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# Effects of dapagliflozin in combination with insulin on cytochrome P450 activities in a diabetes type 1 rat model

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**ABSTRACT.** Previous studies reported that diabetes alters the activities of hepatic cytochrome P450 (CYP) enzymes, which, in turn, affects the disposition of some drugs. We herein examined and compared the effects of the combination of dapagliflozin with a low insulin dose, a full dose of insulin alone, and dapagliflozin alone for 3 and 8 weeks on CYP activities in a diabetes type 1 rat model. We induced type 1 diabetes in rats using a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ). Daily treatment with the full dose of insulin alone, dapagliflozin alone, or dapagliflozin in combination with a low dose of insulin was then initiated. STZ-induced rats developed marked hyperglycemia and altered CYP2E activities. Dapagliflozin in combination with a low dose of insulin stabilized hyperglycemia and CYP1A, 2D, 2E and 3A activities. However, dapagliflozin alone did not improve blood glucose levels or CYP activities. These results suggest that the effects of dapagliflozin in combination with a low dose of insulin are similar to those of a full dose of insulin, and stabilize CYP activities in type 1 diabetes.

**KEY WORDS:** cytochrome P450, dapagliflozin, insulin, type 1 diabetes

Hepatic cytochrome P450 (CYP) is a large enzyme family involved in the first phase of drug metabolism and plays a major role in the detoxification of a wide range of xenobiotics and endogenous substances prior to further detoxification using conjugation reactions in the second phase of drug metabolism. Enzymes in CYP families 1, 2, and 3 are crucially involved in the metabolism and pharmacokinetics of numerous xenobiotics [17, 22]. Changes in CYP activities may alter the elimination of drugs metabolized by CYP. Furthermore, the co-administration of drugs that are metabolized by the same CYP enzymes may increase the risk of side effects and drug toxicity or decrease drug efficacy [9].

Changes in physiological and pathophysiological conditions, such as diabetes mellitus, have been shown to affect drug metabolism and disposition [14]. Diabetes mellitus is a commonly occurring disease in which plasma glucose control is defective because of an insulin deficiency or decreased target cell responsiveness to insulin [30]. Type 1 diabetes (T1D) is characterized by the destruction of pancreatic β-cells and generally leads to absolute insulin deficiency [6]. Previous studies demonstrated that uncontrolled diabetes mellitus altered the disposition of a number of drugs [33], potentially increasing the risk of drug toxicity and side effects or decreasing drug efficacy. Diabetes has been reported to affect the expression and activities of CYP1A, CYP2B, CYP2E1, CYP3A, CYP4A, and other drug-metabolizing enzymes [10]. Due to insulin alterations in diabetes mellitus, insulin may mediate changes in the expression of drug-metabolizing enzymes. The administration of insulin to chemically induced or spontaneously diabetic rats was shown to restore the activities and expression of drug-metabolizing enzymes.

Insulin has been approved for the treatment of T1D, but with a black box warning for hypoglycemia. The kidneys may become a therapeutic target with the advent of insulin-independent, selective, orally active glucosuric agents [18]. Dapagliflozin, a highly selective, orally active inhibitor of sodium–glucose cotransporter 2 (SGLT2), has been shown to attenuate hyperglycemia by inhibiting renal glucose reabsorption independently of insulin [31]. SGLT2 is predominantly expressed in the renal proximal tubes.

We previously reported the stronger hypoglycemic effects of combination therapy with dapagliflozin and a low dose of insulin than monotherapy with dapagliflozin or insulin. Our findings also showed that dapagliflozin in combination with a low dose of insulin significantly attenuated hyperglycemia, hypercholesterolemia, and hypertriglyceridemia [23].

Since diabetes mellitus requires long-term treatment, its effects on the drug-metabolizing enzyme CYP need to be elucidated.

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Therefore, the aim of the present study was to examine and compare the effects of dapagliflozin in combination with a low dose of insulin, dapagliflozin alone, and a full dose of insulin alone on CYP activity in liver microsomes collected from a rat model of T1D in our previous study [23].

