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Possible Hepatoprotective Effect of Aliskiren on Acute Ischemia-reperfusion Liver Injury in Rats

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ABSTRACT

Background: hepatic Ischemia/reperfusion (IR) injury, which can be seen in various clinical settings such as liver transplantation, may lead to local and remote organ damage; yet the precise pathogenesis is not fully defined. Despite the improvements in liver preservation and surgical techniques, hepatic IR injury remains an important clinical complication. To control this problem, further studies are required to find out a novel therapeutic approach. It has been shown that the renin-angiotensin system (RAS) may play roles in diseases of chronic inflammation. However, whether the RAS also can mediate acute inflammation in liver is unclear. Aim: determining the effect of the direct rennin inhibitors (Aliskiren) on acute liver damage and inflammation caused by hepatic ischemia -reperfusion. Methods: IR was induced by the hepatic left lateral and median lobes clamping at its base using a traumatic clip for 60 minutes; then, the clip was removed after 6hrs, initiating hepatic reperfusion. Aliskiren was given 7 days before the onset of IR. Its effect is compared with the effect of preconditioning. Results: Both Aliskiren and preconditioning produced a significant decrease in Serum glutamic-pyruvic transaminase and malondialdehyde levels.It also increased the activities of superoxide dismutase and catalase levels. Furthermore, they produced a significant decrease in serum TNF-α, hepatic nitric oxide and hepatic tissue myeoperoxidase. Conclusions: Aliskiren has an anti-inflammatory and antioxidant effects similar to preconditioning, proving its benifial effect on acute inflammatory liver damage. This indicates the use of Aliskiren on acute inflammatory conditions especially acute IR injury.

Keywords: ***Aliskiren, Liver, Ischemia, Reperfusion, Preconditioning, Rats***

Introduction:

Ischemia-reperfusion injury (IRI) occurs when the blood supply to an organ is cut off and later restored. In the liver, IRI remains a major factor contributing to organ failure after hepatic surgery for liver cancer. It is also a

challenging problem for other types of hepatobiliary surgery. *[1]*

The pathophysiology of hepatic ischemia-reperfusion includes a number of mechanisms that contributes to various degrees in the overall injury. This insult can lead to hepatocellular damage and organ dysfunction through the initiation of a biphasic inflammatory response. *[2]* The initial phase of this response is characterized by activation of Kupffer cells and their subsequent production and release of reactive oxygen species. This causes mild injury to the hepatic parenchyma that regulates the expression of many proinflammatory mediators. Moreover, tumor necrosis factor-α (TNF- α), is critically involved in promoting the second phase of liver injury. *[2]*

So, IRI represents a complex series of events including release of reactive oxygen species, nitric oxide imbalance, cytokine cascades, neutrophil accumulation and cell death, resulting in cellular and tissue damage. *[3]* However, hepatic IRI, which can be seen in various clinical settings such as liver transplantation, hepatectomy, and hemorrhagic shock, may lead to local and remote organ damage; yet the precise pathogenesis is not fully defined. For instance, massive accumulation of neutrophils in the lung, the development of interstitial pulmonary edema and increased expression of proinflammatory mediators are major features of lung injury induced by hepatic IR. *[4]*

Finally, despite the improvements in liver preservation and surgical techniques, hepatic IRI remains an important clinical complication. To control this complicated physiological and pathological process, further studies are required to find out the key pathway and a novel therapeutic approach.

From another view, it has been suggested that the expression of angiotensinogen in liver increased fivefold, 3 hours after reperfusion. Indices of liver damage and inflammation (e.g., alanine aminotransferase levels, pathological features, tumor necrosis factor-alpha levels, and intercellular adhesion molecule-1 expression) were all significantly elevated in vehicle-treated animals after hepatic ischemia and subsequent reperfusion.*[5]* Angiotensin II is produced by enzymatic cleavage of angiotensinogen by renin.Angiotensin II has been demonstrated to regulate adipocyte growth and differentiation, lipid metabolism, and expression and release of adipokines and RAS components, and to promote oxidative stress. *[6]*

Also, renin -the first enzyme in the renin-angiotensin-aldosterone system- cleaves angiotensinogen to angiotensin I which is in turn converted by angiotensin-converting enzyme (ACE) to angiotensin II.*[7]*

Aliskiren is the first in a new class of orally effective rennin inhibitors and is a potent inhibitor of human rennin both in hypertensive patients and in healthy

volunteers. *[8]* Furthermore, it has been shown that the renin-angiotensin system (RAS) may play roles in diseases of chronic inflammation. However, whether the RAS also can mediate acute inflammation in liver is unclear.

