Effects of Exercise on Plasma Concentrations of Caffeine and Its Metabolites in Horses

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Received February 27, 1995; accepted July 5, 1995

The effects of exercise on the metabolism of caffeine (CA) were studied 3 h after administration of the drug to race horses which then underwent exercise sets (1000-m gallop). Analysis was made of pharmacokinetics of CA, changes in its plasma concentrations, its metabolites, i.e., theophylline (TP), theobromine (TB) and paraxanthine (PX), and the molar concentration ratios of CA to these metabolites. After exercise, \( A_{UC} \) and \( t_{1/2} \) tended to decrease, and the concentration of CA decreased, while the concentrations of TP and TB significantly increased. The TP/CA ratio and TB/CA ratio significantly increased from 6 to 74 h and from 25 to 50 h after drug administration, respectively. This indicated promotion of metabolism of CA into TP and TB. The effects on PX were minimal.

Key words caffeine; exercise; metabolite; horse plasma

We previously reported,\(^1\) on the pharmacokinetics of theophylline (TP), theobromine (TB) and paraxanthine (PX), all of which are primary metabolites of caffeine (CA), after i.v., i.m. and p.o. administration of CA to horses. Samples taken to ensure there has been no doping are generally collected from race horses after racing, that is, after plenty of exercise. Because exercise affects physiological factors (hemodynamics, metabolism, pH, temperature, gastro-intestinal functions, etc.) of the body, it is known that pharmacokinetics of a drug is altered by exercise.\(^2\) For this reason, there have been many studies on \( \beta \)-blockers given in association with kinesitherapy in humans.\(^3\) It has been reported that, in horses, exercise causes an increase in the plasma concentration of propranolol but does not cause a change in plasma antipyrene concentration.\(^4\)

Kamimori et al.\(^5\) and Collomp et al.\(^6\) reported the effects of moderate exercise on pharmacokinetics of CA in humans, but they did not analyze changes in its metabolites. To elucidate the effects of exercise on the metabolism of CA, we administered the drug orally to trained race horses, made them gallop 1000-m, and analyzed the effects of exercise on changes in the plasma concentrations of CA and its metabolites and their molar concentration ratios.

MATERIALS AND METHODS

Reagents Powder CA was prepared by recrystallization (mp 238 to 240 °C) of CA from Japan Pharmacopoetia (Sanko Seiyaku Co.) with ethanol. TP, TB and PX were products of Wako Junyaku Co., Tokyo Kasei Co. and Sigma Co., respectively. Other reagents and solvents used were those of guaranteed grade and those for liquid chromatography.

Administration of CA and Collection of Samples The horses employed in the study were given ordinary feed for race horses. Powder CA (2.5 mg/kg) was orally administered using a gastric catheter together with 21 of water 3 h after feeding. Blood samples (20 ml) were collected from the cervical vein prior to drug administration and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 25, 50, 74 and 96 h after dosing. Blood was immediately centrifuged to separate plasma, which was reserved frozen until the time of analysis.

Exercise Loading Eight horses trained as race horses were employed. They were female Anglo-Arab and Thoroughbreds three years of age weighing 386 to 463 kg. On a 1100-m circular race track, the horses underwent 1800-m warm-up exercises, 1000-m gallop exercises and 1200-m cooling-down exercise 3 h after drug administration (Fig. 1).

Determination by High-Performance Liquid Chromatography (HPLC) The concentrations of CA and its metabolites were determined by HPLC.\(^3\)

Pharmacokinetic Analysis of Data Pharmacokinetic analysis was performed in accordance with the method described.\(^3\) Data obtained at rest and after exercise were tested for significant differences by Student's \( t \)-test.

RESULTS

The pharmacokinetic parameters after the administration of CA at rest and exercise are shown in Table 1. Except for a decreasing tendency \( (p<0.1) \) in \( A_{UC} \) and \( t_{1/2} \) in exercise, there were no differences in the other parameters.

