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COMMENTARY

Does the breakdown of the detoxification system for aldehydes as a result of aldose reductase upregulation lead to alcohol-induced liver injury in humans and mice?

Alcohol-induced liver disease (ALD) is one of the major causes of chronic liver disease globally. The pathogenesis of alcohol-induced hepatic injury is characterized by steatosis, inflammation and fibrosis, which can eventually progress to cirrhosis and hepatocellular carcinoma[1]. Recently, the mechanism of both ALD and non-alcoholic fatty liver disease (NAFLD) has been fairly well studied, but a successful treatment for ALD and NAFLD is not available yet. However, recent increasing evidence has suggested that hepatic aldose reductase (AR), a rate-limiting enzyme of the polyol pathway, is dynamically regulated under a variety of conditions, including alcohol consumption. These data show that the inhibition of AR might lead to a marked amelioration in NAFLD and ALD.

In addition, it is well known that either ALD[2] or NAFLD[3] can contribute to markedly affecting blood glucose levels. NAFLD is one of the major causes of insulin resistance, whereas chronic liver disease can lead to the fluctuation of blood glucose levels with alternating hyperglycemia and hypoglycemia[4], making it difficult to maintain steady blood glucose levels at times. In any case, both ALD and NAFLD are closely related with diabetes, making it desirable to develop an effective medicine to treat them. Recent studies of ALD using animals[5–7] and humans[7] give us a ray of hope. These studies approach the mechanisms of alcohol-induced hepatic injury from the perspective of the polyol pathway. NAFLD has also been studied by using animals from the viewpoint of the polyol pathway[8–10]. These studies observed that the inhibition of AR, a key enzyme of the polyol pathway, was useful to prevent the development of NAFLD. In the present authors' previous article in *J Diabetes Investig*, the pathogenesis of NAFLD from the perspective of this pathway was discussed[11]. Therefore, in the following, the cause of and measures against ALD from the viewpoint of AR activity in the liver are considered.

The polyol pathway consists of two steps regulated by two reactive enzymes; namely, AR in the first reaction and then sorbitol dehydrogenase (SDH) in the second step, as shown in Figure 1. In general, diabetic complications based on polyol pathway hyperactivity are caused by complex mechanisms involving osmotic stress, reductive stress, pyruvate kinase C stress, glycative stress, oxidative stress and other factors[11-13]. The role of AR in diabetes has been well clarified by using either AR inhibitors in animals and humans or AR knockout (ARKO) mice.

Recently, Wang *et al.*[7] showed the role of AR in ALD involving therapeutic targets by using human specimens, cultured cells and animal models, including ARKO mice. In liver specimens from alcoholic hepatitis (AH) patients, they found AR upregulation and increased AR metabolites (sorbitol, fructose and uric acid). These observations were also confirmed in studies using animal models. Furthermore, the pharmacological inhibition of AR in mice prevented alcohol-induced hepatic steatosis, apoptosis and liver injury. Therefore, their study provides us a new perspective for the future from the viewpoints of both the mechanisms of and medicines for alcohol-induced liver injury. Therefore, the results of their research are briefly introduced in the present article.

The first novel observation of Wang *et al.*[7] in their study using liver specimens from AH patients found that marked upregulation of AR corresponded with the elevation of AR metabolites (hepatic sorbitol, fructose and uric acid). More importantly, they observed a strong positive correlation between AR upregulation with AR metabolites and the endoplasmic reticulum (ER) stress markers activating transcription factor 3 (ATF3) and CCAAT/enhancer-binding protein homologous protein (CHOP), and a significant negative correlation with the protective chaperone proteins glucose-regulated protein (GRP)78 and GRP94 in the AH patients' livers. The authors also clearly stated that AR upregulation and increased AR metabolites showed a positive correlation with hepatocyte cell death, liver injury and disease severity. Notably, similar results were observed in their experiments using alcohol-fed mice, as shown in the following.

