evaluated by collecting luminal specimens and determining their drug concentrations.

In this study, we clarified to what extent the intramuscularly administered compound permeates from the administration site into the surroundings *in vivo* with the microdialysis method in rats. For this purpose, we monitored the intramuscular drug concentration at various distances from the administration site. As a model compound, acetaminophen was chosen because of its well-known characteristics and pharmacokinetics, and it was intramuscularly administered in a pinpoint manner at an administration site in the abdominal muscle. The intramuscular distribution profile of acetaminophen was then numerically analyzed and the extent of the compound's horizontal permeation from the administration site into the surroundings was quantitated.

2. Materials and methods

2.1. Materials

Acetaminophen, or paracetamol (INN), was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ringer's solution was prepared according to the Japanese Pharmacopeia XIV using reagent grade chemicals obtained from local distributors. All other chemicals used in this study were of the finest grade available.

2.2. Animals

Male Wistar rats (260–320 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were housed at 20–25 °C and 40–50% humidity, and were allowed free access to a standard laboratory diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water prior to the experiments. All animal experiments were performed in accordance with the guidelines for animal experimentation of Okayama University.

2.3. Determination of the permeation coefficient of the microdialysis probe

Prior to each animal experiment, the permeation coefficient of the microdialysis probe was determined *in vitro* (Kurosaki et al., 1998). In brief, the microdialysis probe assembly, which was equipped with a semi-permeable membrane (CMA/20, CMA Microdialysis AB, Solna, Sweden), was immersed in 2 μg/ml acetaminophen Ringer's solution. The semi-permeable membrane used was 4 mm in length and 500 μm in diameter, and its permeation threshold was 20,000 Da. The inlet and outlet of the probe assembly were connected to a syringe infusion pump (CMA/102, CMA Microdialysis AB, Solna, Sweden) and a fraction collector (CMA/142, CMA Microdialysis AB, Solna, Sweden), respectively. Then, the probe's lumen began to be perfused with Ringer's solution at a rate of 2 μl/min, and the perfusion effluent from the outlet was collected for 30 min. The collected effluent was used to determine the acetaminophen concentration, as described later. The permeation coefficient was calculated as the concentration ratio between the effluent and the original solution (2 g/ml).

2.4. In vivo evaluation of the intramuscular acetaminophen concentration

After being anesthetized with ethyl carbamate (1 g/kg, i.p.), each rat was fixed on its back and the abdominal skin was gently removed to expose the muscle underneath. Then, three microdialysis probes were embedded in the abdominal muscle. The first probe was used to release the drug solution in the muscle. For this purpose, we employed the retrodialysis method (Westerink and De Vries, 2001), by which it can be achieved to release the drug solution in a pinpoint manner and to keep the drug concentration constant at the administration site. The second and third probes were used to monitor the intramuscular drug concentration. To embed the first probe, the guide cannula with the microdialysis probe was placed at a distance of 20 mm rightward from the midline, and it was inserted 2 mm deep into the abdominal muscle. Then, leaving its outer split tubing in the muscle, the needle of the guide cannula was replaced by the microdialysis probe. After that, by removing the split tubing, the microdialysis probe was firmly placed there. The inlet of the probe assembly was connected to the syringe infusion pump (CMA/102), and the outlet was connected to a drainage bottle. The location where the first probe was embedded is hereafter referred to as the administration site. Then, the second and third probes were embedded in the same manner as the first probe. The second one was embedded at 2 mm, 5 mm, 10 mm, or 15 mm leftward from the administration site. The third one was embedded at 40 mm leftward from the administration site, which was 20 mm leftward from the midline. Each inlet of these two probes was connected to another infusion pump (CMA/102), and each of their outlets was connected to the fraction collector (CMA/142).

