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Research Article
Evaluating Cytochrome P-2E1 Induction in Diabetic Rats and Rabbits

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Abstract

Background and Objective: Diabetes is one of the most complicated health problems worldwide. Alteration in the amount of CYP450 enzymes during this disease may affect the metabolism of drugs and xenobiotics. In this study, the severity of CYP2E1-induction in different weeks after the onset of diabetes is compared in two different but useful species of experimental animals, rats and rabbits. **Materials and Methods:** Thirty male Wistar rats and 24 adult male New Zealand rabbits were randomly divided into two groups, diabetics and non-diabetics. Streptozocin (50 mg kg^{-1}, i.p.) and alloxan (100 mg kg^{-1}, i.v.) were injected as diabetogenic agents respectively in rats and rabbits. In 3 subsequent weeks (3, 4 and 5 weeks post diabetes induction in rats and 5, 6 and 7 weeks post diabetes induction in rabbits) animals were sacrificed and CYP2E1 proteins levels were determined by western blotting. **Results:** The volume of CYP2E1 protein in diabetic rabbits was significantly higher at 6 weeks post diabetes induction than 5 and 7 weeks post diabetes induction ($p<0.01$, $p<0.05$, respectively). Moreover CYP2E1 level was higher in the 7th week compared to the 5th week ($p<0.05$). The CYP2E1 protein levels in rats who were sacrificed 4 and 5 weeks after diabetes induction were significantly higher than the rats that were killed in the 3rd week ($p = 0.05$). Moreover, the complications in inducing diabetes in these two experimental animals are elaborated. **Conclusion:** The present results showed that diabetes could induce the amount of CYP2E1 proteins in rats and rabbits by the optimum value in the 4th and 6th week after diabetes induction.

Key words: Cytochrome P-450 CYP2E1, diabetes mellitus, western blotting

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The high morbidity and mortality rate made diabetes one of the most important health problems all over the world[1]. Different complications in diabetic patients could be controlled in favour of the extended use of drugs[2]. CytochromeP450 (CYP450) enzymes expression, which has a major role in the drug metabolism pathways, may alter due to diabetes, affecting the pharmacokinetics of these drugs[3]. So it seems necessary to evaluate each drug metabolism in diabetic patients, find its specific kinetic and optimize the dose adjustments.

The CYP2E1 plays a decisive role in the metabolism of a great number of drugs and other exogens. Different investigations show the induction of CYP2E1 due to diabetes[4,5], some of them are managed in rats[6,7] and a few studies on rabbits[5,7]. Moreover, it has been proved that this enzyme protein is not elevated in diabetic mice livers[8], elucidating its species-dependent regulation. Moreover, the CYP2E1 enzyme has been assessed in the different durations after diabetes induction[6,9-11].

While rabbits are preferred to be used in pharmacokinetic studies[12], rats are more selected as diabetic experimental animals[6]. So in the case of analyzing pharmacokinetics of a drug used in diabetes, which experimental animal should be selected for establishing primary phases?

Streptozotocin and alloxan were used to induce diabetes, respectively in rats and rabbits[13]. The challenges faced in the use of rats and rabbits as diabetic samples have been noted too. In this study, the intensity of the CYP2E1 induction in different weeks after diabetes induction was evaluated in rats and rabbits.

MATERIALS AND METHODS

Study area: This study was conducted from January, 2019 to February, 2020 in the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Chemicals: Most of the biochemical and substrates such as Alloxan monohydrate (A7413-25G), Streptozocin (S0130-1G), bovine serum albumin, phenylmethylsulfonyl fluoride (PMSF), sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich Chemical Company (Germany). The HRP-linked, anti-rabbit IgG and Polyclonal antibodies against CYP2E1 were obtained from Abcam (ab28146).

All other chemicals were taken from mercantile sources at the highest degree of purity accessible.

Animals: Thirty male Wistar rats (250-350 g) and 24 adult male New Zealand rabbits (1.5-2.5 kg) were obtained from the animal house of medical university, Mashhad, Iran. Animals were housed in plastic cages covered by wood chips, fed a standard laboratory diet and water *ad lib* and exposed to a 12 hrs light: 12 hrs dark cycle, at a room temperature of 18-22°C. The experiment was approved by the Animal Welfare Committee of the Mashhad University of Medical Science.

