Pharmacokinetic Estimation of Residual Time at the Injected Muscle after Intramuscular Administration of a Water soluble Drug in Swine

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ABSTRACT. The drug residue at the injected site after intramuscular administration of the water solution of sodium sulfamonemethoxine (SMM, 10 mg/kg) was estimated by means of pharmacokinetic analysis of plasma concentration time data in 10 Goettingen miniature swine. With the pharmacokinetic analysis of the changes in plasma concentration of the drug following intramuscular and intravenous injection, it was found that the drug was completely absorbed from the injection site, i.e. F (bioavailability) was about 1. The overall absorption process obeyed a first order kinetics and the rate constant of absorption (ka) was calculated to be $2.87 \pm 2.14$ (hr$^{-1}$; n=8) by Loo-Riegelman method. Accordingly, the mean value of fraction-unabsorbed at the injection site was expressed as following equation; $1 - (1 - e^{-2.87})$. The ratio of drug concentration at the non-injected muscle to that of plasma during the elimination phase was about $0.428 \pm 0.0331$ (hr$^{-1}$; n=6). Assuming 1 ml of the tissue at the injection site was the minimum distribution volume for 1 ml of the drug solution and employing the lower 95% confidential limit of ka value, a possible highest concentration of the drug at the injection site became identical to that in the non-injected muscle 13.3 hr after the drug injection. On the other hand, an absorption ending time determined by the chemical assay of the muscle samples collected from serially slaughtered animals after intramuscular injection of the drug around 5 hr. Accordingly, in the case of such a water soluble drug as SMM, the time of absorption ending after the intramuscular injection could be predicted by means pharmacokinetic analysis of plasma drug time concentration data conservatively.

In veterinary medicine, an intramuscular injection of drug has been a widely recommended and adopted route. Some drugs injected intramuscularly, however, may remain for a long time at the injection site and become drug residue in an edible tissue [1].

The time when the concentration at the injected site becomes identical with that of non-injected site can be defined as time of absorption ending (AET). The determination of AET has been commonly carried out by a chemical and/or biological determination of the drug residue. This method is, however, time-consuming and costly because a large number of animals has to be killed to determine an exact persistent time [2, 3, 4]. If the drug concentration of injected and non-injected site can be estimated by the plasma kinetic analysis without slaughtering animals, the determination of AET would be greatly simplified and economical. Although there have been several reports on the mathematical approach to this subject [5, 6, 7], none of them described how to estimate the drug concentration at the injected muscle.

In the present paper methods have been described to estimate the drug concentration at the injected and non-injected muscles and to predict the AET by
a pharmacokinetic calculation of plasma concentration time data. Chemical determination of the drug residue at the injected and non-injected muscles was conducted simultaneously and compared with the result of plasma kinetic analysis. A water soluble form of sulfamonomethoxine-Na, one of representative sulfadrug in Japan, was used.

**Materials and Methods**

Ten Gettigen miniature swine, weighing 12–55 kg (6 months—1.7 years old), were used after withholding feed for 24 hrs. Sulfamonomethoxine-Na (SMM, Daimon Soda: Daiichi Seiyaku Co., Ltd, Japan) was used as 20% water solution after it had been confirmed that the SMM was miscible freely without precipitation with a physiological saline. SMM solution (10 mg/kg) was intravenously injected into antetrior vena cava, or intramuscularly in the buttocck or in the lateral neck. Each animal at first received the drug intravenously and about a month later intramuscularly.

Under a light halothane anesthesia, heparinized blood was collected at 5, 15, 30, 45, 60, 90, 120 min, with further 1 hr interval until 8 hr after an intravenous injection, and at 5, 10, 20, 30, 40 min, with further 20 min intervals until 3 hr and 1 hr interval up to 8 hr after an intramuscular injection. Plasma obtained after centrifugation at 3,000 r.p.m. for 15 min was frozen at −20°C until assay.

Eight animals were employed for the plasma kinetic data of both intravenous and intramuscular injections. Six animals out of the 8 and 2 animals without plasma kinetic data were killed to determine the actual drug residue in the injected site at an appropriate time. Evans blue was added to the dosage solution to identify the injected site. The muscles stained by Evans blue as well as those of opposite site (non-injected) were collected and weighed immediately after the animal was slaughtered. They were homogenized and mixed with Polytron (CH-6010 Kriens/Luzern Switzerland) and frozen at −20°C until assay. SMM in the tissue was extracted by NaOH solution, cleaned up by ethylacetate extraction and assayed. Each value was multiplied by the sample weight and the difference of the residual amounts in injected and non-injected sites was divided by the administered dose.