## MATERIALS AND METHODS

### *Materials*

*Animals*: Forty 7-week-old male Sprague-Dawley rats were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). They were kept for one week for adaptation prior to the initiation of experiments. Rats were housed in stainless steel cages at a controlled temperature of 21 ± 1°C with a 12-hr light/dark cycle and *ad libitum* access to a standard commercial diet from Sankyo Labo Service Corporation, Inc. and water. All animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, and the present study was approved by the Ethics Committee of the Faculty of Agriculture, Tokyo University of Agriculture and Technology (approval number 28-37).

*Chemicals*: Resorufin, ethoxy resorufin, and bufuralol hydrochloride were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). 1-Hydroxybufuralol, 4-hydroxymidazolam, and 1-hydroxymidazolam were obtained from Daiichi Pure Chemicals (Tokyo, Japan). P-aminophenol and aniline sulfate were purchased from Wako Pure Chemicals (Osaka, Japan).

*Diabetes induction*: Streptozotocin (STZ) was dissolved in 0.1 M citrate buffer (pH=4.5). Rats were pretreated with a single intraperitoneal injection of 60 mg/kg STZ to induce experimental diabetes. The manifestations of diabetes mellitus were confirmed by measuring blood glucose levels 72 hr after the STZ injection.

*Experimental design*: After a diabetic state had been confirmed in STZ-injected rats, animals were grouped as follows: 3-week groups: group 1 (n=4), normal animals; group 2 (n=4), untreated diabetic animals; group 3 (n=4), diabetic animals treated with 0.1 mg/kg of dapagliflozin orally once a day [13]; group 4 (n=4), diabetic animals treated with insulin. Insulin doses were individually adjusted to maintain a normoglycemic state, varied between 3–5 U/rat, and were subcutaneously administered once a day. In group 5 (n=4), diabetic animals received a combination of 0.1 mg/kg of dapagliflozin orally once a day and insulin at a dose of 1.5–2.5 U/rat that was administered subcutaneously once a day (half the insulin dose of group 4) [23].

The 8-week groups consisted of group 1 (n=4), normal animals; group 2 (n=4), untreated diabetic animals; group 3 (n=4), diabetic animals treated with 0.1 mg/kg of dapagliflozin orally once a day; group 4 (n=4), diabetic animals treated with insulin. Insulin doses were individually adjusted to maintain a normoglycemic state, varied between 3–5 U/rat, and were subcutaneously administered once a day. In group 5 (n=4), diabetic animals received a combination of 0.1 mg/kg of dapagliflozin orally once a day and insulin at a dose of 1.5–2.5 U/rat that was administered subcutaneously once a day (half the insulin dose of group 4).

Blood glucose levels were measured once a week from a drop of blood obtained by tail vein puncture using Glutest Neo Alpha and Glutest New Sensor purchased from Sanwa Kagaku Kenkyusho Co., Ltd. (Nagoya, Japan). Animals in the 3- and 8-week studies were sacrificed after 3 and 8 weeks of treatment, respectively.

At the end of each study, animals were anesthetized by inhalation anesthesia. The liver was perfused with microsome buffer via the portal vein to remove all blood, cut into pieces, and kept at −80°C for the preparation of microsomes.

*Preparation of hepatic microsomes*: The microsomal fractions were prepared from the liver specimens using the differential centrifugation method described before [28]. The obtained microsomal suspension was stored at −80°C until being used. The concentrations of protein and contents of CYP were measured as reported before, [3] and [21], respectively.

### *Microsomal enzyme assays*

CYP1A, 2D, 2E, and 3A enzyme activities were assessed using ethoxy resorufin O-deethylation, bufuralol 1'-hydroxylation, aniline hydroxylation, and midazolam 4-hydroxylation as the metabolic reactions of specific substrates, respectively.