Following a 30-min equilibration period, the probes simultaneously began to be perfused at a rate of 2 μl/min. The first probe was perfused with 10 mg/ml acetaminophen Ringer's solution, and the second and third probes were perfused with unmodified Ringer's solution. The collection of the effluents from the second and third probe outlets also began. The effluent was collected every 30 min until 2 h, and every 60 min until 6 h. Acetaminophen in the collected effluents was determined by means of HPLC as described later, and the intramuscular concentration was then calculated taking account of the probe's permeation coefficient that was evaluated prior to the experiment. In addition, a 200-μl blood sample was drawn from the jugular vein at 30, 60, 90, 120, 240, and 360 min during the experimental period. Blood samples were centrifuged, and plasma was collected. The acetaminophen concentration in the plasma specimens was also determined as described later. During the experiment period, the rat was maintained to be anesthetized, and it was kept warm with heating apparatuses.

2.5. Determination of the plasma protein binding ratio of acetaminophen

With freshly prepared rat plasma specimens, acetaminophen plasma solution was prepared at a final concentration of 1, 2, and 5 μg/ml, 400 μl of which was applied to the MPS® micropartition ultrafiltration device (Millipore, Billerica, MA, USA). The unbound fraction of acetaminophen was separated by centrifugation (1500 × *g*, 10 min) at room temperature (Nakayama et al., 1997). The acetaminophen concentration was determined as described later.

2.6. Analytical procedure

To evaluate the relationship between the intramuscular and plasma concentration of acetaminophen, an interpolation of the observed plasma concentration data was carried out using the following equation:

$$C_p(t) = C_{p_{ss}}(1 - e^{-k_e t}) \quad (1)$$

where $C_p(t)$ is the plasma concentration of acetaminophen at time t. The elimination rate constant and the plasma acetaminophen concentration at a steady-state are designated as k_e and $C_{p_{ss}}$, respectively. These two parameters were determined with non-linear least square calculation based on the observed concentration data (Yamaoka et al., 1981).

The intramuscular distribution profile of acetaminophen was characterized in a model-dependent manner with the following equations:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - K(C - f_u C_p(t)) \quad (2)$$

$$C = 0 \quad (t = 0) \quad (3)$$

$$\begin{cases} C = C_0 & (x = 0) \\ \dfrac{\partial C}{\partial x} = 0 & (x = \infty) \end{cases} \quad (4)$$

where C is the intramuscular concentration of acetaminophen at time t and at distance x from the administration site. D is the apparent diffusion coefficient of acetaminophen in muscle and K is the rate constant at which the concentration C changes according to the concentration gradient between muscle and plasma. The plasma concentration of acetaminophen and its plasma unbound fraction are denoted as $C_p(t)$ and f_u, respectively. The parameters D and K were estimated based on the observed intramuscular concentration profiles with non-linear least square calculation utilizing the fast inverse Laplace transform algorithm (Yano et al., 1989).

2.7. Assay method

The acetaminophen concentration was determined by means of HPLC equipped with an octadodecyl silica column (4 μm, 4.6 mm × 150 mm, Synergi Fusion-RP, Phenomenex, Torrance, CA, USA). The collected effluents and the unbound fraction of the acetaminophen were directly applied to HPLC. The plasma specimen was deproteinized before application, in which it was mixed with three volumes of methanol followed by vigorous agitation and centrifugation, and 20 μl of each sample was applied to HPLC. As a mobile phase, 20% methanol–80% phosphate buffer (pH 3.5) was used. The flow rate was set at 1.0 ml/min. Acetaminophen was spectrometrically determined at a wavelength of 245 nm.

2.8. Statistics

Data are shown as the mean ± S.E.M. for three to five experiments. Multiple comparisons against a single control were performed by Dunn's non-parametric test, and $p < 0.05$ was considered to indicate significance.