The time-concentration changes in the plasma CA and its metabolites are shown in Fig. 2 together with the data at rest. After exercise, the concentration peaked 2–3 h for CA, 12–25 h for TP and TB and 6–12 h for PX.

Fig. 1. Description of Exercise Protocol

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Compared with the data during rests, CA concentration began to decrease 12 h after the administration, and it was significantly lower 50 h afterward. With respect to the metabolites, a significant increase was recorded 5—9 h after the administration for TP and 9 h afterward for TB.

Table 2 compiles the change in the molar concentration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>C_{max} (µg/ml)</td>
<td>2.81±0.03</td>
<td>2.77±0.22</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>1.92±0.46</td>
<td>2.34±0.90</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>14.7±1.0</td>
<td>12.8±1.2*</td>
</tr>
<tr>
<td>AUC (µg h/ml)</td>
<td>65.34±4.61</td>
<td>58.10±6.14*</td>
</tr>
<tr>
<td>K_{p} (h^{-1})</td>
<td>2.13±0.62</td>
<td>1.75±0.83</td>
</tr>
</tbody>
</table>

Mean values (±S.D.) for 5 horses. * p<0.1.

DISCUSSION

The effects of exercise on pharmacokinetics of CA have been studied in recent years. Kamimori et al. administered CA orally to lean and obese persons and had them exercise for 90 min thereafter. They found significant decreases in V_{d} and decreases in C_{max} and AUC. Collomp et al. administered CA to heavy and light coffee drinkers and immediately had them exercise. They reported a significant increase in C_{max} and a significant decrease in t_{1/2} and V_{d}. Metabolites of CA were not included in these studies, however. In the present experiment using horses the effect of exercise on CA pharmacokinetics was reflected only in a tendency for a drop in AUC and t_{1/2}, with no difference in the other parameters. The concentration curves for CA and its metabolites in plasma, in contrast, indicated a decrease in CA and an increase in TP and TB, its metabolites, following exercise. In terms of molar concentration ratios of CA to its metabolites, TP/CA and TB/CA proved to increase significantly over 6—74 h and 20—50 h after the administration of CA, respectively, and the metabolism of CA to TP and TB was accelerated due to exercise. No change in PX/CA due to exercise was noted. The correlations in CA pharmacokinetics, as shown by the present study and its pharmacokinetics in plasma seem to be ascribable to the exercise loading performed 3 h after CA administration. Namely, C_{max} and t_{max} at exercise loading are completely equal to those at rest, but less influence was given to the pharmacokinetics because of exercise performed following t_{max}. Exercise-induced significant increase in metabolites has been reported for isosorbide dinitrate and sulfadimidine.

It is thought that exercise-induced changes in physiology and metabolic functions of the liver alter pharmacokinetics of a drug. In horse races, it is said that most...
exercise-induced changes in blood components return to the pre-exercise levels within one hour after exercise. It has been reported that the metabolic function of the liver of trained athletes differs from that of ordinary persons. As changes in the enzyme system with training are also known in race horses, we employed trained race horses in the present study. The formation of metabolites 1-2 h after exercise did not differ from that observed during rest, but began to differ beginning 3 h after exercise. It is therefore difficult to think that the metabolism of CA was directly affected by a decrease in the hepatic blood flow.

CA is metabolized into dimethylxanthine by the mono-oxygenase system in the liver microsome in the presence of cytochrome P450. Tassaneeyakul et al. reported that due to differences in the affinity of P450 isoforms, the amount of formation of metabolites in man is larger in order of PX > TB > TP. In the present study using horses, the order was reversed to TP > TB > PX, indicating a marked difference between different species. Collomp et al. ascribed the changes in the pharmacokinetics of CA to induction of relevant enzymes by exercise. With regard to the results of the present study, it is also surmised that exercise induced P450, resulting particularly in promotion of metabolism of CA into TP, whose absolute amount is especially large in horses.

Acknowledgments  The authors thank Mr. T. Tanaka, Laboratory of Racing Chemistry, for his encouragement. This work was sponsored by the Japan Racing Association.

REFERENCES