In their study to definitely establish the causal role of AR upregulation in ALD, Wang *et al.*[7]investigated using AR-deficient ARKO mice or the pharmacological inhibition of AR. Alcohol-mediated AR upregulation with increased AR metabolites (hepatic sorbitol and fructose, and serum uric acid) in alcohol-fed wild type (WT) mice was reduced in ARKO mice. Furthermore, the elevation of stress markers, ATF3 and CHOP, in response to alcohol-induced AR upregulation observed in WT mice was prevented in ARKO mice. Corresponding to these

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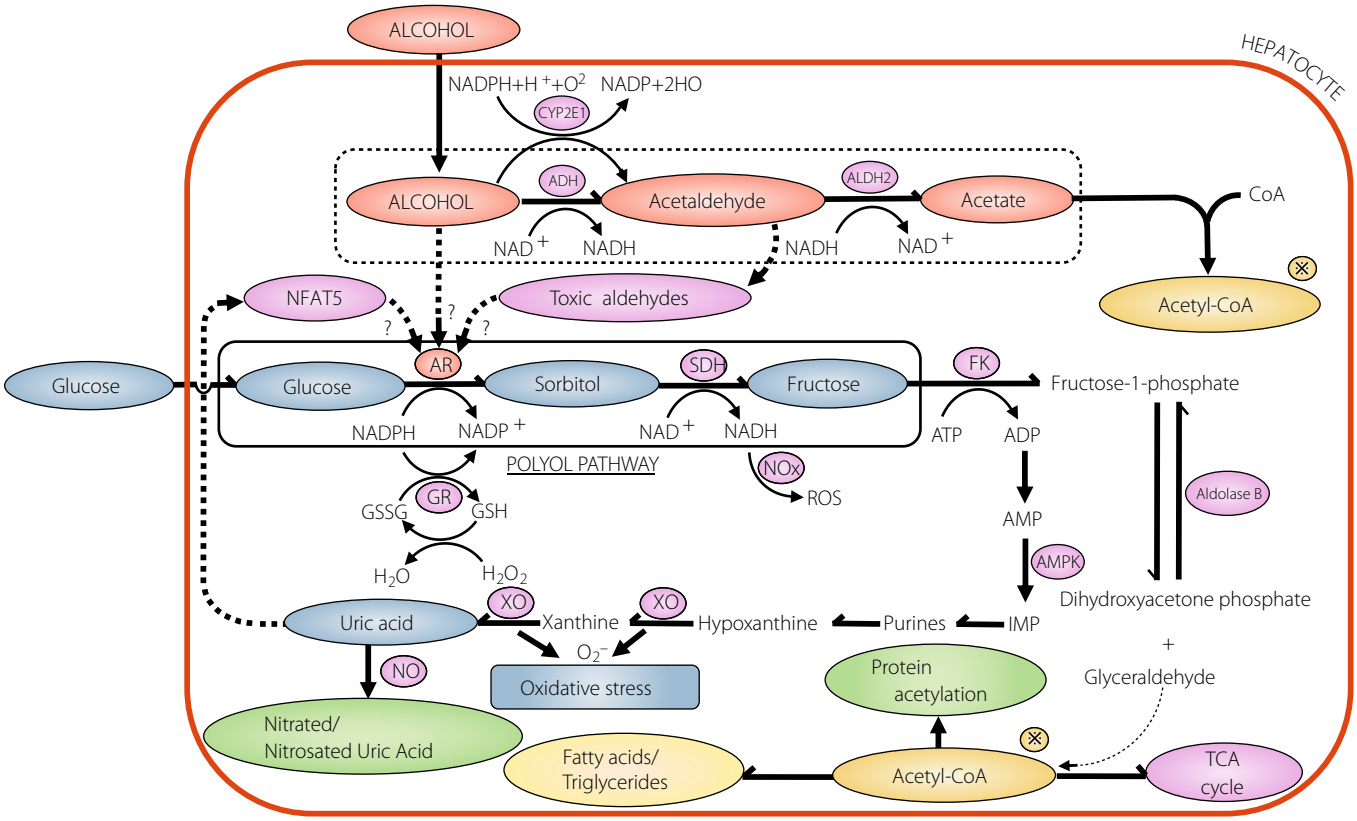

