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# Pharmacokinetic Changes of Intravenous Diltiazem in Rabbits with Alloxan-Induced Diabetes Mellitus

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# 알록산으로 유도된 당뇨가토에서 정맥투여된 딜티아젬의 약물동태 변화

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당뇨환자가 합병증으로 고혈압이 있을경우 항고혈압약물인 딜티아젬을 투여시 딜티아젬의 동태학적 측면에서 투여계획을 설계하기 위해서 토끼에 알록산으로 당뇨병모델을 만들었다. 알록산으로 유도된 급성 및 만성 당뇨 토끼에서 딜티아젬의 약물동태 변화에 대한 결과는 다음과 같다.
1. Alloxan 45 mg/kg을 토끼의 귀정맥에 투여시 혈당농도는 control군에서는 112±20.6 mg/dl, acute DM군에서는 260±87 mg/dl, chronic DM군에서는 331±85 mg/dl으로 당뇨가 유발되었음이 확인되었다.
2. Alloxan에 의한 당뇨병 유발토끼에서 딜티아젬의 혈중농도곡선하면적(AUC)값은 대조군(21.6±2.45 μg/ml) 보다 chronic DM군(31.91±379 mg/ml · hr)에서 유의성($p<0.05$) 있게 증가하였다.
3. 딜티아젬의 요중누적배설량은 대조군에 비해 acute 및 chronic DM군에서 감소되었으나 유의성은 없었다
4. 당뇨병 유발 토끼에서 딜티아젬의 토탈바디클리어런스($CL_t$) 값과 β-소실속도정수값이 대조군에 비해서 유의성($p<0.05$)있게 감소되었다. 실험적 당뇨 토끼에서 딜티아젬의 생체이용률의 증가는 딜티아젬의 토탈바디클리어런스($CL_t$) 값과 베타의 소실속도정수 값이 대조군에 비해서 유의성있게 감소되었기 때문으로 사료된다.

□ Key words – Diltiazem, Pharmacokinetics, Alloxan-Induced, Diabetes Mellitus(DM)

Many diabetic patients develop serious complications during the course of the disease, including cardiovascular disorders, nephropathy, neuropathy, and retinopathy.[1)] Some physiolosical disorders such as gastroparesis, decreased plasma albumin level, elevated plasma free fatty acid level, glycosylation of plasma proteins, and changes in the cytochrome P-450 contents were reported to occur in diabetes mellitus patients.[1,2)] Such physiological changes could alter the pharmacokinetics and hence the pharmacodynamics of drugs in such patients. Animal models of insulin-dependent diabetes mellitus, induced by administration of several chemicals, principally alloxan, streptozotocin, or zinc chelators, have been reported.[3)] Effects of diabetes mellitus on the pharmacokinetics and/or pharmacodynamics of some drugs in the patients or rats with diabetes mellitus induced by alloxan have been reviewed.[1,2)] Changes in the pharmacokinetics and/or pharmacodynamics of furosemide,[4)] azosemide,[5)] DA-1131, a new carbapenem,[6)] DA-125, a new anthracycline,[7)] and adriamycin[8)] in rats with diabetes mellitus induced by alloxan have recently been reported. Some drugs are used to treat above mentioned secondary hypertention which result from diabetic complications. Diltiazem is one of these drugs. Diltiazem has no effects on glucose tolerance, insulin secretion, and platelet aggregation in patients with type II diabetes mellitus and is therefore useful in the treatment of hypertension induced in diabetic patients. Diltiazem inhib-

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its voltage-dependent L-type calcium channels and leads to vascular smooth muscle relaxation and negative inotroic and chronotropic effects in the heart. Diltiazem is almost completely absorbed after oral administration, but its bioavailability is reduced because of considerable first-pass hepatic metabolism.[9,10] Diltiazem is able to dilate renal vasculature and can increase the glomerular filtration rate and renal sodium excretion.[11,12,13]

Diltiazem has a large volume of distribution because of its liphopilicity and is rapidly and extensively distributed into body tissues.[14] Diltiazem is rapidly and almost completely metabolized in the liver deacetylation,[15,16] N-demethylation and O-demethylation to several active and at least 5 inactive metabolites via cytochrome P-450 enzyme system.[17] The drug and its also glucuronide and sulfate conjugation.[18] Diltiazem is excreted in urine unchanged from approximately 2-4% with renal failure patients.[19,20] The purpose of this study was to report the pharmacokinetic changes of AUC, Kel and total body clearance after intravenous administration of diltiazem to control rabbits, and acute and chronic alloxan-induced diabetes mellitus rabbits (AIDRs).