Induction of diabetes mellitus in rats and rabbits by streptozotocin or alloxan injection, respectively: After 1 week of acclimatization, the animals were randomly divided into two equal groups, diabetics and non-diabetics.

At first, to determine the exact dose, we injected streptozocin (60 mg kg^{-1} b.wt., intraperitoneally) in three rats but they could not tolerate and survive. In three other rats, we reduce the dose to 50 mg kg^{-1} b.wt. and all of them survived and had persistent hyperglycemia after three days. Moreover, 40 mg kg^{-1} streptozocin could not induce diabetes in our animals.

Diabetes was induced by a single intraperitoneal injection of a buffered solution (0.1 mol L^{-1} citrate, pH 4.5) of streptozocin at a dosage of 50 mg kg^{-1} b.wt., in fasting rats. Non-diabetic rats received vehicles alone. The animals were considered diabetic when their blood glucose values exceeded 200 mg dL^{-1} (range 200-500). The non-diabetic and diabetic rats were caged separately and housed under the same conditions. An equal number of animals (5 rats) were sacrificed in 3 subsequent weeks (3, 4 and 5 weeks) and their livers were separated immediately and kept at -80°C. Selected weeks were chosen according to the previous studies[9].

Rabbits were given a single dose of alloxan (100 mg kg^{-1}) solved in saline solution (5%) by intravenous injection into the lateral ear after overnight fasting. They have sedated with an intramuscular injection of 30 mg kg^{-1} ketamine and 3 mg kg^{-1} xylazine just before the injection of alloxan[14]. Control animals were injected with a similar volume of saline. Complete care including heart rate, respiratory rate and body temperature monitoring during the anaesthetic phase, was considered. To avoid hypoglycemic shock after alloxan injection, 10 mL glucose 5% was subcutaneously injected and for 1 day the rabbits received 2 g kg^{-1} glucose in water orally. Blood was collected 3 and 5 days after injection from an ear vein to measure the glucose concentrations of both the control and the alloxan-treated rabbits. The animals were considered diabetic if the blood glucose level was >200 mg dL^{-1}. If the rabbits were not diabetic, after 10 days, they received another reduced dose of alloxan (75 mg kg^{-1}). This procedure continues at most to four times, every time with a lower

concentration. The glucose levels and body weight were measured up to the end of the investigation. The treated and the age-matched control rabbits (4 in each) were euthanized at the end of the 5th, 6th and 7th week after diabetes induction and the livers were prepared and kept at -80°C. Selected weeks were chosen according to the previous studies[8].

The animals in all groups were weighed before the administration of streptozocin and alloxan and also before their sacrifice.

Western blot analysis: The protein extracts from the liver tissue were prepared using a lysis buffer (50 mM Tris-HCl, pH 7.6, 0.5% Triton X-100 and 20% glycerol). The extracts were then centrifuged (15,000 g, 15 min at 4°C). The supernatant fractions were tested to evaluate protein concentration using a Bradford reagent (Bio-Rad, Richmond, CA). Liver homogenates from non-diabetic and diabetic animals were checked by Western blot analysis of CYP2E1 (Abcam, USA) as described in previous work[15]. Horseradish peroxidase-conjugated IgG (Abcam, USA) (diluted 1/3000 with TBST) was used as a secondary antibody.

Statistical analysis: Data were expressed as Mean±SEM. Statistical analysis was performed using GraphPad Prism software 6.0. (GraphPad, San Diego, CA, USA) by one-way analysis of variance (ANOVA), followed by Tukey *post hoc* analysis. The $p<0.05$ was chosen as the significant level.

RESULTS

During the experimental study, the animals from the normal control group and streptozocin or alloxan-treated groups remained alive. The animals in the control groups appeared healthy, active and gained body weight while the ill-looking animals in various diabetic groups had symptoms such as polydipsia and polyphagia with increased plasma glucose level and loss of body weight.