3. Results and discussion

3.1. Evaluation of intramuscular concentration–time and concentration–distance profiles of acetaminophen in vivo

The permeation coefficient of the microdialysis probe that was determined prior to each experiment was 38.9 ± 3.0% on the average. When the acetaminophen concentration was examined in the muscle surrounding the administration site, it was clearly detected at 2, 5, 10, 15, and 40 mm distance from the administration site (Fig. 1A). At a 2 mm distance, the concentration in the muscle was increased to 17.52 ± 0.95 μg/ml for 75 min, and then it became nearly constant at that value (Fig. 1A). The concentration value at 5 mm varied in a similar manner to that at 2 mm, being increased to a constant level of 0.35 ± 0.01 μg/ml for 105 min. On the other hand, the concentration values at 10, 15, and 40 mm varied in an indistinguishable manner from each other, and they reached a constant level of 0.090–0.098 μg/ml around 150 min (Fig. 1A). This finding indicated that acetaminophen gradually permeates into the surroundings after administration, and thereby the acetaminophen concentration in the muscle is increased, as observed. However, the impact of permeation on the acetaminophen concentration in the muscle tended to become unnoticeable as the measurement distance was increased. This tendency was clearly shown when the concentration–distance profile was examined at the end of the experimental period (Fig. 1B). That is, although the concentration rapidly decreased when the distance was increased from 2 mm to 5 mm, it was not further decreased when the distance was increased from 10 mm to 15 mm or 40 mm. The permeation seemed to influence the acetaminophen concentration in the muscle only in the area adjacent to the administration site.

3.2. Evaluation of the relationship between the intramuscular and plasma acetaminophen concentrations

Since compounds which are introduced into the blood stream distribute to whole tissues including muscle, as reported (Araki et al., 2002), we monitored the change in the unbound concentration of acetaminophen in plasma to examine its relationship to the concentration in muscle. The plasma protein binding ratio

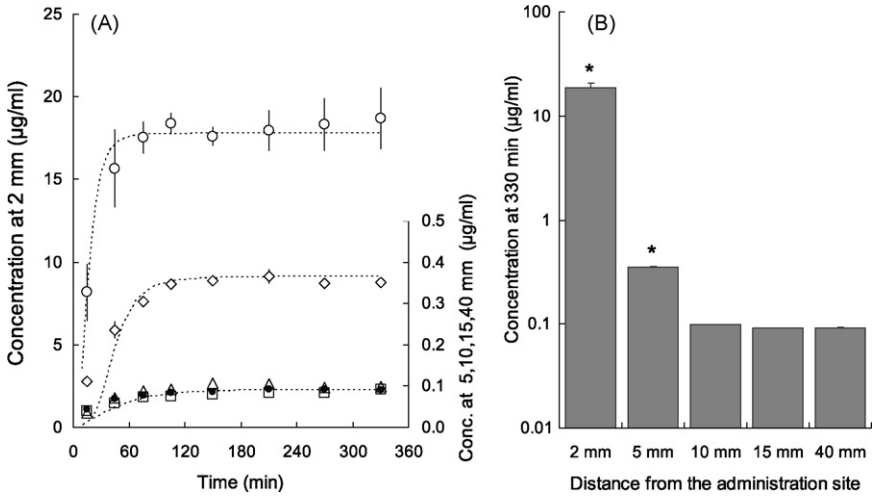

Fig. 1. Intramuscular concentration–time (A) and concentration–distance (B) profiles of acetaminophen in rats. (A) The intramuscular concentration of acetaminophen measured at a 2, 5, 10, 15, and 40 mm distance from the application site are indicated with open circles, open diamonds, open triangles, open squares, and closed circles, respectively. Dotted lines are the best fit lines obtained from numerical analysis of the acetaminophen distribution (see text). (B) The intramuscular concentration values measured at the end of the experiment were compared. Data are shown as the mean ± S.E.M. of three to five experiments. $*p < 0.05$; significantly different from the value at 40 mm.

of acetaminophen was determined to be 33.7 ± 2.3%. During the experiment, the unbound concentration of acetaminophen in plasma was gradually increased (Fig. 2). When the concentration profile in plasma was compared with that in muscle measured at 40 mm, these two profiles were shown to be proportionally related to each other, and the proportional ratio became nearly constant at 0.268 ± 0.001 as the time elapsed (Fig. 2). This finding suggested that the concentration in the muscle becomes to mainly reflect the concentration in plasma as the distance from the administration site is increased.