*Measurement of CYP1A activity*: The metabolite of ethoxy resorufin, resorufin, was measured using the fluorometric method described by Burke *et al.* [4]. Pre-NADP (840 µl) was added to a 20-fold diluted microsomal suspension (100 µl), and the mixture was pre-incubated at 37°C in a shaking water bath for 5 min. β-NADP (50 µl) and the substrate, ethoxy resorufin solution (10 µl) were then added to initiate enzyme reactions. The concentrations of ethoxy resorufin ranged between 0.5 and 10 µM. Reactions were terminated by the addition of 3 ml of methanol 15 min after the addition of the substrate, followed by placement on ice for 5 min. Following centrifugation at 2,000 × *g* for 5 min, 1 ml of the supernatant was taken and diluted with 4 ml of methanol in a clean tube and applied to a spectrofluorometer (RF-1500; Shimadzu Co., Kyoto, Japan). Fluorescence was monitored at 550 nm (excitation) and 586 nm (emission).

*Measurement of CYP2D activity*: The metabolite of bufuralol, 1`-hydroxybufuralol, was analyzed by HPLC, as described by Kronbach *et al.* [13]. Pre-NADP (200 µl) was added to the microsomal suspension (20 µl), and the mixture was pre-incubated at 37°C in a shaking water bath for 5 min. β-NADP (20 µl) and bufuralol solution (10 µl) were then added to initiate enzyme reactions. The concentrations of bufuralol ranged between 4 and 60 µM. The enzyme reaction was performed for 10 min, and then terminated by the addition of perchloric acid (30 µl) to the sample. The sample was centrifuged at 12,000 rpm/2 min/4°C. The denatured protein was precipitated, the supernatant was filtered, and 20 µl of the filtrate was injected into a C18 HPLC column (TSK-gel ODS-120T, 4.6 × 250 mm; TOSO Co., Tokyo, Japan). The mobile phase was 1 mM perchloric acid and acetonitrile (65:35) and the flow rate was 1 ml/min. Excitation and emission wavelengths were 252 and 302 nm, respectively.

*Measurement of CYP2E activity*: The metabolite of aniline sulphate was measured using HPLC as previously reported by Noguchi *et al.* [19]. Pre-NADP (200 µl) was added to the microsomal suspension (20 µl), and the mixture was pre-incubated at

37°C in a shaking water bath for 5 min. β-NADP (20 µl) and aniline sulphate solution (10 µl) were then added to initiate enzyme reactions. The concentration of aniline sulphate ranged between 0.5 and 10 mM. The enzyme reaction was performed for 10 min, and was then terminated by the addition of acetonitrile (250 µl) to the sample. The sample was centrifuged at 15,000 rpm/6 min/4°C. The supernatant was filtered and 20 µl of the filtrate was immediately analyzed using a $C_{18}$ HPLC column (Mightysil RP-18GP 4.6 × 250 mm, Kanto Chemical Co., Tokyo, Japan) to assess the concentrations of aniline sulphate metabolites. The mobile phase consisted of disodium hydrogen phosphate pH 7.5 and acetonitrile. The column effluent was monitored by UV absorbance at 300 nm. The flow rate was 1 ml/min.

*Measurement of CYP3A activity*: The metabolite of midazolam, 4-hydroxymidazolam was measured using HPLC as previously reported by Von Moltke *et al.* [29]. Pre-NADP (200 µl) was added to the microsomal suspension (20 µl), and the mixture was pre-incubated at 37°C in a shaking water bath for 5 min. β-NADP (20 µl) and midazolam solution (10 µl) were then added to initiate enzyme reactions. The concentration of midazolam ranged between 7 and 250 µM. The enzyme reaction was performed for 10 min, and was then terminated by the addition of acetonitrile (250 µl) to the sample. The sample was centrifuged at 15,000 rpm/6 min/4°C. The supernatant was filtered and 20 µl of the filtrate was immediately analyzed using a $C_{18}$ HPLC column (TSK-gel ODS-120T, 4.6 × 250 mm; TOSO Co., Tokyo, Japan). The mobile phase consisted of sodium acetate trihydrate pH 4.7 and acetonitrile. The column effluent was monitored by UV absorbance at 254 nm. The flow rate was 1 ml/min.