Comparison of blood glucose levels in different groups: The blood glucose level in the diabetic groups increased significantly 3-5 days after injection of alloxan or streptozocin and remained increased for the rest of the investigation. Although no significant difference ($p<0.05$) was observed in the initial plasma glucose levels between different diabetic groups and their age-matched control groups, a significant difference ($p<0.05$) was observed in the final values of the plasma glucose levels in diabetic groups in various weeks. The mean plasma glucose level in non-diabetic rabbits at the end of the 5th, 6th and 7th weeks were 114±3.55, 121.2±3.12 and 115.8±4.5, respectively, while it was correspondingly 290.5±25.21, 360.2±30.74, 285±32.56 in their age-matched diabetic rabbits. All the values are expressed in mg/100 mL. A significant elevation occurred in diabetic rabbits' blood glucose levels in all the selected weeks in comparison to non-diabetics in Table 1. The differences between the final value of plasma glucose levels in various diabetic groups and their age-matched control groups in rats are elaborated in Table 2. The mean plasma glucose level in non-diabetic rats at the end of the 3rd, 4th and 5th weeks were 94.6±3.4, 104.6±4.73 and 91.4±4.58, respectively, while it was in a parallel manner 276±6.189, 408±43.82 and 375.6±37.38 in their age-matched diabetic rats. All the values are expressed in mg/100 mL. Like what happened in rabbits, a significant increment in diabetic rats' blood glucose levels appeared in all the selected weeks in comparison to non-diabetics (Table 2).

Table 1: Statistical analysis of final plasma glucose levels (mg/100 mL) among various groups of rabbits (data are represented as Mean±SEM)

Plasma glucose levels (mg/100 mL)/experimental duration after Alloxan (vehicle) injection (weeks)	Non-diabetic (final value, n = 4)	Diabetic (final value, n = 4)	Difference between means
5 weeks	114.0±3.55	290.5±25.21**	176.5±25.46
6 weeks	121.2±3.12	360.2±30.74**	239.1±30.90
7 weeks	115.8±4.5	285.0±32.56**	169.2±32.87

Adult male New Zealand white rabbits were injected with Alloxan solved in saline solution (100 mg kg^{-1} b.wt.) after overnight fasting, extra doses were used if necessary, age-matched controls were injected with a similar volume of saline solution, each value represents the Mean±SEM of duplicate determination from four rabbits in each group and **Significantly different from the respective control value ($p\leq0.01$)

Table 2: Statistical analysis of final plasma glucose levels (mg/100 mL) among various groups of rats (data are represented as Mean±SEM)

Plasma glucose levels (mg/100 mL)/experimental duration after Streptozocin (vehicle) injection (weeks)	Non-diabetic (final value, n = 5)	Diabetic (final value, n = 5)	Difference between means
3 weeks	94.60±3.400	276.0±6.189**	181.4±7.061
4 weeks	104.6±4.739	408.0±43.82**	303.4±44.07
5 weeks	91.40±4.589	375.6±37.38**	284.2±37.66

Adult male Wistar rats were injected intraperitoneally with Streptozocin solved in 0.1 mol L^{-1} citrate buffer (50 mg kg^{-1} b. wt.) after overnight fasting, age-matched controls were injected with a similar volume of citrate buffer, each value represents the Mean±SEM of duplicate determination from five rats in each group and **Significantly different from the respective control value ($p\leq0.01$)

Comparison of body weights in different groups: There was no significant reduction in the diabetic rabbit body weight (g) when the initial and final weights were compared (data not shown). Moreover, diabetic rats' initial weights (g) were 333.33±12.5, 343.75±10.4 and 343.83±12.42 which decreased into 312.5±6.25, 291.69±14.56 and 233.33±12.6 at the end of 3rd, 4th and 5th weeks, respectively, after the onset of diabetes. The weight loss was significant at the end of weeks four ($p<0.05$) and five ($p<0.01$), after streptozocin injection in Fig. 1.