Thus; the aim of this work is to determine the effect of the rennin inhibitors (Aliskiren) on acute liver damage and inflammation caused by hepatic ischemia and subsequent reperfusion. Furthermore; this work compares between Aliskiren's effect and hepatic preconditioning. Preconditioning procedures is proven to have enhanced liver regeneration in the experimental model of reduced-size rat liver transplantation. *[9]*

Materials & Methods:

Animals

Adult male Sprague Dawley rats (250-300 g) were obtained from the animal house of the research unit at faculty of medicine, Mansoura University. Animals were handled with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health and the approval from Animal Ethic Committee of the institution (Egypt).

Induction of ischemia/reperfusion

The animals underwent either sham surgery or ischemia-reperfusion. A model of 70% partial hepatic ischemia for 60 min was used as previously reported *[10].* Briefly, mice were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg), and a midline laparotomy was performed. Then, the left lateral and median lobes of the liver were clamped at its base using a traumatic clip. After 60 min of ischemia, the clip was removed, initiating hepatic reperfusion. Sham control rats underwent the same protocol without vascular occlusion. Rats were sacrificed at 6 hrs after reperfusion, and then blood and liver samples were collected for analysis.

Preconditioning

Preconditioning induced by 10 minutes of ischemia, followed by 15 minutes of reperfusion. This preconditioning period has been demonstrated to be the most effective against the hepatic injury in the same experimental model shown in the present study. *[11]*

***Drugs investigated*:**

- Aliskiren 10 mg/kg *[12]* was supplied by NOVARTIS in the form of Rasilez 150 mg tablet.

Experimental design:

50 male Sprague-Dawley rats were randomly divided into five groups:

a) Group (1) :(10 rat) Normal control group.
b) Group (2) :(10 rat) Sham- operated group.
c) Group (3) :(10 rat) Ischemia-reperfusion group.

d) Group (4) :(10 rat) as group 3 but with previous preconditioning.

e) Group (5) :(10 rat) as group 3 but with previous administration of Aliskiren for 7 days before ischemia.

All protocols were approved by our local committee of Animal Care and Use Committee.

Collection of Blood Sample

Blood was collected by heart puncture when rats were sacrificed. The blood samples were then centrifuged at 1000 rpm and sera stored at -20°C till biochemical analysis.

The following biochemical parameters were investigated:

a) Determination of Serum glutamic-pyruvic transaminase (SGPT): SGPT was measured using Kits obtained from Biotic Laboratories. It is considered as a parenchymal marker enzymes for the hepatic cell damage, *[13]*

b) Determination of Serum TNF-α: TNF-α was measured using ELISA kits from G enzymes, Cambridge, Ma, USA, by UV spectrophotometer. It is considered as a representative of cytokines production.

c) Determination of Hepatic tissue myeoperoxidase (MPO) activity: MPO activity was assayed according to Mullane,et al.*,[14].* It is considered as a marker for tissue neutrophil content.

d) Determination of Hepatic nitric oxide levels: Hepatic nitric oxide levels was measured by a colorimetric method as described by **Montgomery** and ***Dymock [15]***

e) Determination of Hepatic tissue Malondialdehyde (MDA): Hepatic tissue MDA was estimated according to method of Uchiyama & Mihara *[16].* It is considered as a marker for the overproduction of toxic radicals.

f) Determination of Activity of hepatic antioxidant protective enzymes:

Hepatic Super oxide dismutase (SOD) was estimated according to method of

Stefan Marklund et al. *[17]* and catalase (CAT) according to method of Aebi & Packer *[18]*

Statistical analysis

The statistical analysis of results was done by using SPSS (Statistical Package for Social Science) program, version 13, 2004, for windows XP professional. The biochemical data were expressed as Mean ± SD. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by *post-hoc* multiple comparison. *P* value < 0.05 was considered statistically significant at confidence interval 95 %.

RESULTS

Effect of ischemia-reperfusion on tested parameters:

As shown in table (1): Ischemia-reperfusion produced a significant increase in SGPT, hepatic MDA levels, serum TNF-α, hepatic

NOx and hepatic MPO; as compared to sham-operated group.It also, produced a significant decrease in catalase and SOD levels, as compared to sham-operated group.

