Stability of Spironolactone in Rat Plasma: Strict Temperature Control of Blood and Plasma Samples Is Required in Rat Pharmacokinetic Studies

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The stability of spironolactone (SPN) in rat plasma was studied and its degradation was found to be an apparent first-order reaction. The apparent first-order rate constant (kapp) at 37, 23.5, and 0 °C were 3.543±0.261 (h−1, mean±S.D., n=3), 6.278±0.045 (×10−1 h−1), and 7.336±0.843 (×10−2 h−1), respectively. The half-lives were 0.20 h, 1.10 h, and 9.53 h. The degradation rate of SPN in rat plasma was markedly decreased when NaF, an esterase inhibitor, was added to the plasma, and the degradation was catalyzed by esterase in the plasma. These results indicated that not only plasma but also blood and serum samples in rat pharmacokinetic studies should be cooled to 0 °C, the temperature maintained, and treated as soon as possible. In pharmacokinetic studies reported previously, the temperature control of plasma, blood, and serum samples was not described. The pharmacokinetic study in rats after intravenous administration of SPN at 20 mg/kg was performed with strict temperature control of plasma and blood samples. The AUC, MRT, CL, and Vdss values (mean±S.E., 4 rats) for SPN were 4100.8±212.9 ng h/ml, 0.29±0.01 h, 4915.7±248.0 ml/kg, and 1435.4±48.4 ml/kg, respectively. The AUC value was much larger than that previously reported. The AUC, MRT, Cmax and Tmax values (mean±S.E., 4 rats) of canrenone, an active metabolite of SPN, after the administration of SPN were 4196.1±787.5 ng h/ml, 1.99±0.13 h, 1546.3±436.4 ng/ml and 1.0±0.0 h, respectively. This AUC value was almost identical to the value previously reported.

Key words spironolactone; stability; rat plasma; determination method; pharmacokinetics; intravenous administration

Measurement of Stability in Rat Plasma SPN was dissolved in ethanol to make a 1 mg/ml solution. Male Sprague-Dawley rat plasma previously prepared and stored at −80 °C was used to measure the kinetics of SPN degradation. The pH value of the rat plasma was 7.8 at the start and the end of each experiment. The concentration of NaF in the plasma was 20 mg/ml. Immediately before each experiment, suitable aliquots of the plasma with or without NaF were pre-incubated for 15 min at 0 °C, 23.5 °C, and 37 °C. The experiments were performed at 0—1 °C, 23.5 ±0.5 °C, and 37 ±0.5 °C. Those temperatures were employed as the lowest temperature for treatment of plasma samples, usual room temperature, and body temperature, respectively. The experiments were initiated by adding the SPN solution to produce a final concentration of 20 μg/ml. A 200 μl aliquot of methanol containing 0.2% HClO4 was added to 100 μl of sample solution immediately after it was taken from the reaction mixture, and cooled in an ice-bath. The mixture was stirred in a vortex mixer for 30 s and centrifuged at 3000 rpm for 10 min; 50 μl of the supernatant was injected into the chromatograph.

Determination of SPN in Rat Plasma by HPLC The concentration of SPN in plasma was determined with an HPLC assay consisting of a Model LC-9A pump, equipped with a Model SCL-6B system controller, a Model SPD-6A UV spectrophotometric detector, a Model CTO-6A column oven, a Model C-R4AX Chromatopac, and a Model SIL-6B autoinjector, all from Shimadzu (Kyoto, Japan). The mobile phase was acetonitrile–water– perchloric acid (60%)-sodium perchlorate monohydrate (485:515:1.5, v/v/v/w). The chromatographic column was a YMC Pack AM312 ODS (150 mm×6 mm I.D., particle diameter 5 μm) obtained from YMC Co. Ltd. (Kyoto). The flow rate, determination wavelength, and temperature of the column were 1 ml/min, 254 nm and 40 °C, respectively.

Determination of CAN in Rat Plasma by HPLC The...
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Figure 1 shows the relationship between the residual percentage of SPN on a logarithmic scale and time at three different temperatures. This result indicates that the degradation rate of SPN in rat plasma at 37 °C was very rapid and that the rate decreased with decreasing temperature, although the degradation reaction was observed even at 0 °C. Each plot in Fig. 1 for 37, 23.5 and 0 °C is linear. The degradation reaction of SPN within the range of 0 to 37 °C is an apparent first-order reaction. The apparent first-order rate constant, \( k_{obs} \), was calculated from the slope of each straight line shown in Fig. 1. The results are shown in Table 1. The main degradation product of SPN in rat plasma was observed at about 4 min on the chromatogram when the retention times of SPN and CAN were 12.6 and 13.4 min, respectively. The concentration of the degradation product could not be found due to an unidentified peak and not having the standard compound for determination. The peak area of the degradation product at 5 min at 37 °C corresponded to 3.17 ± 0.09 µg/ml as SPN, which well agreed with the value at 5 min at 37 °C. From this result, the reduction of the degradation rate of SPN with decreasing temperature was confirmed by the decrease in rate of producing of the degradation product.