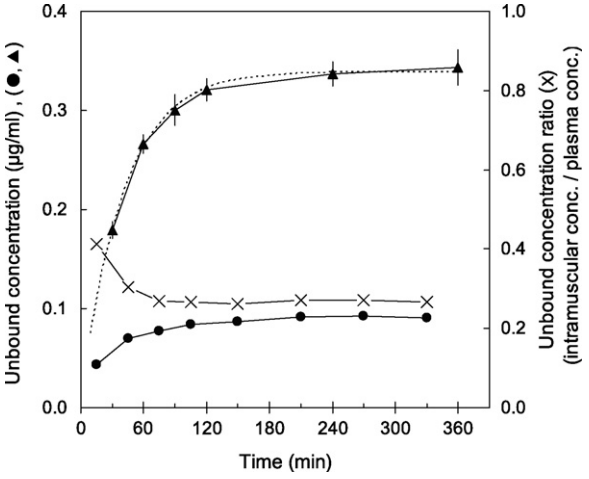

Fig. 2. Relationship between the plasma and intramuscular concentration profiles of acetaminophen. The plasma unbound concentration profile of acetaminophen is shown with closed triangles, and the intramuscular concentration profile measured at 40 mm is shown with closed circles. The concentration ratio is indicated with the symbol '×', which was calculated by dividing the intramuscular concentration by the corresponding value in the unbound plasma concentration profile that was obtained by interpolation. The dotted line is the best fit line calculated to interpolate the plasma concentration profile. Data are shown as the mean ± S.E.M. of four experiments. Some error bars are covered over by symbols.

3.3. Numerical analysis of intramuscular distribution of acetaminophen

To determine to what extent acetaminophen horizontally permeates into the surroundings from the administration site, the concentration profiles in muscle were numerically analyzed, taking account of the influence of the plasma concentration. The observed concentration profiles were well explained with the diffusion equation based on Fick's law (Fig. 1A), and the diffusion coefficient D and the rate constant K were calculated to be $0.0382 \pm 0.0013\ mm^2/min$ and $0.0737 \pm 0.0030\ min^{-1}$, respectively. It was reported that the diffusion coefficient of glucose, which has a similar molecular weight to acetaminophen (180.16 versus 151.17, respectively), is $0.0546\ mm^2/min$ (Pratt, 1990). This value is comparable to that of acetaminophen calculated in this study, suggesting that the intramuscular distribution of therapeutic compounds can be adequately evaluated *in vivo* with our microdialysis approach. However, further investigations may be necessary to precisely determine the diffusion parameters for the intramuscularly administered compounds, taking into account of a variation of the muscle blood flow rate and/or influences of the concentration of the applied compound.

Then, the concentration–distance profile of acetaminophen in muscle was calculated with the determined values of D and K (Fig. 3A). It was indicated that the concentration in muscle was rapidly decreased to 0.36 μg/ml at 5 mm, being 0.12% of the initial value at the administration site (Fig. 3A). To evaluate the extent of horizontal permeation, we defined the adjacent area as that where horizontal permeation from the administration site influences the concentration in the muscle more dominantly than a change in the plasma concentration. Based on this definition, we calculated the distance from the administration site at which the concentration in the muscle was two times higher than the background concentration level calculated from the plasma concentration (0.091 μg/ml). The distance, namely the radius of