The modified method of Bratton & Marshall [8] was employed to determine the concentration of SMM both in plasma and muscle.

**Analysis of the data**: The time data of plasma concentration of SMM after intravenous injection were regressed to Equation 1. The optimum parameters of the equation, the intercept A and B, and the rate constant α and β were obtained from the calculation by means of nonlinear least square regression program with an iterative computer analysis, using program SALS [9]. The initial estimate for the iterative computer fitting was obtained by the feathering technique.

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (Eq. 1) \]

where \( C_p \) is the plasma concentration of the drug at time \( t \), \( \alpha \) and \( \beta \) are the rate constants of distribution and elimination phases. \( A \) and \( B \) are the zero time intercepts associated with the respective phases, and \( e \) represents the base of the natural logarithm.

The data obtained by an intramuscular injection were regressed to Equation 2.

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} + Pe^{kat} \quad (Eq. 2) \]

where \( k_a \) is the first order rate constant of absorption phase and \( P \) is the zero
time intercept. Absolute P is equaled theoretically the sum of A and B, because the lag time of absorption onset was set up to zero in the present calculation.

The bioavailable fraction (F) was calculated by Equation 3-A.

\[ F = \frac{\text{AUC(i.m.)}}{\text{AUC(i.v.)}} \]  
(Eq. 3-A)

where ACU is the area under concentration time curve. The value of AUC(i.v.) is obtained from the best fitted parameters according to Equation 3-B. The value of AUC(i.m.) is the sum of estimation by trapezoidal rule and the area from the last sampling time to infinity which is the last concentration devided by \( \beta \) (Equation 3-C).

\[ \text{AUC(i.v.)} = A/\alpha + B/\beta \]  
(Eq. 3-B)

\[ \text{AUC(i.m.)} = \int_0^\infty C_p + C_p/\beta \]  
(Eq. 3-C)

The fraction absorbed and the fraction unabsorbed at the injected site of time t were expressed as Equations 4 and 5, respectively. In these calculations the lag time was set up to zero.

Fraction absorbed = \( F(1-e^{-kt}) \)  
(Eq. 4)

Fraction unabsorbed = \( 1 - F(1-e^{-kt}) \)  
(Eq. 5)

For the analysis of data, equation 1 was employed according to Jones [11] and the others (Eq. 2-5) were employed according to Wagner[12].

RESULTS AND DISCUSSION

Pharmacokinetic parameters after intravenous and intramuscular administration of SMM are presented in Table 1. The data of plasma concentration after intravenous injection in 8 pigs were best fitted to the biexponential equation (Eq. 1), and therefore a 2-compartment open model with a linear kinetics was chosen to describe the pharmacokinetics of SMM in the pig.

The value of F (bioavailability) after intramuscular injection was about 1 (0.997±0.180; n=8) which was not significantly different from population mean (1) by Student t’s test (P<0.05).

The time data of plasma concentration were analysed to know the kinetics of absorption process by the method of Loo-Riegelman [10]. The representative 5 examples of this analysis as the semilog plots of ln (Ca*-Ca) versus time were shown in Fig.1, where (Ca*-Ca) represents the concentration of the dose re-

| Table 1. Plasma kinetic parameters after intravenous and intramuscular administration of SMM (8 pigs) |
|----------------------------------|---------------|-----------------|------------|---------------|-----------------|
|                                   | Intravenous administration | Mean ± (SD)     | Intramuscular administration | Mean ± (SD)  |
| Parameter (unit)                 | A (μg/ml)      | 30.6(7.22)      | A (μg/ml)      | 7.94(18.2)  |
|                                  | α (hr⁻¹)       | 7.10(4.32)      | α (hr⁻¹)       | 6.88(4.06)  |
|                                  | B (μg/ml)      | 31.7(6.60)      | B (μg/ml)      | 32.9(6.52)  |
|                                  | β (hr⁻¹)       | 0.241(0.0352)   | β (hr⁻¹)       | 0.241(0.0352)|
|                                  | k12 (hr⁻¹)     | 3.25(2.29)      | k12 (hr⁻¹)     | 24.9(16.4)  |
|                                  | k21 (hr⁻¹)     | 3.65(1.99)      | k21 (hr⁻¹)     | 2.87(2.14)  |
|                                  | kel (hr⁻¹)     | 0.445(0.0857)   | kel (hr⁻¹)     | 141(73.9)   |
|                                  | AUC (μg·hr/ml) | 145(37.9)       | AUC (μg·hr/ml) | 141(73.9)   |
|                                  | V1 (litre/kg)  | 0.171(0.0395)   | V1 (litre/kg)  | 0.997(0.180) |
|                                  | Vdarea (litre/kg) | 0.313(0.0663)  | Vdarea (litre/kg) | 0.313(0.0663) |
|                                  | TBCL (litr·hr/kg) | 0.0732(0.0161) | TBCL (litr·hr/kg) | 0.0732(0.0161) |

