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Circadian Changes in the Pharmacokinetics of Acebutolol Orally Administered to Rabbits

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ABSTRACT – Circadian variations of acebutolol and its main metabolite, diacetolol pharmacokinetics were studied after a single oral administration of acebutolol (10 mg/kg) to eight rabbits at 10 : 00 AM (in the morning) and 10 : 00 PM (at night). The plasma concentration profiles of acebutolol were significantly different ($P < 0.05$) between 10 : 00 AM and 22 : 00 PM, suggesting circadian variations of pharmacokinetic behaviors. A significant circadian rhythm of pharmacokinetic parameters was noted in rabbits, showing higher total body clearance (CL/F), and lower the area under the plasma concentration-time curves (AUC) of acebutolol than that at night. The half-life ($t_{1/2}$) of acebutolol and diacetolol were also significantly shorter in the morning than at night ($P < 0.05$). Metabolite-parent AUC ratio at night significantly decreased compared to in the morning, implying that night time could inhibit acebutolol metabolism than in the morning. From this study there was an administration-time difference of acebutolol pharmacokinetics in the rabbits. The optimized dosing regimen of acebutolol can be decided by considering circadian rhythm so that the effective therapies are established for patients.

Key words – Acebutolol, Diacetolol Pharmacokinetics, Circadian rhythm, Dosing regimen

It has been well recognized that nearly all the functions of the body show significant daily variations and circadian phase dependency. Circadian rhythmic variations have been demonstrated for cardiovascular, antiasthmatic, anticancer, analgesic, psychotropic and antibiotic medication.[1-3] Many physiologic changes such as blood pressure, heart rate, acid secretion, gastric emptying time, liver metabolism, glomerular filtration, renal plasma flow, urine production and pH vary as circadian rhythms with potential influences on absorption, distribution, metabolism and elimination. In addition, the onset and symptoms of diseases such as asthma, myocardial infarction, stroke and angina pectoris are also highly circadian phase dependent.[3-6] For these reasons, circadian time has to be taken into account as an important parameter affecting pharmacokinetics, bioavailability as well as positive and side effects of various drugs.[3,5,7-10] For example, the time to reach a peak concentration and half-lives of theophylline,[11] valproic acid[12] and gentamycin[13] were shorter when the drugs were administered in the morning than those in the afternoon, and the urinary excretion of cisplastin[9] and acetaminophen[14] increased when the drugs were administered in the morning. The plasma concentrations and the area under the plasma concentration-time curve from time zero to time infinity of propranolol,[15,16] triamcinolone,[17] aspirin,[18] midazolam,[19] nortriptyline,[20] antipyrine[21] and cyclosporine[22] increased and urinary excretion of the drugs decreased when the drugs were administered in the morning.

Acebutolol is beta-adrenergic blocking agent, which is widely used in the treatment of hypertension and cardiac arrhythmias.[22-24] Acebutolol is a drug that is well absorbed from the small intestine following oral administration. It undergoes significant hepatic first-pass metabolism being converted first to the primary amine,[25,26] then acetylated to diacetolol. Acebutolol's main metabolite, diacetolol, has pharmacologic properties similar to those of the parent compound.[27,28] Both acebutolol and diacetolol are excreted in bile and urine.[29-31] Although the chronopharmacokinetics of other beta-adrenergic blocking agent, propranolol, has been studied in humans and rats, the circadian variations of acebutolol pharmacokinetics have not been investigated in humans and animals. The purpose of this study was to investigate administration-time differences of acebutolol pharmacokinetics after oral administration to rabbits at 10 : 00 h and 22 : 00 h.

Materials and Methods

Materials

Acebutolol, diacetolol and triamterene (an internal standard) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Phosphoric acid, sodium hydroxide and potassium phosphate, monobasic were purchased from Shinyo Pure

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Chemicals Co. (Osaka, Japan) and ether and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Other chemicals were reagent grade and used without further purification.

Animals

The white male New Zealand rabbits weighing 2.0-2.5 kg were fasted at least 24 h before experiment and were given water freely. 25% urethane (4 mL/kg) anesthesia, the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling at room temperature. The protocol of this animal study was approved by the Animal Care Committee of the Chosun University.