*Michaelis-Menten kinetic analysis*: The formation of each metabolite was consistent with single-enzyme Michaelis-Menten kinetics. Therefore, the following equation was fit to the observed data using the non-linear least-squares regression to calculate maximum velocity (Vmax) and the Michaelis constant (Km):

$$V=\frac{Vmax\times S}{Km+S}$$

*Statistical analysis*: Data are presented as the mean ± standard deviation (SD). An analysis of variance (ANOVA) with Tukey's *post hoc* multiple comparison test was used in the statistical analysis of data. *P* values <0.05 were considered to be significant. Statistical analyses were conducted using GraphPad Prism 7 (GraphPad software, San Diego, CA, USA).

## RESULTS

Hyperglycemia was more severe in the untreated diabetic and dapagliflozin-treated groups than in the control group. However, the daily administration of insulin alone or in combination with dapagliflozin significantly decreased blood glucose levels in the 3- and 8-week studies (Table 1). Changes in glucose concentrations over time were similar to those previously reported [23].

In the 3-week study, CYP concentrations were significantly higher in the dapagliflozin-treated group than in the control group, but were significantly lower in the insulin-treated and combination-treated groups than in the dapagliflozin-treated group. Hepatic CYP concentrations did not significantly differ among the groups in the 8-week study (Table 2).

As shown in Fig. 1, CYP2E activities increased in the untreated diabetic and dapagliflozin-treated groups in the 3- and 8-week studies. However, CYP2E activities were similar between the insulin-treated and combination-treated groups and the control group. CYP1A activities increased in the untreated diabetic and dapagliflozin-treated groups in the 3- and 8-week studies. However, CYP1A activities were similar between the insulin-treated and combination-treated groups and the control group. In the 3-week study, CYP3A activity decreased in the untreated diabetic group. However, CYP3A activities were similar between the dapagliflozin-treated, insulin-treated, and combination-treated groups and the control group. CYP2D activities were similar in all

**Table 1.** Blood glucose levels after the administration of dapagliflozin alone, insulin alone, or a combination of both for 3 and 8 weeks

| | | Control | Untreated diabetic | Dapagliflozin | Insulin | Combination of dapagliflozin and insulin |
|---|---|---|---|---|---|---|
| Blood glucose (mg/dl) | 3-week | 118 ± 15.12 | 537* ± 93.98 | 400* ± 76.33 | 93.25$^{\#\$}$ ± 8.26 | 178.5$^{\#\$}$ ± 85.49 |
| | 8-week | 135 ± 18.38 | 596* ± 8.00 | 501* ± 145.35 | 117$^{\#\$}$ ± 52.84 | 114 $^{\#\$}$ ± 44.35 |

Each value is represented by the mean ± SD (n=4). *$P$<0.05 versus the control group, $^{\#}P$<0.05 versus the untreated diabetic group, $^{\$}P$<0.05 versus the dapagliflozin-treated group.

**Table 2.** Microsomal cytochrome P450 concentrations after the administration of dapagliflozin alone, insulin alone, or a combination of both for 3 and 8 weeks

| | | Control | Untreated diabetic | Dapagliflozin | Insulin | Combination of dapagliflozin and insulin |
|---|---|---|---|---|---|---|
| CYP concentration (nmol/mg protein) | 3-week | 0.90 ± 0.07 | 1.05 ± 0.23 | 1.22* ± 0.15 | 0.83$^{\$}$ ± 0.09 | 0.93$^{\$}$ ± 0.12 |
| | 8-week | 0.79 ± 0.13 | 1.09 ± 0.27 | 1.08 ± 0.26 | 0.88 ± 0.12 | 0.89 ± 0.14 |

Each value is represented by the mean ± SD (n=4). *$P$<0.05 versus the control group, $^{\$}P$<0.05 versus the dapagliflozin-treated group.

groups in the 3- and 8-week studies.

Table 3 shows the Vmax values of reactions catalyzed by CYP1A, CYP2D, CYP2E, and CYP3A in hepatic microsomes obtained from rats treated for 3 to 8 weeks. In comparisons with the control group, Vmax values in the untreated diabetic group at 8 weeks were significantly higher for aniline hydroxylation. However, Vmax values for aniline hydroxylation were similar between the insulin-treated and combination-treated groups and the control group. The values of the other reactions were also not significantly different from those in the control group.