The apparent first-order rate constant at 40 °C in pH 7.3 buffer solutions at 40 °C in pH 7.3 and at 37 °C in pH 7.8 seemed to be small, and in the comparison of the degradation rates the value at 40 °C in pH 7.3 was considered to be large. Therefore, the degradation rates were directly compared. The \( k_{obs} \) values at 37 °C, 23.5 °C and 0 °C were about 4300, 760, and 88 times larger than the value at 40 °C in pH 7.3 buffer solutions. The \( k_{obs} \) at 37 °C in rat plasma was markedly larger than the value at 40 °C in pH 7.3 buffer solutions in spite of the higher temperature of the buffer solution. It was found from the results that the degradation reaction of SPN in rat plasma was catalyzed enzymatically. Figure 2 shows the effect of NaF on the degradation rate of SPN in rat plasma at 37 °C. The effect of NaF on the \( k_{obs} \) values at 0, 23.5, and 37 °C is summarized in Table 1. This result showed that the degradation of SPN was catalyzed by esterase in rat plasma. We previously reported the degradation rate of NT-16.7 and
nikkomycin Z\(^8\)) in the plasma of several animal species. The \(k_{\text{obs}}\) values of \(\text{NT-1}\)\(^6\) and nikkomycin Z\(^8\)) are 7.26 and 1.74×10\(^{-3}\) min\(^{-1}\), respectively, while the \(k_{\text{obs}}\) of SPN was smaller than NT-1 and nikkomycin Z. NT-1 and nikkomycin Z are catalyzed by esterase in plasma and SPN is also catalyzed by esterase. A detailed study of the degradation rates in plasma among different animal species is required in the future.

**Pharmacokinetics of SPN in Rats** In a previously reported pharmacokinetic study of SPN using rats, the temperature control for blood and plasma or serum samples was not described clearly in the experimental section.\(^3\) Accordingly, the plasma concentration of SPN might have been evaluated below the real concentration. We attempted to obtain real plasma concentrations and real pharmacokinetic parameters. Figure 3 shows the plasma concentration of SPN and CAN after an intravenous administration of SPN at 20 mg/kg. The pharmacokinetic parameters for SPN and CAN are summarized in Table 2. The plasma levels of SPN appeared to decrease with time with a biexponential pattern. The elimination rate of SPN is very rapid, which was reflected in the small value of \(MRT\) (0.29 h) and in the large value of \(CL\) (4915.7 ml/kg). The \(AUC\) value and the concentration at 5 min, 4100.8 ng h/ml and 16065.3 ng/ml, were much higher than the values reported, 1628 ng h/ml and 6425 ng/ml,\(^3\) respectively. On the other hand, CAN was detected in the plasma, and the \(AUC\) value for CAN was 4196.1 ng h/ml which agreed with the reported value, 4700 ng h/ml.\(^3\)

The plasma concentration of SPN in our result is much higher and that of CAN is almost equal compared with the reported values,\(^3\) suggesting that SPN in the samples was degraded, and that its determined concentration was lower than the real concentration. However, the reason for this difference is not clear, as there were some differences in the experimental conditions, for example, differences in the rats used and the administered solutions. We will study the pharmacokinetics of SPN in rats with plasma and blood samples under strict temperature control.

In conclusion, SPN was degraded in rat plasma, and this reaction was catalyzed by esterase in the plasma. The degradation rates at 37 and 23.5 °C were rapid, which indicated that blood and plasma samples in rat pharmacokinetic studies should be cooled to 0 °C, the temperature maintained, and treated as soon as possible. We also demonstrated the pharmacokinetic data obtained under strict temperature control for plasma and blood samples. A possibility of the degradation of SPN in samples of pharmacokinetic studies previously reported was suggested from these results.

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### REFERENCES


### Table 2. Pharmacokinetic parameters of SPN and CAN after Intravenous Administration of SPN at 20 mg/kg to Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SPN</th>
<th>CAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AUC) (ng h/ml)</td>
<td>4100.8±212.9</td>
<td>4196.1±787.5</td>
</tr>
<tr>
<td>(MRT) (h)</td>
<td>0.29±0.01</td>
<td>1.99±0.13</td>
</tr>
<tr>
<td>(CL) (ml/kg)</td>
<td>4915.7±248.0</td>
<td>—</td>
</tr>
<tr>
<td>(V_d) (ml/kg)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(C_{max}) (ng/ml)</td>
<td>—</td>
<td>15463±436.4</td>
</tr>
<tr>
<td>(T_{max}) (h)</td>
<td>—</td>
<td>1.0±0.0</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 4 rats.