Effect of Preconditioning on tested parameters in ischemia-reperfusion group:

As illustrated in table (2): Preconditioning produced a significant decrease in SGPT,hepatic MDA levels, serum TNF-α, hepatic NOx and hepatic MPO; as compared to Ischemia-reperfusion group. It also, produced a significant increase in catalase and SOD levels, as compared to Ischemia-reperfusion group.

Effect of Aliskiren on tested parameters in ischemia-reperfusion group:

As illustrated in table (2): Aliskiren produced a significant decrease in SGPT,hepatic MDA levels, serum TNF-α, hepatic NOx and hepatic MPO as compared to Ischemia-reperfusion group. It also, produced a significant increase in catalase and SOD levels, as compared to Ischemia-reperfusion group.

Comparison between the effects of Aliskiren and Preconditioning on tested parameters in ischemia-reperfusion group

As shown in Table 2, there was a non significant difference between Aliskiren-treated ischemia-reperfusion group and ischemia-reperfusion group with previous preconditioning. This was as regards to all tested parameters.

Table (1): Effect of Ischemia-reperfusion on Serum glutamic-pyruvic transaminase (SGPT), Malondialdehyde (MDA), Catalase, Super oxide dismutase activity(SOD), Serum TNF- α, HEPATIC NOx and HEPATIC MPO activity.

Groups / Parameter	Normal control group	Sham-operated Group	Ischemia-reperfusion group
SGPT (units/L)	21.3±1.5	20.5± 0.3	92.16 ± 0.3 #
MDA (nmol/mg protein)	25.84±2.11	26.94±2.21	232.22±50.15 #
CATALASE (u/mg protein)	8.81±.72	8.79±.62	3.61±1.09#
SOD (u/mg protein)	4.1±.68	4.2±.66	1.55±.32 #
TNF-α (pg/mL)	90.98 ± 4.91	91.40 ± 4.67	400.55 ± 90.58 #
HEPATIC NOx (umol/mg protein)	15.85±1.97	16.24±1.93	80.18±9.01 #
HEPATIC MPO (u/gr tissue)	3.2±0.3	3.8±0.2	6.5±0.5 #

Data represented as mean + SD (/group).
Significance when p<0.05
* Comparison between Control group and sham-operated group.
# Comparison between Ischemia-reperfusion group and sham-operated group.

Table (2): Effect of Aliskiren 10 mg/kg on, Serum glutamic-pyruvic transaminase (SGPT), Malondialdehyde (MDA), CATALASE, Super oxide dismutase (SOD) Serum TNF- α, HEPATIC NOx and HEPATIC MPO activity in hepatic ischemia-reperfusion rats

Groups / Parameters	Ischemia-reperfusion groups		
	Non-treated group	Preconditioning group	Aliskiren –treated group
SGPT	92.16 ± 0.3	60.72 ± 0.4 *	48.7 ± 0.66 #
MDA (nmol/mg protein)	232.22±50.15	53.21±1.2 *	46.21±5.57 #
CATALASE (u/mg protein)	22.264±1.1	39.81±1.5 *	39.84 ±1.6 #
SOD (u/mg protein)	1.55±.32	3.40±.31 *	4.3±.62 #
Serum TNF-α (pg/mL)	400.55 ± 90.58	150.51±20.35 *	148.18±22.15 #
HEPATIC NOx (umol/mg protein)	80.18±9.01	51.65±3.55 *	37.4±1.39 #
HEPATIC MPO (u/gr tissue)	6.5±0.5	4.3±0.3 *	3.99±0.6 #

Data represented as mean + SD (/group).
Significance when p<0.05
* Comparison between non-treated Ischemia-reperfusion group and preconditioning group.
# Comparison between non-treated Ischemia-reperfusion group and Aliskiren-treated group.
^ Comparison between preconditioning group and Aliskiren-treated group.

Discussion

Ischemia-reperfusion injury is a complex pathophysiology with a number of contributing factors. The ischemic insult can lead to sublethal cell injury, which is aggravated by the formation of reactive oxygen from various intracellular sources during reperfusion. In addition, formation of proinflammatory mediators and the recruitment and activation of macrophages, neutrophils, and lymphocytes, can further enhance the injury. Furthermore, it has been shown that the renin-angiotensin system (RAS) may play roles in diseases of chronic inflammation. However, whether the RAS can also mediate acute inflammation in liver is unclear.