Figure 1 | Pathways of aldose reductase metabolites and alcohol metabolism in the liver. The polyol pathway consists of just two steps. Glucose converts to sorbitol in the first step, then sorbitol is metabolized to fructose in the second step. Aldose reductase (AR) is a key enzyme in the first step. During the hyperglycemic state, approximately 30% of the glucose pool is disposed of through this pathway. Endogenous production of fructose by the polyol pathway can lead to a variety of metabolites, as shown in this figure. Uric acid and acetyl coenzyme A (acetyl-CoA) also can be produced from fructose through the pathway in this figure. In contrast, in the hepatic alcohol metabolism, ethanol is converted to acetaldehyde by alcohol dehydrogenase (ADH) in the cytoplasm. Then the conversion of acetaldehyde to acetate is made by aldehyde dehydrogenase 2 (ALDH2) in the mitochondria. The trigger for the mechanism of AR upregulation initiated by alcohol is unclear, but increased uric acid through polyol pathway hyperactivity can lead to an enrichment of nuclear factor of activated T cells 5 (NFAT5), contributing to induce AR upregulation[7,11]. As another possibility, toxic aldehydes, such as acetaldehyde, might cause AR upregulation in alcohol-induced liver injury. Otherwise, there could be other unknown factors caused by alcohol. ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; CYP2E1, cytochrome P450E1; FK, fructose kinase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione disulfide; IMP, inosine monophosphate; NAD, nicotinamide adenine dinucleotide; NADH, reduced form of nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOx, reduced form of nicotinamide adenine dinucleotide oxidase; ROS, reactive oxygen species; SDH, sorbitol dehydrogenase; TCA, tricarboxylic acid; XO, xanthine oxidase.

in vivo studies, similar results were confirmed by *in vitro* experiments using cultured hepatocytes. Exposure to increasing concentrations of fructose (5–25 mmol/L) or uric acid (50–200 μg/mL) markedly decreased cell viability. In primary hepatocytes, either fructose or uric acid attenuated cell viability, and also markedly upregulated ATF3 and CHOP. It is interesting and valuable that AR upregulation was observed as a result of exposure to fructose or uric acid, indicating a kind of a feed-forward mechanism[7]. The observation of this mechanism induced by AR metabolites seems to be highly significant in consideration of a trigger for AR upregulation caused by alcohol. Furthermore, they found that increases of propidium iodide-positive cells, annexin V-positive cells and the double positive cells from flow cytometry using annexin V–propidium iodide staining on cells were induced by exposure to fructose (10 mmol/L) or uric acid (100 μg/mL). This clearly shows that fructose and uric acid caused by AR upregulation can pull the trigger of ER stress and cell death in cultured hepatocyte-derived cells *in vitro*. Furthermore, they observed that the increased hepatic gene expression of sterol regulatory element-binding protein 1c (a transcription factor that plays a critical role in the control of lipogenesis

gene expression) and fatty acid synthase (a key enzyme in lipogenesis and a target of sterol regulatory element-binding protein 1c) in alcohol-fed WT mice was prevented in ARKO mice.

In the next step, the authors examined the phospho-activation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, because these markers are known to associate frequently with altered expression of pro- and anti-apoptotic proteins, activation of caspases, and apoptosis. The authors found that increased phosphorylation of p38 and c-Jun N-terminal kinase under alcohol-induced AR upregulation in WT mice only occurred to a lesser extent in ARKO mice. Furthermore, it is known that the Bcl-2 family can promote or inhibit apoptosis. However, the authors showed elevated pro-apoptotic Bid levels and low expression of anti-apoptotic Bcl-w in alcohol-fed WT mice, along with proteolytic activation of ER-resident caspase-12 and the executioner caspase-3. Incidentally, it is also known that these alterations are consistent with the known involvement of the mitochondrial death pathway in alcohol-induced liver injury. These apoptotic changes and liver injury observed in alcohol-fed WT mice were prevented or attenuated in ARKO mice. In addition to these observations, they found an accumulation of acrolein, one of the protein adducts of lipid-derived aldehydes, in the AH patients' livers. Also, increased acrolein and 4-hydroxynonenal were observed in alcohol-fed MT mice, but reduced in ARKO mice. This suggests that AR deficiency prevented alcohol-induced hepatic lipid peroxidation and generation of lipid-derived aldehydes. This evidence seems to have great importance in terms of the physiological function of AR. Despite the well-demonstrated harmful effects of AR under hyperglycemia in diabetes[11–13], the physiological role of this enzyme remains obscure. However, the function of AR in detoxifying aldehydes is known as a possible important route[14]. Incidentally, it is known that AR can catalyze the reduction of acrolein and 4-hydroxynonenal. Thus, all of these facts might indicate actions beyond the physiological role of AR in alcohol-induced liver injury.