CYP2E1 expression in rats and rabbits: The effect of diabetes on hepatic CYP2E1 expression in rats and rabbits was studied during various weeks. On day 0, rats were treated with streptozocin (50 mg kg^{-1}, i.p.) to induce diabetes or 0.1 mol L^{-1} citrate buffer (1 mL kg^{-1}, i.p.) as non-diabetics. Rabbits were injected with alloxan (100 mg kg^{-1}, i.v.) in the diabetic group or saline solution (1 mL kg^{-1}, i.v.) as controls. On various weeks after injection, animals were sacrificed and liver samples were collected and prepared for the western blot analysis. The CYP2E1 protein was immunochemically detected with a polyclonal antibody raised against the respective rat or rabbit CYP2E1. The antigen-antibody complex was visualized after the addition of a peroxidase-conjugated anti-rabbit IgG and quantified by scanning with an image analysis system. Representative CYP2E1 blots either in rabbits or rats are shown, respectively in Fig. 2 and 3.

After quantification by densitometry, at first CYP2E1 levels in non-diabetic groups (various weeks) were compared. The enzyme volume in the first group of non-diabetics (3rd week in rats and 5th week in rabbits) was taken to be 100% and the value obtained from the other non-diabetic groups was expressed as a percentage of them. There was no significant difference in enzyme expression, between non-diabetic groups either in rats or rabbits, using the ANOVA test (data not shown).

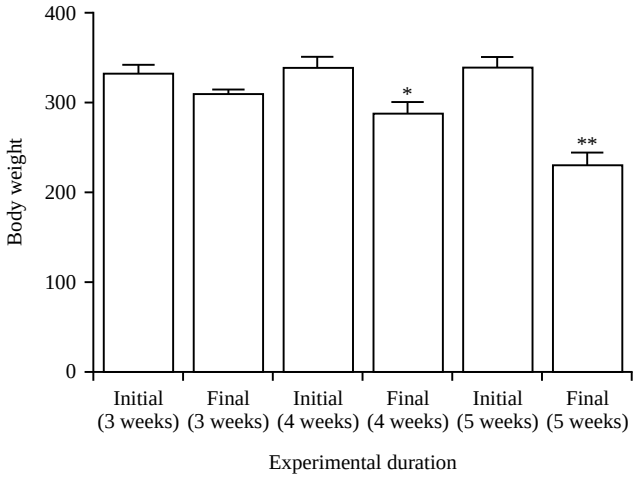

Fig. 1: Comparison between the changes of the diabetic rat's weight (g) at the initial and final point of the experimental period
*$p<0.05$ and **$p<0.01$

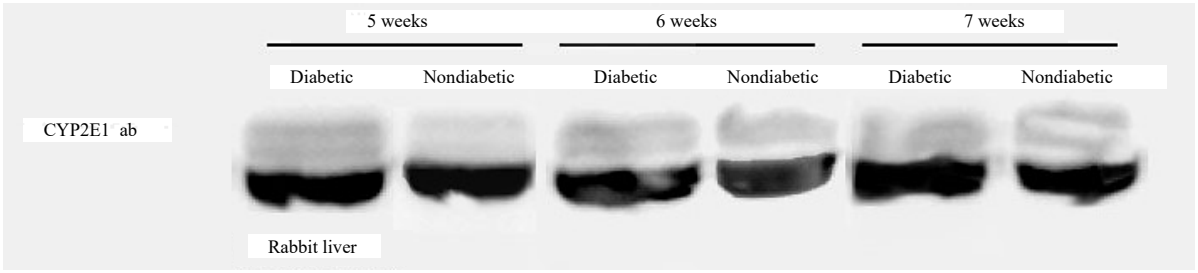

Fig. 2: Comparison of CYP2E1 biotin rabbits throughout various weeks of the experiment after Alloxan injection

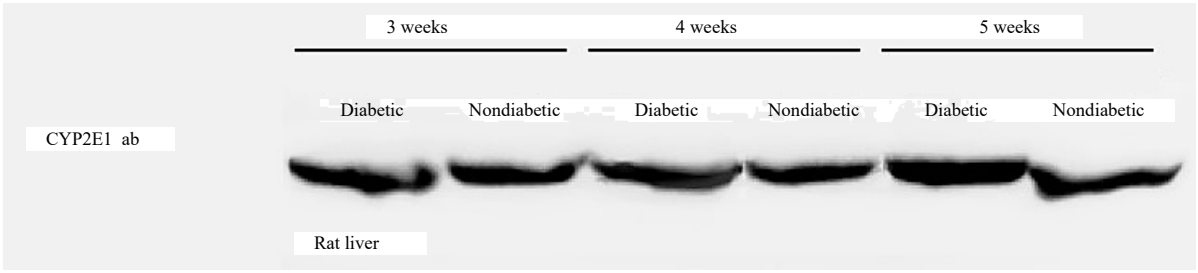

Fig. 3: Comparison of CYP2E1 biotin rats throughout various weeks of the experiment after Streptozocin injection