Aliskiren is the first in a new class of orally effective rennin inhibitors and is a potent inhibitor of human rennin both in hypertensive patients and in healthy volunteers. *[8]*

This study was designed to determine the effect of the rennin inhibitors (Aliskiren) on acute liver damage and inflammation caused by hepatic ischemia and subsequent reperfusion.. Furthermore, this work compares between Aliskiren's effect and hepatic preconditioning. Preconditioning procedures significantly enhanced liver regeneration in the experimental model of reduced-size rat liver transplantation. *[9]*

In the present study, Ischemia-reperfusion produced a significant increase in Serum glutamic-pyruvic transaminase (Table 1). Serum glutamic-pyruvic transaminase is considered as a parenchymal marker enzyme for the hepatic cell damage, indicating liver

damage.This is in consistent with many studies *[2, 19&20].* Also, Table 1 showed that Ischemia-reperfusion produced a significant increase in Hepatic tissue Malondialdehyde (MDA), which is considered as a marker for the overproduction of toxic radicals. Furthermore, in the present study Ischemia-reperfusion produced a significant increase in serum TNF-α, hepatic NOx and hepatic MPO.It also, produced a significant decrease in activity of hepatic antioxidant protective enzymes' (catalase and SOD) levels

Vaghasiya et al., *[21]* showed that Lipid peroxidation, xanthine oxidase activity, myeloperoxidase activity and nitric oxide level in liver tissue, were significantly increased after IR in diabetic rats compared to normal rats. Also, they showed that antioxidant enzymes like glutathione, superoxide dismutase, catalase and glutathione peroxidase, were significantly reduced.

Moreover, the results of study of Xin et al., *[4]* showed that 90-min hepatic ischemia followed by 4-h reperfusion, induced significant lung injury as it was manifested by evidence of polymorph neutrophil (PMN) infiltration. This lung injury was associated with inflammation, as indicated by NF-κB translocation, increase of TNF-α levels and MPO activity.

It has also been reported that the role of macrophages in inducing tissue injuries by releasing reactive oxygen species, nitric oxide, complement factors, and proinflammatory cytokines, or even an opposite role in resolution of inflammation or assisting regeneration. *[22]*

Also, in the present study Preconditioning produced a significant decrease in SGPT and hepatic MDA, serum TNF-α, hepatic NOx and hepatic MPO (table 2). On the other hand, it produced a significant increase in catalase and SOD levels (table 2).

This is in consistent with a study of Yuan et al., *[23]* who observed that preconditioned livers showed a significant reduction in oxidative damage, which could be ascribed to a marked increase in SOD as well other endogenous antioxidants such as catalase (CAT) and glutathione peroxidase.

Ischaemic Preconditioning (IP) also attenuated the production of pro-inflammatory cytokines/chemokines during reperfusion. *[24]* Furthermore, Hepatocyte proliferation in rats subjected to 70% hepatectomy is significantly reduced by 45 min of hepatic ischemia. Such an effect was entirely reverted by pre-exposure to IP. *[25]* Consistently, preconditioning procedures significantly enhanced liver regeneration in the experimental model of reduced-size rat liver transplantation. *[9]*

This could be explained by IP protected mitochondria from oxidative reperfusion damage. *[26]* IP also improved hepatic intracellular oxygenation preserved sinusoidal wall integrity and avoided liver microcirculatory failure induced by IR. *[27]*

Also, it has been suggested that ATP itself could act as an additional trigger of liver preconditioning. The release of ATP from hepatocytes enhanced their tolerance to hypoxia independently from the generation of adenosine. *[28]* Further evidence indicated that during IP, hepatic endothelial cells responded to adenosine stimulation by generating nitric oxide (NO),which have contributed to the modulation of hepatocytes tolerance to IR.*[29]* IP also attenuated the production of pro-inflammatory cytokines/ chemokines during reperfusion.*[24]*

Moreover, in the present study Aliskiren produced a significant decrease in SGPT and hepatic MDA, serum TNF-α, hepatic NOx and hepatic MPO (table 2). On the other hand, it produced a significant increase in catalase and SOD levels (table2). Rashikh et al., *[30]* showed that Pretreatment with Aliskiren 100 attenuated the doxorubusin (DXR)-induced rise in MDA, followed by improvement in GSH, CAT and SOD levels which reflects the possible antioxidant effect of Aliskiren.Furthermore, In vivo treatment with a renin inhibitor, significantly reduced oxidative stress and glomerular filtration injury in association with improvements in hypoalbuminemia, blood pressure and plasma rennin activity (PRA). *[30]*