As shown in Table 4, the Km values for ethoxy resorufin O-deethylation and midazolam 4-hydroxylation were significantly lower in the dapagliflozin-treated, insulin-treated, and combination-treated groups than in the control group, whereas the Km values for the other reactions did not significantly differ. The Km values for ethoxy resorufin O-deethylation and midazolam 4-hydroxylation in control changed from 3-week to 8-week.

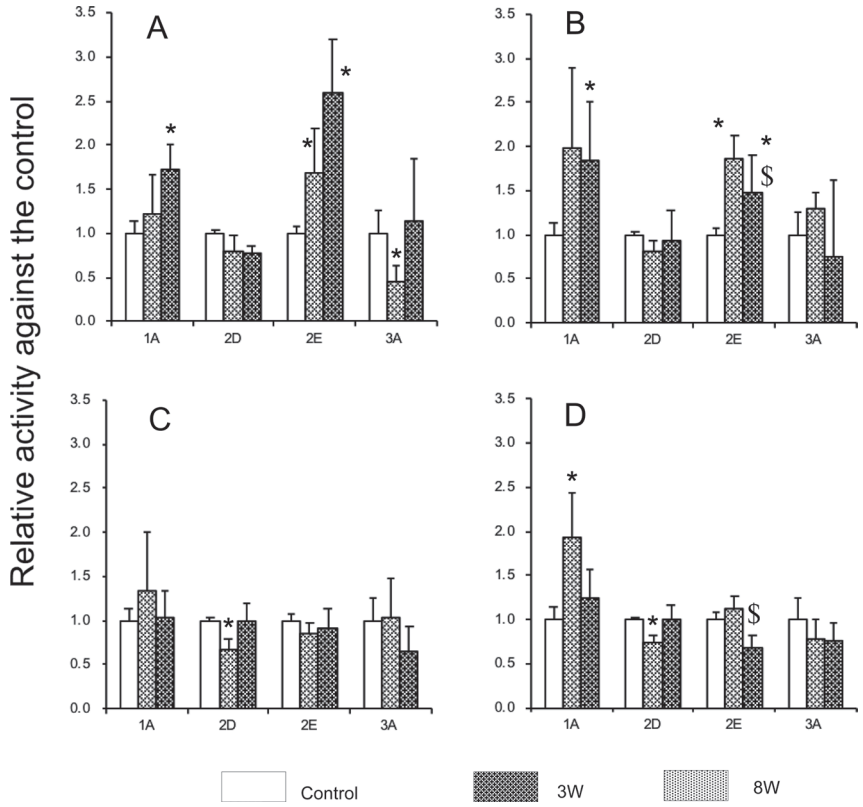


**Fig. 1.** Effects of dapagliflozin alone, insulin alone, or a combination of both on metabolic reactions catalyzed by cytochrome P450 1A, 2D, 2E, and 3A in 3-week and 8-week studies. **A**: untreated diabetic group, **B**: dapagliflozin-treated group, **C**: insulin-treated group, **D**: group treated with a combination of dapagliflozin and a low dose of insulin. Bars represent relative reaction activities against Vmax of the control, error bars indicate SD of the mean (n=4), **P*<0.05, significantly different from the control. $^{\$}P$<0.05 versus 2E activities of the untreated diabetic in 8-week.