Drug administration

Acebutolol 10 mg/kg was administered orally to eight rabbits at 10:00 AM (in the morning) and 10:00 PM (at night), respectively. Blood samples (1.2 mL) were withdrawn from the femoral artery at 0.25, 0.5, 1, 2, 3, 4, 6, 9 and 12 h after the acebutolol administration. Plasma samples were obtained by centrifuging at 3,000 rpm for 10 min. The separated 0.5 mL plasma were stored at −70°C until assayed. Saline was infused at the rate of 1.5 mL/h to ear vein by infusion pump.

Assay and HPLC conditions

Plasma concentrations of acebutolol and diacetolol were determined by a HPLC.[32] A 0.1 mL of triamterene (2 μg/mL) and 0.2 mL of 1 M sodium hydroxide and 4 mL of ether were added to 0.5 mL of the sample. It was mixed for 10 min and centrifuged at 3,000 rpm for 5 min. 3.5 mL of the organic layer was transferred to a clean test tube (Axygen Scientific INC., CA, USA) and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in a 0.2 mL of 0.05% phosphoric acid and centrifuged at 6,000 rpm for 3 min. 50 μL of the solution was injected into the HPLC system.

The HPLC system consisted of a solvent delivery pump (Model CBM-10A, Shimadzu Co., Japan), a variable UV absorbance detector and computing integrator. The detector wavelength was set at 243 nm and the column was used at room temperature. The stationary phase used was a Shin-Pack CLC-ODS column (4.6×250 mm, Shimadzu Co., Japan). Mixtures of acetonitrile:water:0.1 M phosphate buffer (pH=4) (22:68:10, v/v/v/) were used as the mobile phase. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. At a flow rate of 1.0 mL/min, the retention times were as follows: diacetolol, 4.2 minutes, internal standard, 7.8 minutes, and acebutolol, 10.5 minutes.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with a non-linear least square regression using a MULTI program.[33] The parameter value was obtained fitted to simplex method when AIC (Akaike's information criterion) value was the lowest. The area under the plasma concentration-time curves (AUC) was calculated by trapezoidal rule and total body clearance (CL/F) was calculated by CL/F=Dose/AUC. The maximum plasma concentration (C_{max}) and time to reach the maximum plasma concentration (T_{max}) were obtained directly from plasma concentration-time curves. Metabolite-parent AUC ratio (MR) was calculated by AUC metabolite/AUC parent.

Statistical Analysis

The statistical significance of difference in parameters between 10:00 AM and 10:00 PM dosing was compared by the student's paired t-test was used for statistical analysis of pharmacokinetic parameters. Statistical significance was set at $P<0.05$ and $P<0.01$.

Results and Discussion

Plasma concentration

Mean plasma concentration of acebutolol and its major metabolite, diacetolol after a single oral administration of acebutolol (10 mg/kg), respectively, at 10:00 AM and 10:00 PM are shown in Figure 1 and 2. The mean plasma concentration of acebutolol were significantly ($P<0.05$) higher at night than that in the morning from 4 h to 12 h, suggesting administration-time difference of acebutolol pharmacokinetics. Mean

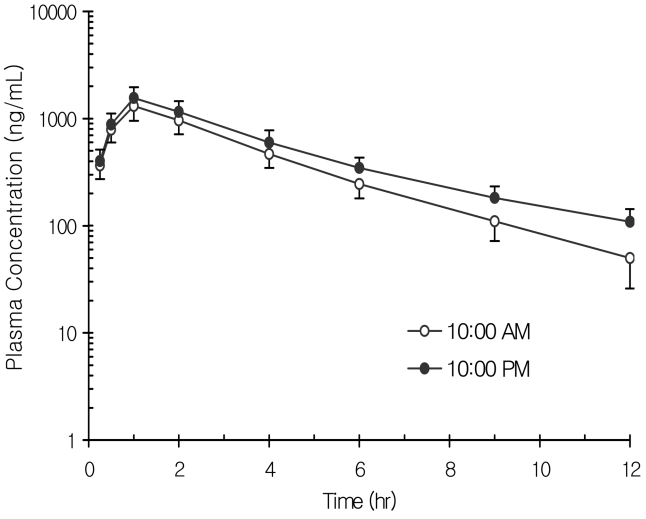

Figure 1—Mean plasma concentration (ng/mL) of acebutolol after oral administration of acebutolol (10 mg/kg) in rabbits at 10:00 AM and 10:00 PM.