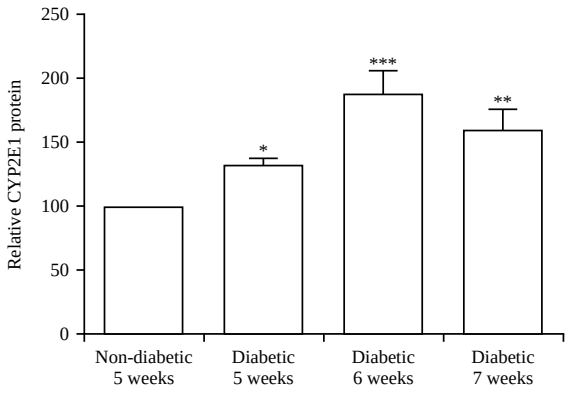

Fig. 4: Elevated cytochrome P450 2E1 in Alloxan-induced diabetic rabbits, 5, 6 and 7 weeks after alloxan injection
All 3 groups showed significant elevation of CYP2E1 concentration compared to the non-diabetic group (5 weeks), using ANOVA test, *p<0.05,**p<0.01 and ***p<0.001

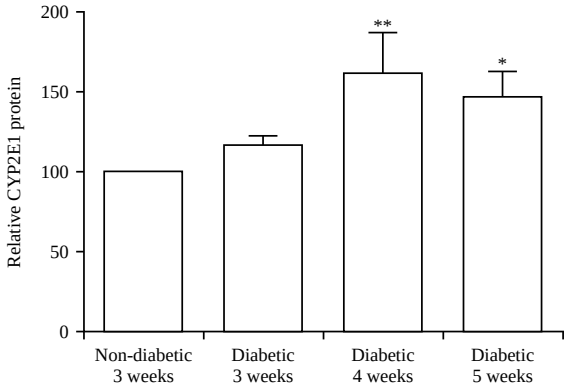

Fig. 5: Elevated cytochrome P450 2E1 in Streptozocin-induced diabetic rats after 3, 4 and 5 weeks
In all 3 various weeks, CYP 2E1 was increased in diabetic groups compared to the non-diabetic group (3 weeks), *p<0.05 and **p<0.01

To compare the diabetic groups, regarding the similar CYP2E1 expression between non-diabetics, the volume obtained from the diabetic groups was expressed as a percentage of the first non-diabetic group in each species which was taken 100%. Five weeks post diabetic, relative CYP2E1 protein percentage in rabbits was 132.75%, after 6 weeks 187.75% and eventually, after 7 weeks it became 159.75%. All were significantly elevated in comparison to non-diabetic rabbits ($p<0.05$). The results are shown in Fig. 4. In rats, at 3 weeks post diabetes induction, CYP2E1 protein percent was 115.81 which was not a significant elevation in comparison with non-diabetic rats ($p>0.05$) while after 4 and 5 weeks it became 160.18 and 146.24%, respectively, both were significantly elevated in comparison with non-diabetic rats ($p<0.05$). The results are shown in Fig. 5.

DISCUSSION

When a novel product in diabetic individuals wants to be approved, a great number of evaluations have to be carried out. In the primary steps, the pharmacokinetics parameters in an experimental animal may be considered. In this case, selecting the best animal and having information about the metabolism pathways in that species is one of the crucial steps. In this study, the CYP2E1 induction in different weeks after the onset of diabetes was studied in two different but useful species of experimental animals, rats and rabbits. The time in which the most value of the enzyme was expressed could be considered as the golden time to analyze the substrate kinetics. Besides, the procedure of preparing diabetic animals with mentioning the pros and cons of using each diabetogenic agent is discussed.