For symbols of parameter unit, see text and Fig. 2.
maining to be absorbed at the injected site. Several plots after the drug injection appeared to be straightly arranged and the monoexponential decline of the drug concentration at the injected site could be estimated in all the cases, suggesting that the overall absorption process would be the first order kinetics. The monoexponential decline represents the absorption rate constant $ka$, the calculated value of which was $2.87 \pm 2.17$ in 8 experiments (Table 1). Fig. 2 indicates the schematic mobilization of SMM after the intramuscular injection in the body.

Practically, with the chemical determination of the drug residue in the muscle, the real time of absorption ending was revealed between 5 and 7 hours after the injection, when there was no significant difference between the drug concentrations of injected and non-injected muscles collected from pigs I.D. YMI, B, Z and YJ. In the Table 2, values of the fraction unabsorbed (residual rate) were presented as a percent of administered dose. When the values of residual rate were serially plotted on the time scale, they went well into the estimated curve introduced from equation 5 with the mean value of absorption rate constant (2.87 hr$^{-1}$) as shown in Fig.3. The result suggested that the time curve of unabsorbed fraction at the injected site could be reasonably calculated from time data of plasma concentration using Equation 5.

Table 2 also shows that the ratio of drug concentration at the non-injected...
Table 2. Chemical determination of drug residue at injected and non-injected muscles

<table>
<thead>
<tr>
<th>Fig. ID</th>
<th>J</th>
<th>Y</th>
<th>K</th>
<th>A</th>
<th>YM1</th>
<th>B</th>
<th>Z</th>
<th>YJ</th>
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<tbody>
<tr>
<td>Slaughtered time (hr)</td>
<td>0.33</td>
<td>0.33</td>
<td>1.56</td>
<td>2.53</td>
<td>4.98</td>
<td>5.15</td>
<td>7.99</td>
<td>8.03</td>
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<tr>
<td>Injected muscle sample weight (g)</td>
<td>210</td>
<td>152</td>
<td>300</td>
<td>350</td>
<td>72</td>
<td>216</td>
<td>201</td>
<td>109</td>
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<tr>
<td>concentration (µg/g)</td>
<td>2550</td>
<td>194</td>
<td>32.0</td>
<td>71.7</td>
<td>7.40</td>
<td>92.1</td>
<td>1.66</td>
<td>2.01</td>
</tr>
<tr>
<td>Non-injected muscle concentration (µg/g)</td>
<td>1.37</td>
<td>9.95</td>
<td>9.84</td>
<td>6.15</td>
<td>8.01</td>
<td>6.58</td>
<td>1.84</td>
<td>1.90</td>
</tr>
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<td>Residual amount at injected site (mg)**</td>
<td>536</td>
<td>280</td>
<td>6.65</td>
<td>22.9</td>
<td>-0.0439</td>
<td>18.5</td>
<td>-0.0362</td>
<td>0.012</td>
</tr>
<tr>
<td>Total dose administered (mg)</td>
<td>550</td>
<td>500</td>
<td>310</td>
<td>340</td>
<td>240</td>
<td>385</td>
<td>235</td>
<td>120</td>
</tr>
<tr>
<td>Residual rate at injected site (%)**</td>
<td>97.5</td>
<td>40.0</td>
<td>2.14</td>
<td>6.74</td>
<td>-0.0183</td>
<td>4.81</td>
<td>-0.0154</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma concentration (µg/ml)</td>
<td>5.40</td>
<td>34.8</td>
<td>21.0</td>
<td>16.0</td>
<td>19.8</td>
<td>14.9</td>
<td>4.00</td>
<td>4.61</td>
</tr>
<tr>
<td>Muscle conc./Plasma conc. (noninjected)</td>
<td>0.254</td>
<td>0.286</td>
<td>0.468</td>
<td>0.384</td>
<td>0.405</td>
<td>0.440</td>
<td>0.460</td>
<td>0.412</td>
</tr>
</tbody>
</table>

* (Drug concentration at injected muscle - drug concentration at non-injected muscle) x sample weight.
** Residual amount at injected site: total dose administered x 100.