## Materials and Methods

### Materials

Diltiazem (DTZ), deacetyldiltiazem (DAD) and imipramine (the internal standard in high-performance liquid chromatographic, HPLC assay) and alloxan were obtained from Sigma Chemical Company (St. Louis, MO., USA).

HPLC grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). Urethane was a product from Junsei Chemical Company (Tokyo, Japan). Other chemicals were of reagent grade or HPLC grade, and therefore used without further purification.

Male New Zealand white rabbits (Dai Han Laboratory Animal Research Center, Eumsung, Korea) weighing 2.0-2.4 kg were fasted except water at least 24 hr before experiment.

### Induction of diabetes mellitus in rabbits

Alloxan (dissolved in normal saline injectable solution), 40 mg/kg, was administered intravenously via the ear vein (total injection volume was approximately 6-7 ml) on first and second days to the overnight-fasted rabbits. The same volume of normal saline injectable solution was administered to control group. On the fifth day, the blood glucose levels were measured and rabbits with blood glucose levels of greater than 230 mg/dl were chosen as an acute group. The same dose of alloxan was further administered on sixth and tenth days for a chronic group. On the thirteenth day, the blood glucose levels were measured and rabbits with glucose levels of greater than 300 mg/dl were chosen as a chronic group.

### Intravenous administration

On the fifth (for control and acute groups) or thirteenth (for chronic group) day, each rabbit was anesthetized with a subcutaneous injection of 25% urethane, 4 ml/kg. The polyethylene tube (Clay Adams, Parsippany, NJ) was cannulated into the right femoral artery and the urethra.

Diltiazem, 4 mg/kg, was administered intravenously (total iv volume was 4 ml) to ear vein of control group (n=6) and acute (n=6) and chronic (n=6) groups using a feeding tube after overnight fasting with water ad libitum. Blood samples (approximately 1.5 ml) were collected via the right femoral artery at 0 (to serve as a control), 0.125, 0.25, 0.5, 1, 2, 4, 6, 9, 12, and 24 h after intravenous administration of diltiazem. Heparinized normal saline injectable solution (0.25 ml; 75 units/ml), was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately at 5000 rpm for 5 min and plasma samples were stored at 30 until HPLC analysis of diltiazem.[25] Urine samples were collected via the exact volume of urine sample from the urethra between 0-2, 2-4, 4-6, 6-12, and 12-24 h after administration of the drug. The aliquot of urine samples were stored at 30 until HPLC analysis of diltiazem[25]. Each rabbit was kept in supine position during the entire experimental period.

### Plasma chemistry

At the end of experiment, plasma was stored at -30°C for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatinine, total proteins, and glucose using corresponding kits and a photometer (Model 5010, Böheringer Mannheim GmBH, Mannheim, Germany).

### HPLC analysis of diltiazem in plasma

The concentration of diltiazem in plasma and urine were analyzed by HPLC method reported previously

(Goebel and Kolle, 1985). Briefly, 0.2 ml aliquot of imipramine (2.5 μl/ml,dissolved in methanol) and a 5 ml aliquot of tert-butyl methyl ether were added to a0.5ml aliquot plasma and a 0.1 ml aliquot of urine samples, and vortex-mixed for 5 min. After centrifugation at 5000 rpm for 5 min, a 4.5 ml aliquot of organic phase was collected and a 0.3 ml aliquot of hydrochlonic acid (0.01 N) was added, mixed for 2 min, and centrifuged for 5 min. After the upper layer was discarded, a 0.2 ml aliquot was collected into micro-centriuge, and a 50 μl aliquot was injected onto the HPLC column.

### Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method,[21] this method employed the logarithmic trapezoidal rule for the calculation of area during the declining plasma-level phase[22] and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

The mean value of terminal half-life[23] was calculated by the harmonic mean method.

### Statistical analysis

A P value of less than 0.05 was considered to be statistically significant using student t- test among three means for paired data. All data were expressed as mean ±S.D.

## Results and Discussion

### Blood chemistry of liver and kidney

In acute and chronic AIDRs, impaired hepatic function was observed; the plasma concentrations of ALT were significantly higher (1.94 and 1.96 times, respectively) than that in control rabbits (Table 1). The plasma concentrations of AST and total proteins were higher and lower, respectively, in acute and chronic AIDRs(not significant difference of the parameters could be due to the limited numbers of rabbits employed, n=6) (Table 1).

In acute and chronic AIDRs, impaired kidney function was also observed; the plasma concentrations of creatinine in chronic AIDRs were significantly higher (1.49 times) than that in control rabbits, and urea nitrogen were higher in acute and chronic AIDRs (Table 1).

### Pharmacokinetic parameters

The mean plasma concentration-time profiles of diltiazem in control rabbits and acute and chronic AIDR are shown in Fig. 1, and relevant pharmacokinetic parameter are listed in Table 2. After intravenous administration, the plasma concentrations of diltiazem

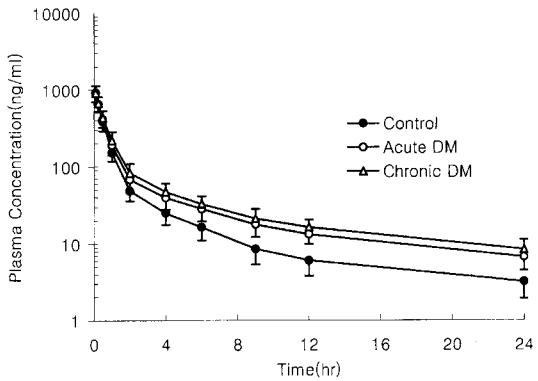


**Fig. 1. Plot of plasma concentration (ng/ml) of diltiazem in rabbits with alloxan-induced diabetes mellitus. Bars represent standard deviation.**

**Table 1. Mean (±S.D.) plasma chemistry data in control rabbits and rabbits with acute and chronic diabetes mellitus induced by alloxan**

| Parameter | Control (n=6) | Acute (n=6) | Chronic (n=6) |
|---|---|---|---|
| Aspartate aminotransferase (IU/l) | 63±16.2 | 74±18.3 | 78±20.6 |
| Alanine aminotransferase (IU/l) | 40±7.20 | 49±10.9 | 56±13.1[b] |
| Urea nitrogen (mg/dl) | 25.3±5.06 | 38.3±8.06 | 45.6±10.09[a] |
| Creatinine (mg/dl) | 1.08±0.21 | 1.27± 0.25 | 1.48±0.27[b] |
| Total proteins (g/dl) | 6.10±0.82 | 5.87±1.82 | 5.75±0.64 |
| Glucose (mg/dl) | 121±20.4 | 276±82.5 | 351±85.5[a] |

[a]Acute and chronic groups were significantly different ($P<0.05$) from control group.
[b]Chronic group was significantly different ($P<0.05$) from control group.
Aspartate aminotransferase (AST)
Alanine aminotransferase (ALT)

**Table 2. Mean (±S.D.) pharmacokinetic parameters of diltiazem after intravenously administration to control rabbits and acute or chronic alloxan-induced diabetes mellitus**

| Parameters \ Rabbits | Control (n=6) | Acute (n=6) | Chronic (n=6) |
|---|---|---|---|
| AUC (ng/ml·hr) | 835±155 | 1111±209 | 1263±236* |
| β ($hr^{-1}$) | 0.184±0.03 | 0.121±0.02* | 0.118±0.03* |
| $t_{1/2}$ (hr) | 3.76±0.65 | 5.70±0.96* | 5.83±1.01* |
| CLt (l/hr) | 0.014±0.015 | 0.013±0.013 | 0.011±0.011* |
| RB (%) | 100 | 133 | 151 |