**Table 3.** Results of maximum velocity (Vmax) values of cytochrome P450 isozymes

| | CYP activity | | Control | Untreated diabetic | Dapagliflozin | Insulin | Combination of dapagliflozin and insulin |
|---|---|---|---|---|---|---|---|
| | | | (nmol/min/mg protein) | | | | |
| Ethoxy resorufin O-deethylation | CYP1A | 3-week | 0.58 ± 0.29 | 0.73 ± 0.43 | 0.65 ± 0.16 | 0.39 ± 0.24 | 0.61 ± 0.20 |
| | | 8-week | 0.39 ± 0.08 | 0.66 ± 0.09 | 0.67 ± 0.26 | $0.34^{\#\$}$ ± 0.11 | 0.44 ± 0.13 |
| Bufuralol 1'-hydroxylation | CYP2D | 3-week | 2.49 ± 0.10 | 1.89 ± 0.41 | 2.13 ± 0.48 | 1.75 ± 0.39 | 1.87 ± 0.33 |
| | | 8-week | 1.65 ± 0.32 | 1.24 ± 0.32 | 1.43 ± 0.75 | 1.61 ± 0.53 | 1.58 ± 0.32 |
| Aniline hydroxylation | CYP2E | 3-week | 1.01 ± 0.09 | 2.52* ± 0.86 | 2.07 ± 1.37 | 0.93 ± 0.27 | 1.55 ± 0.86 |
| | | 8-week | 1.31 ± 0.34 | 3.72* ± 0.76 | 2.74 ± 1.64 | $1.28^{\#}$ ± 0.40 | $1.06^{\#\$}$ ± 0.55 |
| Midazolam 4-hydroxylation | CYP3A | 3-week | 0.99 ± 0.26 | 0.44 ± 0.19 | $1.37^{\#}$ ± 0.25 | $1.05^{\#}$ ± 0.44 | $0.81^{\$}$ ± 0.24 |
| | | 8-week | 1.11 ± 0.22 | 1.26 ± 0.86 | 0.82 ± 0.99 | 0.70 ± 0.31 | 0.82 ± 0.25 |

Each value is represented by the mean ± SD (n=4). **P*<0.05 versus the control group, $^{\#}P$<0.05 versus the untreated diabetic group, $^{\$}P$<0.05 versus the dapagliflozin-treated group.

**Table 4.** Results of Michaelis constant (Km) values of cytochrome P450 isozymes

| | CYP activity | | Control | Untreated diabetic | Dapagliflozin | Insulin | Combination of dapagliflozin and insulin |
|---|---|---|---|---|---|---|---|
| | | | (μM) | | | | |
| Ethoxy resorufin O-deethylation | CYP1A | 3-week | 7.89 ± 3.66 | 7.75 ± 2.94 | 2.07*$^{\#}$ ± 0.34 | 2.02*$^{\#}$ ± 0.46 | 2.46*$^{\#}$ ± 0.47 |
| | | 8-week | 2.89$^{\$}$ ± 0.23 | 2.70 ± 0.25 | 2.43 ± 0.28 | 1.92* ± 0.35 | 2.26 ± 0.53 |
| Bufuralol 1'-hydroxylation | CYP2D | 3-week | 8.32 ± 0.39 | 9.00 ± 0.99 | 16.97 ± 7.64 | 13.82 ± 4.51 | 13.44 ± 3.85 |
| | | 8-week | 12.14 ± 4.46 | 11.45 ± 8.86 | 10.33 ± 6.89 | 11.75 ± 7.44 | 10.02 ± 3.56 |
| Aniline hydroxylation | CYP2E | 3-week | 1.64 ± 0.35 | 4.62 ± 1.96 | 3.94 ± 3.34 | 2.40 ± 1.02 | 6.10 ± 5.19 |
| | | 8-week | 2.77 ± 2.87 | 2.75 ± 1.22 | 3.72 ± 5.09 | 3.08 ± 3.55 | 4.66 ± 5.30 |
| Midazolam 4-hydroxylation | CYP3A | 3-week | 11.07 ± 4.71 | 14.64 ± 1.07 | 22.40* ± 4.42 | 20.53* ± 2.01 | 19.32* ± 5.01 |
| | | 8-week | 27.54$^{\$}$ ± 2.43 | 27.19 ± 21.29 | 22.62 ± 11.44 | 25.95 ± 6.01 | 17.94 ± 5.08 |

Each value is represented by the mean ± SD (n=4). *$P$<0.05 versus the control group, $^{\#}P$<0.05 versus the untreated diabetic group, $^{\$}P$<0.05 versus control group for 3-week.