Finally, to confirm the therapeutic potential of AR inhibition to protect against alcohol-induced ER stress and liver injury in ALD, epalrestat as an AR inhibitor was used in mice *in vivo*. The inhibition of AR decreased alcohol-induced hepatic steatosis and apoptotic cell death, along with a reduction of hepatic fructose and serum uric acid increased by alcohol. Furthermore, epalrestat markedly reduced the expression of ATF3 and CHOP, while largely upregulating GRP78 and GRP94. This study using immunohistochemical staining of liver sections confirms that alcohol-induced hepatic accumulation of acrolein-protein adducts and upregulation of ATF3 and CHOP protein were attenuated by the inhibition of AR. These observations are similar to those observed in ARKO mice, suggesting that the pharmacological inhibition of AR by epalrestat showed marked protective action, and reduced alcohol-induced ER stress and liver injury.

The study by Wang *et al.*[7] gives us hope from the standpoint of taking possible countermeasures against ALD, as well as its pathogenesis. However, there is no information about the exact mechanism for the interaction between alcohol and AR. That is, the authors do not refer to the question of how alcohol induces AR hyperactivity. As shown by Sanchez-Lazada *et al*[10], even if uric acid induces AR upregulation through the nuclear factor of activated T cells 5, as shown in Figure 1, the initial trigger of increased uric acid in the liver of ALD is obscure. This is a question of which came first, the chicken or the egg; or which came first, alcohol or uric acid. As another possibility, the alcohol metabolic acetaldehyde might be a key trigger for AR upregulation. It is known that AR can catalyze the reduction of a variety of aldehydes and carbonyls[14]. In other words, AR takes part in the anti-oxidant defense mechanism in our bodies, being highly effective for the reduction of toxic aldehydes, deriving from pathological connections with oxidative stress. In the *in vivo* study, they observed that the genetic deficiency and pharmacological inhibition of AR markedly attenuated the alcohol-mediated increase in toxic-aldehyde generation[7]. It is shown that one of the functions of AR is detoxifying aldehydes. In addition, a well-known anti-oxidant, the reduced form of glutathion can chemically react with toxic aldehydes, such as acrolein, thus reducing their toxicity. Namely, glutathion contributes to cellular defense against toxic effects. However, under AR upregulation caused by alcohol, this system might not work normally, because AR hyperactivity induced by alcohol causes a substantial depletion of nicotinamide adenine dinucleotide phosphate and consequently a significant decrease in the glutathion level (Figure 1), resulting in the accumulation of toxic aldehydes. Therefore, in the study of Wang *et al*[7]., toxic aldehydes, such as acrolein and 4-hydroxynonenal adducts, were accumulated in the liver under the condition of alcohol-induced AR upregulation in MT mice, but reduced in ARKO mice. In contrast, the increment of nicotinamide adenine dinucleotide caused by AR upregulation induces increased reactive oxygene species production, and then leads to ER stress and mitochondrial dysfunction, contributing to cell death. Thus, all of these facts might indicate actions beyond the physiological role of AR in alcohol-induced liver injury. AR might also be one of the important determinants of tissue injury. Therefore, AR has been considered to serve a cytoprotective function through rapidly detoxifying aldehydes. Incidentally, whereas acetaldehyde is subsequently oxidized to acetate by aldehyde dehydrogenase 2, the kinetics of this reaction are very slow, allowing for the accumulation of acetaldehyde in humans consuming alcohol. In that case, the initial trigger of AR upregulation induced by alcohol would occur as a result of accumulated toxic aldehydes, such as acetaldehyde. Otherwise, other unknown factors caused by alcohol might induce AR upregulation in the liver as an initial trigger. We need to resolve this issue in the future. In any case, we place our hope in the further development of studies based on polyol pathway hyperactivity toward a solution