*P<0.05 compared with control
β:β-phase rate constant
$t_{1/2}$: terminal half-life
AUC: area under the plasma concentration-time curve from time zero to time infinaty
RB: relative bioavailability compared to control rabbits
CLt: total body clearance

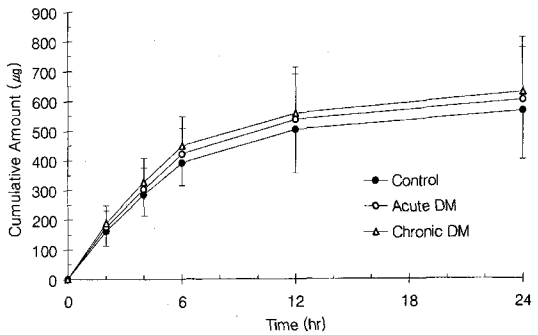


**Fig. 2. Mean cumulative urinary excretion (μg) of diltiazem after intravenously administration to control rabbits (●) and rabbits with acute (○) and chronic (△) Alloxan-Induced Diabetes Mellitus (n=6, each). Bars represent standard deviation.**

increased with reaching its peak at 3-4 h, then declined thereafter in a monoexponential fashion for all groups of rabbits studied (Fig. 1). The plasma concentrations of diltiazem in acute and chronic AIDR were higher than those of control rabbits (Fig. 1), and this resulted in a significantly greater AUC, 1111 and 1263 vs 8351 μg · ml/hr (Table 2). The higher plasma concentrations and significantly greater AUC in acute and chronic AIDRs than that in control rabbits could be due to slower renal excretion of diltiazem because of the impaired kidney function as mentioned earlier. It was reported[24] that kidney is perhaps next in importance to the pancreas as a site of lesion in alloxan diabetes and the severest damage occurs in the convoluted tubules and appears to be generally proportional to the size of the dose. Elimination rate constant and total body clearance were significantly decreased compared to these in control. Biological half-life were significantly prolonged.

**Cumulative urinary excreted amount**

For comparison, the renal clearance of diltiazem was calculated by dividing total amount of diltiazem excreted unchanged in 24-h urine ($Ae_{0\text{-}24h}$) by $AUC_{0\text{-}24h}$ of diltiazem; the renal clearance values in acute and chronic AIDRs were approximately 3 and 2 times slower, respectively, than that in control rabbits. Cumulative amount of urinary excretion of diltiazem kept increased with increasing time for up to 24 hr compared to control but not significant. Although the metabolic clearance was not measured in the present study, the higher plasma concentrations and significantly greater AUC in acute and chronic AIDRs could be at least partly due to slower metabolism of diltiazem than that in control rabbits because of impaired hepatic function in the rabbits. Note that the effects of the diabetes on the pharmacokinetics of intravenous diltiazem were more considerable in chronic AIDRs; the AUC was significantly greater than that in acute AIDRs and Cmax were significantly higher than that in control rabbits.

## Conclusion

Because physiological changes occurring in diabetes mellitus patients could alter the pharmacokinetics of the drugs used to treat hypertension resulting from diabetic complications, the pharmacokinetics of diltiazem were investigated after intravenous administration of the drug (4 mg/kg) to control rabbits and in acute and chronic alloxan-induced diabetes rabbits (AIDR). Impaired kidney and liver functions were observed in acute and chronic AIDRs based on plasma chemistry data. After intravenous administration of diltiazem to rabbits with acute and chronic diabetes, the plasma concentrations were higher and this resulted in a significantly greater area under the plasma concentration-time curve from time zero to time 24hr than control rabbits. This could be due to significantly slower renal clearance because of impaired kidney function. The effects of diabetes on the pharmacokinetics of diltiazem were more considerable in rabbits with chronic diabetes ; the AUC was significantly greater in acute AIDRs and in chronic AIDRs than that in control rabbits. Total body clearace and elimintion rate constant were significantly decreased

compared to these in control. No significant change has been shown in cumulative urinary excretion of diltiazem among acute and chronic AIDRs and control rabbits. These findings suggest that in acute and chronic AIDRs, the hepatic metabolism of diltiazem was inhibited due to liver impairment and elimination rate constant was decreased due to kidney impairment.

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