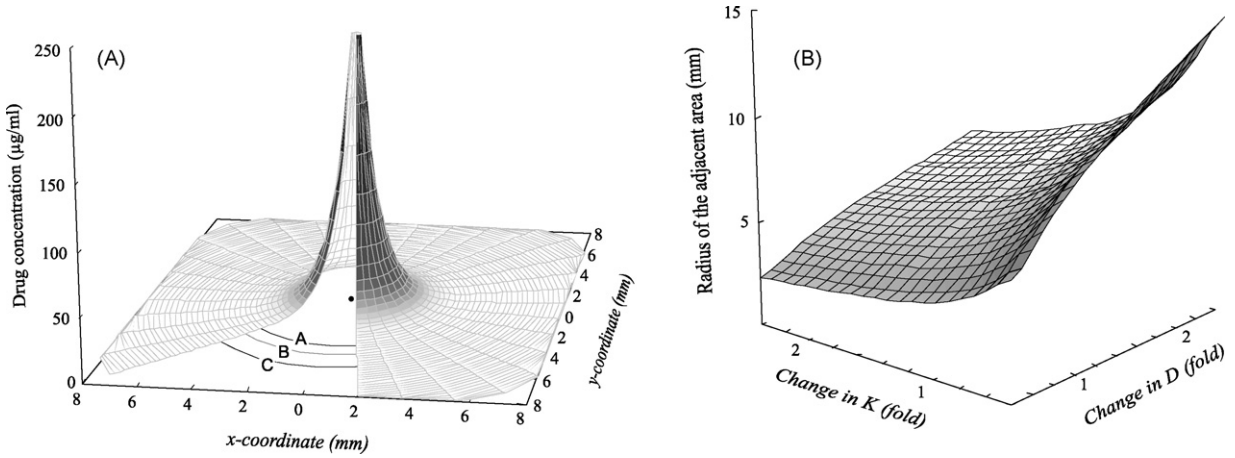

Fig. 3. Numerical characterization of the intramuscular concentration profile of acetaminophen. (A) The intramuscular distribution of acetaminophen at the end of the experimental period was simulated up to 8 mm from the administration site. The administration site is indicated with the symbol '●'. Outline A, B, and C designate the distances at which the acetaminophen concentration is 10, 5, and 2 times higher than the background level (0.091 μg/ml), respectively. The radii of A, B, and C are 4.21 mm, 4.80 mm, and 5.80 mm, respectively. A part of the profile has been removed for explanation. (B) The change in the radius of the outline C is shown. The radius was referred to as the radius of the adjacent area (see text). The calculation was performed with the parameters D and K being changed 0.4–2.4-fold over their original values (0.0382 mm^2/min for D, and 0.0737 min^{-1} for K).

the adjacent area, was determined to be 5.80 mm in the case of acetaminophen (Fig. 3A).

Since the concentration–distance profile varies depending on the apparent diffusion coefficient D and the rate constant K, the change in the radius of the adjacent area was examined with these two parameters being altered. It was indicated that the radius is increased as the parameter D is increased and/or the parameter K is decreased (Fig. 3B). Therefore, when compounds are aimed to be effectively dispersed in the muscle surrounding the administration site, a small compound is more advantageous than a large one from the viewpoint of an increase of the parameter D. It is also preferable to prevent the compound from entering the blood stream because of a decrease of the parameter K. On the other hand, lowering the temperature around the administration site may be preferable to confine the compound within the administration site because the parameter D is decreased, and thus the compound's horizontal permeation is suppressed. These findings provide useful information to increase the efficiency of therapeutic compounds. An increased efficiency of an anti-inflammatory compound which is topically applied to treat muscle pains may be achieved by cooling the application site to confine the compound there, for example. In addition, our approach to evaluate the intramuscular distribution of compounds can be utilized in a process to optimize the design of intramuscularly-embedded drug-releasing devices to minimize their adverse side-effects, in which topical irritations are likely to be reduced by suppressing the compound's horizontal permeation into the muscle surrounding the embedment site.

4. Conclusions

It was demonstrated that acetaminophen horizontally permeated into the muscle surrounding the administration site following its intramuscular administration. In the area adjacent to the administration site, the acetaminophen concentration in the muscle was increased due to horizontal permeation, but it was rapidly decreased as the distance from the administration site was increased. Numerical analysis indicated that the radius of the adjacent area was 5.80 mm for acetaminophen, and the simulation showed that the radius is increased as the compound's diffusion coefficient is increased and/or the rate constant of the compound entering the blood stream is decreased.

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