Aliskiren treatment decreased plasma rennin activity (PRA) and this action was accompanied by a reduction in superoxide production and peroxynitrite levels. Both superoxide and peroxynitrite have been demonstrated to oxidize critical eNOS cofactor, leading to eNOS uncoupling. Also, Aliskiren improved NO bioavailability and protects against spontaneous atherosclerotic change in the Watanabe heritable hyperlipidemic (WHHL) rabbit. The findings in WHHL rabbits suggest that the inhibitory effects of Aliskiren on the peroxynitrite and superoxide production would, at least in part, result in potentiated NO bioavailability through the suppression of NO breakdown. Moreover, Aliskiren treatment significantly up regulated eNOS phosphorylation, which is crucial to eNOS activity. *[31]*

Also, a (pro) renin receptor has been discovered and was detected in the brain, heart, liver, and kidney. Prorenin, when bound to the (pro) renin receptor, displayed enzymatic activity and activation of intracellular signaling pathways without proteolytic removal of the prosegment. Also, studies in animals with diabetes and in vitro conditions with high glucose have shown that Aliskiren reduces the number of (pro)renin receptors in the kidney, mitigates profibrotic activity in the kidney, and nearly abolishes the apoptotic effects on cultured podocytes. *[7]*

Al-Aubaidy et al., *[32]* showed that VCAM-1, MCP-1, TNF-alpha and IL-17 expression is up regulated under hypercholesterolemic conditions. The anti-inflammatory effect of Aliskiren has been supported by many researches; *Ino et al., [33]* demonstrated that renin inhibition by Aliskiren significantly reduced the expression of VCAM-1 and ICAM

in injured arteries, and he found that the expression of NF-$_k$B, was attenuated by Aliskiren.*[33]* Also, Aliskiren down-regulates TNF-alpha-stimulated Tissue Factor expression in human umbilical vein endothelial cells, possibly as a reflection of endothelial renin activation by the cytokine.*[34]*

Also, intraperitoneal (I.P.) administration of Aliskiren (25 or 50 mg/kg) for 14 days in chronic constriction injury (CCI) -subjected rats, significantly attenuated CCI-induced pain-related behavior and rise in TNF-α level. It may be concluded that Aliskiren-mediated anti-inflammatory actions could be responsible for its beneficial effects in neuropathic pain in rats. *[35]* Moreover, Aliskiren (50mg/kg) I.P. reduced levels of TNF-α and IL-6 in hind paw homogenates of formalin-injected mice. *[36]*

Furthermore, Matavelli et al., *[37]* concluded that reduced albuminuria in diabetes is via reduction in renal inflammation, independent of BP changes. This is because Aliskiren reduced renal interstitial fluid TNF-α and IL-6 and the renal expression of TNF-α, IL-6, transforming growth factor beta 1, and nuclear factor kappa B. *[37]*

In the gentamicin-treated kidneys, the levels of inflammatory cytokines (TNF-α, IL-1β and IFN-γ) and adhesion molecules were restored by Aliskiren co-treatment. These findings suggest that Aliskiren attenuates gentamicin-induced nephropathy by suppression of inflammatory factors. *[38]*

Also, Aliskiren may be a useful therapeutic agent in the treatment of type II diabetes and diabetic nephropathy. This may be attributed to the improvement of albumin levels in plasma and suppression of proinflammatory cytokine synthesis viz TNF-α with Aliskiren treatment. *[39]*

In conclusion, the renin inhibitor Aliskiren has an anti-inflammatory effects through the inhibition of inflammatory cytokines (TNF-α), hepatic NOx and hepatic MPO. Moreover, Aliskiren has an antioxidant effect through the attenuation of rise in hepatic MDA followed by improvement in GSH, CAT and SOD levels. This effect is the cause of the hepatoprotective effect of Aliskiren against IR which is similar to hepatoprotective effect of preconditioning. This study proves the benifial effect of Aliskiren on acute liver damage and inflammation caused by hepatic ischemia and subsequent reperfusion. It also indicates the use of Aliskiren on acute inflammatory conditions, especially acute IR injury.

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