Some other investigators have evaluated CYP2E1 concentration at various times after diabetes induction in rats and rabbits. At 5 weeks[9] or 11 weeks[11] post diabetes induction in rats or 6 weeks after the first day of diabetes induction in rabbits[8], the assessment has been done. Some other researchers even appraise their results on the 4th day after the first alloxan administration[10] or the 7 or 10th day[6] after intravenous administration of streptozotocin.

Induction or suppression of metabolizing enzymes are very important in diabetic patients due to their role in the increase or decrease of the efficacy and toxicity of drugs and carcinogens. Although the effect of diabetes mellitus on rats' and rabbits' CYP2E1 activities have been studied till now[5,6,9,10] no data has been available regarding the influence of diabetes on rat and rabbit CYP2E1 in various weeks after diabetes induction. The present study was designed to observe the effects of streptozotocin and alloxan-induced diabetes in the induction of CYP2E1 in different weeks, respectively in rats and rabbits.

According to the results, the most induction rates of CYP2E1 were at 4 and 6 weeks ($p<0.01$) after diabetes induction, respectively in rats and rabbits.

Plenty of studies demonstrated the increase, decrease or unchanged amount of metabolizing enzymes in experimental diabetic animals. These findings are so essential to interpret the pharmacokinetic results of useful drugs in the treatment procedure of diabetes.

Similar to the observation obtained from this study in diabetic rabbits, Arinc and his colleagues investigated the activity of metabolizing enzymes in the liver of diabetic rabbits. Significant induction of CYP2E1 has been reported. It was also stated that there is a strong relationship between the CYP2E1 activity in diabetic rabbits' microsomes with liver aniline4-hydroxylase and para-nitrophenol hydroxylase. Thus, diabetes increases the activity of enzymes associated with

CYP2E1[5]. The researchers also believed that the response of CYP-dependent drug-metabolizing enzymes to diabetes is species-dependent[5].

In one other study, a significant increase of N-demethylase Enzyme activity (NDMA), which is straightly associated with the CYP2E1 enzyme, was observed in the liver, kidney and lung in diabetic rabbits[8]. In both studies, liver microsomes were prepared 6 weeks after alloxan injection[5,8].

In agreement with this study, Sindhu *et al.*[16] conducted a 4 week experiment on male Sprague-Dawley rats to evaluate the expression of major isozymes of the CYP450 family including CYP2E1 in streptozocin-induced diabetic rats. There was a remarkable induction of CYP2E1 proteins in diabetic animals.

Mitochondrial levels of CYP2E1 were 5-8 times higher in streptozocin-induced diabetics than in non-diabetic animals' tissues which were prepared 4 weeks after diabetes induction[9].

In another study done by Kim and his colleagues, two groups of diabetic rats (induced by streptozocin or by alloxan) were examined. They evaluated the pharmacokinetic parameters of theophylline which is one of the CYP2E1 substrates. Their results showed 3 times higher expression of CYP2E1 in diabetic rats compared to the normal group. Four days after alloxan administration and seven days after streptozocin injection, rats with blood glucose levels more than 250 mg dL^{-1} were considered diabetic rats[10].

In one other study done by Karimani *et al.*[15] CYP2E1 was induced in diabetic rats and the toxicodynamic of acrylamide as a CYP2E1 substrate has been altered.

In various investigations, different doses of this drug were used. Furman suggested 65 mg kg^{-1} b.wt., of streptozocin intraperitoneally in rats to induce diabetes[17]. In some other studies, it is shown that intraperitoneally injection of streptozocin even in a dose of 45 mg kg^{-1} b.wt., of rats, could efficaciously produce hyperglycemia[18,19]. Ojewole[20] used 90 mg kg^{-1} b.wt., streptozocin intraperitoneally. Similar to our study, Oscika used streptozocin in a dose of 50 mg kg^{-1} b.wt., intravenously in rats for producing hyperglycemia[21]. In the present study, streptozotocin was used in a dose of 50 mg kg^{-1} b.wt.

There are also investigations in which alloxan has been used to induce diabetes in rabbits. Various doses of alloxan were used in these studies. Mushtagh[22] used 150 mg kg^{-1} b.wt. while some other investigations suggested using lower doses due to the narrow window of the diabetogenic efficacy of alloxan. Even light overdosing may cause severe toxicity and loss of the rabbits which make the researchers carefully manage and monitor them during the experiments[23,24]. In this study, alloxan was used 100 mg kg^{-1} b.wt., intravenously.