## DISCUSSION

Dapagliflozin, a competitive and highly selective inhibitor of SGLT2 [8], reduces renal glucose reabsorption, increases renal glucose excretion, and attenuates hyperglycemia [12]. Since dapagliflozin acts independently of insulin, it may provide additional glycemic control when used in combination with insulin [5]. In the present study, the insulin-treated and combination-treated groups showed marked decreases in blood glucose levels that were similar to those in the control group (Table 1).

Hepatic CYP concentrations did not significantly differ between any of the groups in the 8-week study (Table 2). These results are consistent with the findings reported by Oh *et al.* [20], who found no significant differences in CYP concentrations between diabetic and control rats.

CYP2E is constitutively expressed not only in the liver, but also in many other tissues, and promotes the generation of reactive oxygen species, which, in turn, enhance oxidative stress in animals. Therefore, CYP2E is clinically and toxicologically important. CYP2E1 metabolizes a wide variety of chemicals with different structures, particularly low-molecular-weight compounds and hydrophobic compounds, such as fatty acids, lipid hydroperoxides, and ketone bodies [16]. In the present study, the untreated diabetic and dapagliflozin-treated groups showed increased CYP2E activities against control groups, as shown in Fig. 1 and Table 3. However, CYP2E activities were similar between the insulin-treated and combination-treated groups and the control group. Hepatic CYP2E1 expression was previously shown to be up-regulated in diabetes [32], and was attributed to elevated ketone body levels in rats [2]. Furthermore, the expression of CYP2E1 was down-regulated by insulin in primary cultured rat hepatocytes [32]. Treatment of dapagliflozin alone for 8 weeks slightly suppressed the increase in aniline hydroxylation compared to untreated diabetic (Fig. 1), although the improvement in blood glucose level was insufficient (Table 1). Further research may be needed to confirm this interesting finding.

CYP1A, which is constitutively expressed in the liver, is primarily involved in the oxidative metabolism of xenobiotics and is capable of metabolically activating numerous procarcinogens [24]. The hepatic expression and activity of CYP 1A2 were previously reported to be up-regulated in chemically induced diabetic animals [11]. These findings are consistent with the present results showing that CYP1A activity increased in the untreated diabetic and dapagliflozin-treated groups in the 8-week study. However, similar activities were observed between the insulin-treated and combination-treated groups and the control group (Fig. 1). The present results appear to agree with the findings reported by Sindhu *et al.* [26], who showed that ethoxy resorufin O-deethylase activity was significantly induced in diabetic rats and decreased to control levels after a treatment with insulin.

The CYP3A subfamily contributes to the biotransformation of 55% of marketed medications [7] as well as many endogenous substrates (cortisol, estradiol, progesterone, and testosterone) [1]. In the 3-week study, CYP3A activity decreased in the untreated diabetic group (Fig. 1). Discrepancies have been reported for the activity of CYP3A2 in rats with experimental diabetes. Thummel and Schenkman *et al.* [27] noted an increase in enzyme activity, whereas Shimojo *et al.* [25] found a decrease in diabetic rats. However, in the present study, CYP3A activities were similar between the insulin-treated and combination-treated groups and the control group. In the 8-week study, CYP3A activities were similar in all groups, and, unfortunately, we cannot currently provide an explanation for these results.

CYP2D activities were similar in all groups in the 8-week study. This was consistent with the findings of another study, which demonstrated that the protein expression of hepatic CYP2D1 was not changed in a rat model of diabetes mellitus induced by STZ [15].

In summary, the present results showed that hyperglycemia increased CYP2E activity. The attenuation of hyperglycemia with a full dose of insulin or dapagliflozin in combination with a low dose insulin prevented changes in CYP2E activity. Regarding CYP1A, 2D, 2E, and 3A activities examined in the present study, no changes were observed in CYP activity when hyperglycemia was ameliorated. Therefore, CYP activity did not change with the long-term administration of a full dose of insulin or dapagliflozin in combination with a low dose of insulin, and, thus, there was no risk of alterations in the metabolism of drugs administered at the same time. However, further investigations are required.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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