Muscle to plasma concentration is almost constant (0.428±0.0331) in 6 animals slaughtered 1 hr after the drug administration. In pharmacokinetics, the transfer of drug between tissues and plasma will be theoretically equilibrium for an elimination phase, that is, the ratio of drug concentration of tissue and plasma is constant during the phase. The concept has been well demonstrated in many drugs and animals by many authors [6, 13, 14, 15].

The mathematical prediction of the time of absorption ending (AET) at the injected muscle would be possible from the plasma kinetic parameters. In this study, a total dose of 300 mg SMM was given to a pig (10 mg/kg), a possible minimum volume of distribution of the drug solution at the injection site was 1.5 ml (1 g of the tissue for 1 ml of the drug solution) and the ratio of drug concentration at the non-injected muscle to that of plasma was 0.428, respectively. The time profile of drug residue of injected and non-injected muscles was presented in Fig. 4, in which lines 1 and 2 represent the time course of concentration of SMM at injected and non-injected sites estimated from the plasma kinetic analysis. Line 1: time course of concentration at the injected site with mean absorption rate constant of 2.87 hr⁻¹. Line 2: that with lower value of mean absorption rate constant at 95% confidence (0.961 hr⁻¹). Cp: the mean plasma concentration time curve with the equation, \( Cp = -7.94 e^{-0.88t} + 32.9 e^{-0.24t} - 24.9 e^{-2.87t} \). Cm: the estimated concentration time line at non-injected muscle drawn parallel to Cp curve with a distance 0.428 times below.
sented the estimated decline of drug concentration at the injected muscle and Cm was a time curve of drug concentration in non-injected muscle which was drawn pararell 0.428 times below to the time curve of Cp (mean plasma concentration). The line 1 which was calculated by the equation of $300,000 \times (1 - (1 - e^{-2.87 t})) / 1.5$ crossed Cm curve at about 3.6 hr after the injection. The line 2 which was clucedy by the equation $300,000 \times (1 - (1 - e^{-0.961 t})) / 1.5$ crossed the curve at about 13 hr after the drug administration. On the other hand, the real time of absorption ending by the chemical assay of injected and non-injected muscles was between 5 and 7 hr.

From the result obtained, the possibilith of the estimation of drug residue at the injected site by the calculation of plasma kinetic parameters would be reasonable in a water soluble intramuscular dosage form as SMM, when the lower 95% confidence value of absorption rate constant was used. Since there is a considerable number of intramuscular dosage forms of water or oil suspension, whether or not the plasma kinetic parameter would be applicable to estimate safely the drug residue at the injection site in all dosage forms remains to be elucidated.

References


要　約

水溶性筋注用剤の投与部位筋肉残留時間の血中動態解析による予測について：田村淳子・小久江栄一・吐山豊秋（東京農工大学農学部家畜薬理学科）——薬剤の筋肉内投与後の投与部位筋肉における吸収終了時間について、血漿中薬物濃度を薬物動態論の解析によって推定する方法を検討した。動物はゲッチャンゲクラブ07頭、薬剤はスルファモノメトキシン（SMM、10mg/kg）の水溶液を用いた。SMM静注および筋注後の血漿中薬物濃度を薬物動態論によって解析した結果、筋注後の吸収は完全であった。またLoo–Riegelman解析によると、吸収過程は一次速度で、吸収速度定数（ka）は2.87±2.14(hr⁻¹)（n=8）と計算された。したがって平均投与部位残留率は1-(1-e⁻².87)として表わされた。消失相における非投与部位筋肉と血漿中の薬物濃度は0.428±0.033(hr⁻¹)（n=6）であった。また薬物投与後のある時点における投与部位での最大局所薬物濃度は、局所薬物濃度を考える最低の注射部位薬物分布容積である、1ml/gで割ることによって得られる。kaの平均値の95%信頼限界下限値（0.961）を使用すると、注射部位薬物が注射部位筋肉濃度と等しくなる時間、つまり吸収終了時間は投薬約13時間後と算出された。同時に得られたAUCは筋注後の逐次増減による筋肉残留時間を実測した結果、吸収終了は投薬後5～7時間間であった。以上から筋注後の投与部位筋肉の薬物吸収終了時間は、薬物動態論による解析によって、安全に、一定の規準をもって推定できると考えられた。