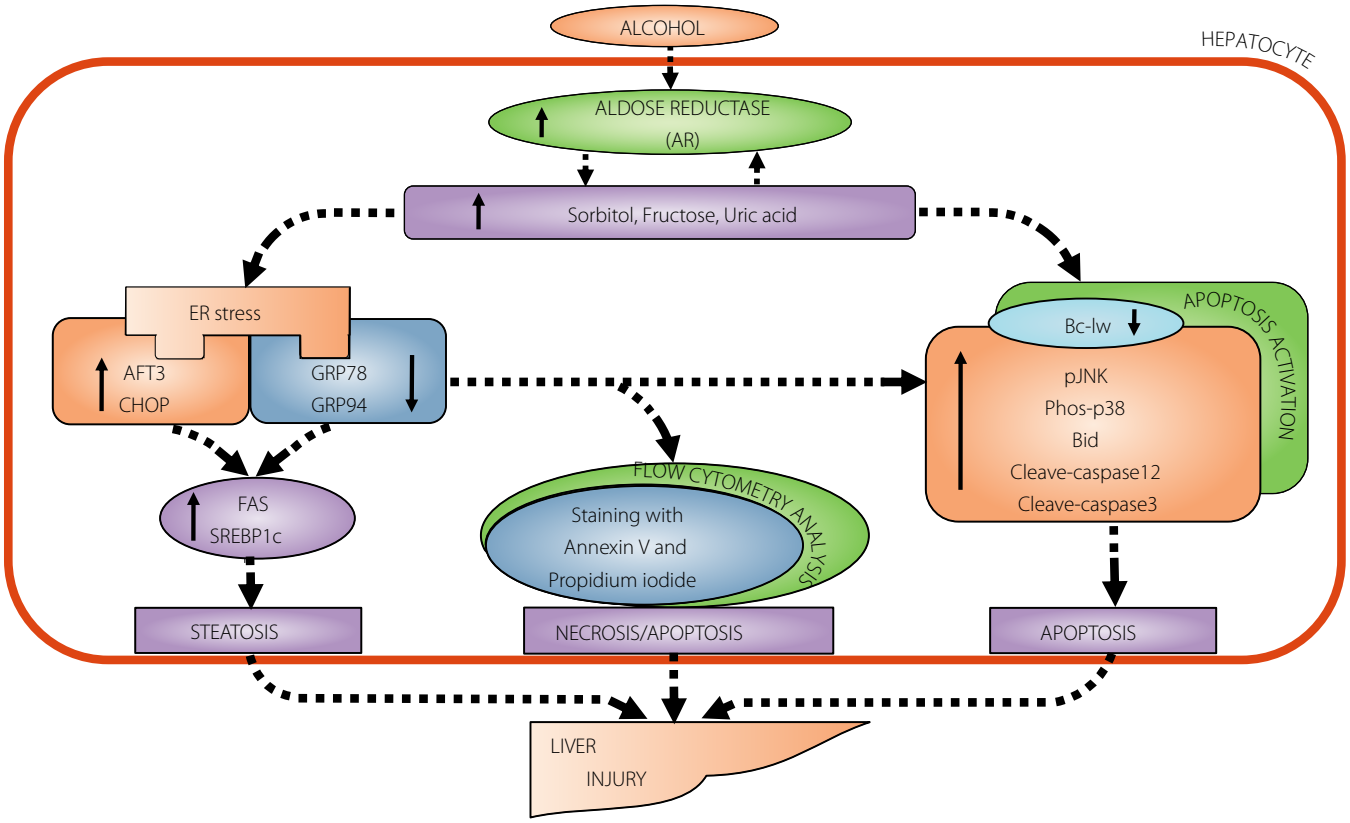

Figure 2 | Elevated fructose and uric acid as a result of aldose reductase upregulation contribute to alcoholic liver disease in humans and mice. On the basis of observations using humans and mice[7], hepatic aldose reductase (AR) upregulation, and consequent elevation of sorbitol, fructose and uric acid are important factors contributing to alcohol-induced steatosis, apoptosis and liver injury in both mouse and human alcohol-induced liver disease. In the alcohol-feeding state, AR upregulation and increased AR metabolites (sorbitol, fructose and uric acid) affect a positive correlation with the endoplasmic reticulum (ER) stress markers, activating transcription factor 3 (ATF3) and pro-apoptotic CCAAT/enhancer-binding protein homologous protein (CHOP), and a negative correlation with the protective proteins glucose-regulated protein (GRP)78 and GRP94, contributing to increased hepatic gene expression of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (FAS) with subsequent hepatic steatosis. Furthermore, this alcohol-induced ER stress becomes a trigger for cell death with hepatic necrosis and apoptosis. In contrast, AR upregulation and increased AR metabolites in the alcohol-mediated hepatic injury induce the low expression of anti-apoptotic Bcl-w and increased phosphorylation of c-Jun N-terminal kinase (JNK) and p38, and elevated pro-apoptosis Bid levels along with proteolytic activation of ER-resident caspase-12 and the executioner caspase-3, leading to hepatic apoptosis. This apoptosis activation is also induced in the context of ER stress. Some modification from Wang *et al.*[7] *Hepatology* 2020. https://doi.org/10.1002/hep.31197

for the pathogenesis of and a cure for ALD. Regardless of the precise pathogenesis, AR upregulation for the development of alcohol-induced hepatic injury might be one of the important factors in ALD[5–7]. This new insight is summarized in Figure 2 according to these observations of alcohol-induced liver injury in mice and humans[7].