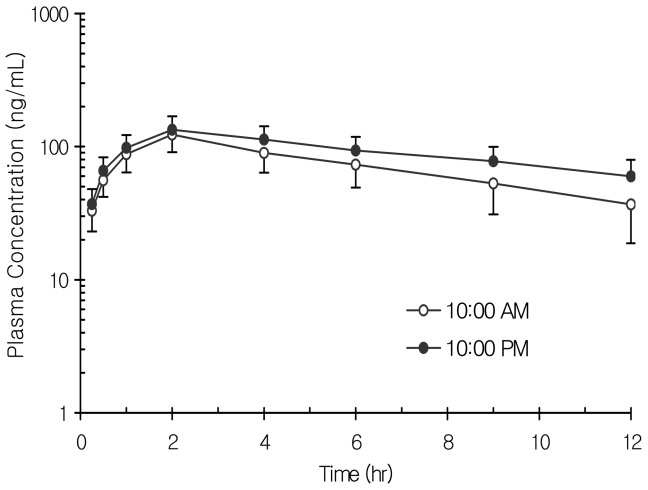

Figure 2–Mean plasma concentration (ng/mL) of diacetolol after oral administration of acebutolol (10 mg/kg) in rabbits at 10:00 AM and 10:00 PM.

Table I–*Pharmacokinetic Parameters of Acebutolol after Oral Administration of Acebutolol (10 mg/kg) in Rabbits at 10:00 AM and 10:00 PM*

Parameters	10:00 AM	10:00 PM
K_a (h^{-1})	1.14 ± 0.28	1.09 ± 0.25
C_{max} (ng/mL)	1208 ± 282	1456 ± 379
T_{max} (h)	1.30 ± 0.36	1.36 ± 0.35
$t_{1/2}$ (h)	5.67 ± 1.60	8.06 ± 2.20*
CL/F (mL/min)	1.72 ± 0.41	1.07 ± 0.27*
AUC (ng/mL·h)	5819 ± 1248	8410 ± 1982*

Mean ± S.D.(n=8), Significantly different from the value of 10:00 h group($^*P<0.05$,$^{**}P<0.01$)

K_a: absorption rate constant

C_{max}: maximum concentration

T_{max}: peak time reached to maximum concentration

$t_{1/2}$: terminal half-life

CL/F: total body clearance

AUC: area under the plasma concentration-time curve

plasma concentration of diacetolol at night was increased compared to that in the morning, but not significant.

Pharmacokinetic parameters

The pharmacokinetic parameters of acebutolol after a single oral administration at 10:00 AM and 10:00 PM are summarized in Table I. A significant circadian rhythm of acebutolol pharmacokinetics as a function of drug-administration time of day was noted with lower CL/F and higher AUC after the night dose. The pharmacokinetic parameters showed differences among rabbits. The $t_{1/2}$ was significantly ($P<0.05$) shortened to in the morning (5.67 ± 1.60 h) when compared to

Table II–*Pharmacokinetic Parameters of Diacetolol after Oral Administration of Acebutolol in Rabbits at 10:00 AM and 10:00 PM*

Parameters	10:00 AM	10:00 PM
C_{max} (ng/mL)	121 ± 28.1	133 ± 34.7
T_{max} (h)	2.54 ± 0.58	2.57 ± 0.46
$t_{1/2}$ (h)	7.14 ± 2.22	9.62 ± 2.42*
CL/F (mL/min)	3.72 ± 0.82	2.71 ± 0.43*
AUC (ng/mL·h)	2786 ± 528	3287 ± 698
MR	0.48 ± 0.091	0.39 + 0.082*

Mean ± S.D.(n=8), Significantly different from the value of 10:00 h group($^*P<0.05$,$^{**}P<0.01$)

C_{max}: maximum concentration

T_{max}: peak time reached to maximum concentration

$t_{1/2}$: terminal half-life

CL/F: total body clearance

AUC: area under the plasma concentration-time curve

MR: metabolite-parent AUC ratio, AUC metabolite/AUC parent

that at night (8.06 ± 2.20 h). The CL/F was significantly ($p<0.05$) increased in the morning (1.72 ± 0.41 mL/min) versus at night (1.07 ± 0.27 mL/min). The AUC was significantly ($P<0.05$) decreased in the morning (5819 ± 1248 ng/mL·h) than that at night (8410 ± 1982 ng/mL·h).