Anaphylactic shock, severe weight loss, seizure and some other complications occurred during the investigation due to hyper- or hypoglycemia, especially in alloxan-induced diabetic rabbits.

During the investigation, we had confronted some complications to induce diabetes in rabbits by the use of alloxan. Although even we injected alloxan 2, 3 or 4 times according to plenty of articles, some of them were not diabetic yet[13,14,25,26]. Moreover, some days after the alloxan injection, checking the blood glucose level and proving the diabetes induction, in some rabbits the blood glucose came back again to the non-diabetic range, making the situation so unstable and unpredictable. In addition, some of the rabbits passed away throughout the experiment and were excluded.

Bacevic *et al.*[24] have suggested a practical protocol for decreasing mortality rates in alloxan-induced diabetic rabbits by monitoring rabbit behaviour and checking blood glucose levels hourly after alloxan injection, for up to 36 hrs. Indeed, administrating subcutaneous glucose to prevent hypoglycemic shock is necessary.

In the contrast, there was no problem when using streptozocin to induce diabetes in rats. The diabetes was extremely permanent and all the animals were alive until the end of the investigation. Seems that the streptozocin diabetogenic agent is much safer. Regarding experience, it seems that the use of diabetic rats for these sorts of studies could be much easier than rabbits, even if you need to perform a pharmacogenetic study.

CONCLUSION

In conclusion, findings demonstrate that CYP2E1 isozyme of the big family of CYP450 first phase metabolism enzymes are induced in both species of diabetic rats and rabbits and the most induction appeared 4 weeks after streptozocin injection in rats and 6 weeks after alloxan injection in rabbits. Moreover, more challenges in using rabbits as a diabetic sample were encountered in comparison with rats. It seems that the use of diabetic rats for these sorts of studies may be a better choice, even if you need to perform a pharmacogenetic study.

SIGNIFICANCE STATEMENT

This study compared the induction of CYP2E1 in diabetic rats and rabbits which can be beneficial for further researchers to choose the best experimental animal in the first steps of evaluating and approving a new drug for diabetic patients. The CYP2E1 induction may impact therapeutic properties or deteriorate the side effects of drugs that are metabolized by

this enzyme. By optimizing the time for checking the pharmacokinetic parameters, researchers may find the best dose adjustment in diabetic patients.