There are a variety of proposals of the possible mechanisms of ALD, including AR upregulation. However, the recent investigation of both experimental and human ALD[7] suggests that hepatic AR upregulation accompanied by the increment of fructose and uric acid is one of the important causes contributing to alcohol-induced ER stress, apoptosis and liver injury. Furthermore, it showed that the pharmacological inhibition of AR markedly reduced the aforementioned changes observed in mice *in vivo*[7]. This observation seems to signify the great importance of these results. It is a new insight that the polyol pathway and its key enzyme, AR, might be a novel therapeutic target for ALD, because the AR inhibitor, epalrestat (developed in Japan), is currently marketed in Japan, China and India, and also widely used for the treatment of diabetic neuropathy.

DISCLOSURE

The authors declare no conflict of interest.

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REFERENCES

1. Chao X, Wang S, Zhao K, *et al*. Impaired TFEB-mediated lysosome biogenesis and autophagy promote chronic ethanol-induced liver injury and steatosis in mice. *Gastroenterology* 2018; 155: 865–879.
2. Creutzfeldt W, Hartmann H, Nauck M, *et al*. Liver disease and glucose homeostasis.In: Blanchi L, Gerok W, Landmann L, Sickinger K, Stalder GA (eds). Liver in Metabolic Diseases. Boston: MTP Press Ltd., 1983; 221–234.
3. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease, insulin resistance, and ceramides. *N Engl J Med* 2019; 381: 1866–1869.
4. Honda F, Hiramatsu A, Hyogo H, *et al*. Evaluation of glycemic variability in chronic liver disease patients with type 2 diabetes mellitus using continuous glucose monitoring. *PLoS One* 2018; 13: e0195028.
5. Shi C, Wang Y, Gao J, *et al*. Inhibition of aldose reductase ameliorates alcoholic liver disease by activating AMPK and modulating oxidative stress and inflammatory cytokines. *Mol Med Rep* 2017; 16: 2767–2772.
6. Guo C, Chen L, Huang J, *et al*. Aldose reductase inhibitor protects mice from alcoholic steatosis by repressing saturated fatty acid biosynthesis. *Chem Biol Interact* 2018; 287: 41–48.
7. Wang M, Chen W-Y, Zhang J, *et al*. Elevated fructose and uric acid via aldose reductase contributed to experimental and human alcoholic liver disease. *Hepatology* 2020. https://doi.org/10.1002/hep.31197
8. Qiu L, Lin J, Xu F, *et al*. Inhibition of aldose reductase activates hepatic peroxisome proliferator-activated receptor-α and ameliorates hepatosteatosis in diabetic db/db mice. *Exp Diabetes Res* 2012; 2012: 789730.
9. Qiu L, Guo C. Natural aldose reductase inhibitor: a potential therapeutic agent for non-alcoholic fatty liver disease. *Curr Drug Targets* 2020; 21: 599-609.
10. Sanchez-Lozada LG, Andres-Hernando A, Garcia-Arroyo FE, *et al*. Uric acid activates aldose reductase and the polyol pathway for endogenous fructose and fat production causing development of fatty liver in rats. *J Biol Chem* 2019; 294: 4272–4281.
11. Hotta N, Kawamura T, Umemura T. Are the polyol pathway and hyperuricemia partners in the development of non-alcoholic fatty liver disease in diabetes? *J Diabetes Investig* 2020; 11: 786–788.
12. Umemura T, Kawamura T, Hotta N. Pathogenesis and neuroimaging of cerebral large and small vessel disease in type 2 diabetes: a possible link between cerebral and retinal microvascular abnormalities. *J Diabetes Investig* 2017; 8: 134–148.
13. Hotta N, Kawamori R, Fukuda M, *et al*. Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on progression of diabetic neuropathy and other microvascular complications: multivariate epidemiological analysis based on patient background factors and severity of diabetic neuropathy. *Diabet Med* 2012; 29: 1529–1533.
14. Srivastava S, Watowich SJ, Petrash JM, *et al*. Structural and kinetic determinants of aldehyde reduction by aldose reductase. *Biochemistry* 1999; 38: 42–54.

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