The pharmacokinetic parameters of diacetolol after a single oral administration of acebutolol at 10:00 AM and 10:00 PM are summarized in Table II. The $t_{1/2}$ was significantly shortened to in the morning when compared to that at night. The CL/F was significantly increased in the morning versus at night. Metabolite-parent AUC ratio at night (0.39 ± 0.072) significantly ($P<0.05$) decreased compared to that in the morning (0.48 ± 0.081), implying that night time could be inhibit acebutolol metabolism than that in the morning.

The results of this study clearly demonstrate that the pharmacokinetics of oral acebutolol in rabbits are dependent on the time of drug intake within the 24 h span of a day. In this way the investigation has added to the observations of periodic and predictable changes in the actions of drugs as a function of their time of administration.[34,35]

Fujimura et al[16] found that the mean K_a and C_{max} of propranolol, other beta-adrenergic blocking agent, in healthy humans were significantly higher in the morning (9:00 AM) than after the night dose (9:00 PM), whereas in the present study the K_a and C_{max} of acebutolol was not significant differences. Acebutolol is well absorbed from the small intestine following oral administration, daily variation in drug absorption can only be attributed to daily variation in gastrointestinal perfusion. The faster hepatic elimination of acebutolol during the day-time hours could have been brought about by

increased liver perfusion due to the higher cardiac output in the activity period.[34,35] It undergoes significant hepatic first-pass metabolism being converted first to the primary amine,[25,26] then acetylated to diacetolol.

Langner et al[15] found that the $t_{1/2}$ of propranolol, other beta-adrenergic blocking agent, after a morning dose was shorter than that following an evening dose in humans, whereas in the previous study in rats[36] the $t_{1/2}$ of propranolol was shorter during in the dark period than during in the light period. The present data revealed significant daily variation in the $t_{1/2}$ of acebutolol, which was shortened to in the morning when compared to that at night. The total body clearance of acebutolol was significantly increased in the morning versus at night so that the AUC was significantly ($P<0.05$) decreased in the morning than that at night. Metabolite-parent AUC ratio at night significantly ($P<0.05$) decreased compared to that in the morning, implying that night time could be inhibit acebutolol liver metabolism than that in the morning.

Although the rabbit is an almost classical laboratory animal, chronobiological research in this species is in its infancy. It appears not even clear, whether the rabbit is a predominantly diurnal or nocturnal animal. When the hours of external noise coincided with the light period displayed a predominantly diurnal pattern. In contrast, in a properly sound-isolated laboratory locomotor activity was significantly higher during the dark period which is typically in nocturnally active animals. The present chronopharmacokinetic data go well with findings about acebutolol in diurnally active rabbits.[37] In them a significantly higher total body clearance (CL/F), a higher metabolite-parent AUC ratio (MR), a shorter half-life and a lower AUC of acebutolol was found in the morning than at night. Thus, both in humans and in the rabbits hepatic elimination of acebutolol is enhanced in the activity span of the species. This observation supports the notion that animal data can adequately be compared with findings in humans only when species-dependent differences in circadian phase taken into account.[35]

From this study, there was an administration-time difference of acebutolol pharmacokinetics in rabbits. The optimized dosing regimen of acebutolol can be decided by considering circadian rhythm and rest-activity routine so that the risk of toxicity is minimized and the effect of therapy optimized for patients. The right time and the right dose are required for the safe and effective drug treatment as mentioned previously.[1,3,6] The current findings can be also applied to other drugs with circadian rhythms of pharmacokinetics and narrow therapeutic windows in clinical chronotherapeutics so that the safe and effective drug therapy can be established.

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