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REFERENCES

1. Shaw, J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 4-14.
2. Forbes, J.M. and M.E. Cooper, 2013. Mechanisms of diabetic complications. Physiol. Rev., 93: 137-188.
3. Zanger, U.M. and M. Schwab, 2013. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities and impact of genetic variation. Pharmacol. Therapeut., 138: 103-141.
4. Wang, Z., S.D. Hall, J.F. Maya, L. Li, A. Asghar and J.C. Gorski, 2003. Diabetes mellitus increases the *in vivo* activity of cytochrome P450 2E1 in humans. Br. J. Clin. Pharmacol., 55: 77-85.
5. Arinc, E., S. Arslan and O. Adali, 2005. Differential effects of diabetes on CYP2E1 and CYP2B4 proteins and associated drug metabolizing enzyme activities in rabbit liver. Arch. Toxicol., 79: 427-433.
6. Wang, T., K. Shankar, M.J.J. Ronis and H.M. Mehendale, 2000. Potentiation of thioacetamide liver injury in diabetic rats is due to induced CYP2E1. J. Pharmacol. Exp. Ther., 294: 473-479.
7. Sakuma, T., R. Honma, S. Maguchi, H. Tamaki and N. Nemoto, 2001. Different expression of hepatic and renal cytochrome P450s between the streptozotocininduced diabetic mouse and rat. Xenobiotica, 31: 223-237.
8. Arinc, E., S. Arslan, A. Bozcaarmutlu and O. Adali, 2007. Effects of diabetes on rabbit kidney and lung CYP2E1 and CYP2B4 expression and drug metabolism and potentiation of carcinogenic activity of n-nitrosodimethylamine in kidney and lung. Food Chem. Toxicol., 45: 107-118.
9. Raza, H., S.K. Prabu, M.A. Robin and N.G. Avadhani, 2004. Elevated mitochondrial cytochrome P450 2E1 and glutathione S-transferase A4-4 in streptozotocin-induced diabetic rats: Tissue-specific variations and roles in oxidative stress. Diabetes, 53: 185-194.
10. Kim, Y.C., A.K. Lee, J.H. Lee, I. Lee and D.C. Lee *et al*., 2005. Pharmacokinetics of theophylline in diabetes mellitus rats: Induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. Eur. J. Pharm. Sci., 26: 114-123.
11. Nosti-Palacios, R., J. Gómez-Garduño, D. Molina-Ortiz, R. Calzada-León, V.M. Dorado-González and A. Vences-Mejía, 2014. Aspartame administration and insulin treatment altered brain levels of CYP2e1 and CYP3a2 in streptozotocin-induced diabetic rats. Int. J. Toxicol., 33: 325-331.
12. del Amo, E.M. and A. Urtti, 2015. Rabbit as an animal model for intravitreal pharmacokinetics: Clinical predictability and quality of the published data. Exp. Eye Res., 137: 111-124.
13. Alam, S., A.H. Khan, G. Sirhindi and S. Khan, 2005. Alloxan induced diabetes in rabbits. Pak. J. Pharmacol., 22: 41-45.
14. Wang, J., R. Wan, Y. Mo, Q. Zhang, L. Sherwood and S. Chien, 2010. Creating a long-term diabetic rabbit model. Exp. Diabetes Res., Vol. 2010. 10.1155/2010/289614.
15. Karimani, A., H. Hosseinzadeh, S. Mehri, A.H. Jafarian, S.A. Kamali, A.H. Mohammadpour and G. Karimi, 2021. Histopathological and biochemical alterations in non-diabetic and diabetic rats following acrylamide treatment. Toxin Rev., 40: 277-284.
16. Sindhu, R.K., J.R. Koo, K.K. Sindhu, A. Ehdaie, F. Farmand and C.K. Roberts, 2006. Differential regulation of hepatic cytochrome P450 monooxygenases in streptozotocin-induced diabetic rats. Free Radical Res., 40: 921-928.
17. Furman, B.L., 2015. Streptozotocin-induced diabetic models in mice and rats. Curr. Prot. Pharmacol., 70: 5.47.1-5.47.20.
18. Zafar, M., S.N. Naqvi, M. Ahmed and Z.A. Kaimkhani, 2009. Altered kidney morphology and enzymes in streptozotocin induced diabetic rats. Int. J. Morphol., 27: 783-790.
19. Zafar, M., S.N.U.H. Naqvi, M. Ahmed and Z.A. Kaimkhani, 2009. Altered liver morphology and enzymes in streptozotocin induced diabetic rats. Int. J. Morphol., 27: 719-725.
20. Ojewole, J.A.O., 2002. Hypoglycaemic effect of *Clausena anisata* (Willd) Hook methanolic root extract in rats. J. Ethnopharmacol., 81: 231-237.
21. Osicka, T.M., Y. Yu, S. Panagiotopoulos, S.P. Clavant and Z. Kiriazis *et al*., 2000. Prevention of albuminuria by aminoguanidine or ramipril in streptozotocin-induced diabetic rats is associated with the normalization of glomerular protein kinase c.. Diabetes, 49: 87-93.
22. Mushtaq, M.N., 2017. Effect of aqueous extract of *Thymus serpyllum* on lipid profile and some liver enzymes in alloxan-induced diabetic rabbit. Bangladesh J. Pharmacol., 12: 58-62.
23. Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., 50: 537-546.
24. Bacevic, M., E. Rompen, R. Radermecker, P. Drion and F. Lambert, 2020. Practical considerations for reducing mortality rates in alloxan-induced diabetic rabbits. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e04103.
25. Jain, D.K. and R.K. Arya, 2011. Anomalies in Alloxan-induced diabetic model: it is better to standardize it first. Indian J. Pharmacol., 43: 91-91.
26. Misra, M. and U. Aiman, 2012. Alloxan: An unpredictable drug for diabetes induction? Indian J. Pharmacol., 44: 538-539.