Research Article

"Possible Effects of Mirabegron on Rat Liver in Renal Ischemia Reperfusion Injury: Histological and Immunohistochemical Studies"

Mahmoud M. Montaser, Ibrahim K. Ragab, Esam O. Kamel
Department of Medical Histology and Cell Biology, Faculty of Medicine, Al-Azhar University in Assuit

Abstract
Liver and kidney are both critical controller organs in our body and any liver or kidney damage including organ ischemia, may affect the other. Renal ischemia reperfusion injury diminishes antioxidant protein and increases neutrophils and lymphocytes amassing in liver tissue. Mirabegron is a medication used to treat overactive bladder. It works by activating the β_3 adrenergic receptor in the bladder, resulting in its relaxation. We aimed in this study to investigate the possible histological and immmunohistochemical effects of mirabegron on the rat liver in cases of induced renal ischemia reperfusion injury. Fifteen rats were used and randomly classified into 5 groups, 10 rats each. Control group in which the rats received the vehicle; CMC without any surgical procedures. Sham group in which the rats received the vehicle then undergone laboratomy without ligation of renal pedicle. Mirabegron group in which the rats received 0.3mg/kg mirabegron without surgical procedures. Renal Ischemia Reperfusion group (RIR), rats administered the vehicle, after which ischemia induced (30 min) followed by reperfusion (3 hours). Mirabegron + RIR group in which the rats pretreated with 0.3 mg/kg mirabegron then RIR induced using the same procedures as that for the RIR group. The liver tissues from all groups were collected and then processed for histological and iNOs immunohistological studies.Liver sections from RIR rats showed loss of normal architecture of hepatic lobules in the form of congested central vein, restricted inflammatory cell infiltration and dilated hepatic sinusoids. Most of hepatocytes looked vacuolated with eccentric nuclei and others showed pyknotic dense nuclei. In comparison, pretreating the RIR rats with mirabegron showed restoration of the normal architecture of the hepatic lobules to great extent. Positive iNOS immunostaining in hepatocytes of RIR rats was noticed and pretreating the RIR rats with mirabegron reversed this positivity**.** We can conclude that mirabegron has a protective role on the rat liver in induced renal ischemia reperfusion injury.
Keywords; Liver, RIR, Mirabegron, iNOs

Introduction
Liver and kidney are both critical controller organs in our body and any liver or kidney damage may affect the other (Park, et al., 2011). Acute kidney injury and ischemic renal illnesses are common clinical records (Thakar, et al., 2009). Renal Ischemia-reperfusion injury (RIR) depicts the tissue ischemia with lacking oxygen supply taken after by reperfusion which starts a wide cluster of incendiary reactions (Malek and Nematbakhsh., 2015). Remote organ damage is an oxidative harm which will be seen in different organs away from the tissue uncovered to RIR (Zhu, et al., 2013). Farther organ disappointment like liver, brain and lung commonly coexists with intense renal damage in the intensive care units and it increments the mortality chance [Golab, et al., 2009) and (Grams and Rabb, 2012). Despite the progressed renal substitution treatment, mortality among patients who support intense renal injury complicated by multi-organ dysfunction, remained unaltered and was evaluated at around 50% (Thakar, et al., 2009).

RIR diminishes antioxidant protein exercises (Serteser, et al., 2002) and increases neutrophils and lymphocytes amassing in liver tissue (Golab, et al., 2009). Furthermore, it causes an increment in provocative cytokines like tumor necrosis factor-alpha (Serteser, et al., 2002). Additionally

advances oxidative stress and lipid peroxidation (Kaçmaz, et al., 2005). which in turn causes hepatic auxiliary and functional derangements and cell passing eventually (Kadkhodaee, et al., 2009). This complex pathophysiology results from a number of contributing variables, so it is troublesome to attain successful treatment or security by targeting only person arbiters or components in spite of the endeavors made to deal with this issue (Lameire, et al., 2013). The expanding mortality rate in cases with acute renal damage requires planning methodologies to evaluate the impact of renal damage on far off organ. In this manner, a few considers (Kaçmaz, et al., 2005 & Kim, et al., 2011) tried to secure liver from inaccessible damage actuated by renal RIR for the prevention and constriction of intense renal harm related horribleness. RIR is a cause of acute renal injury and leads to multi-organ dysfunction especially liver injury [El-Tahawy N G & Ali A H, 2017).

Mirabegron, sold under the brand name Myrbetriq among others, is a medication used to treat overactive bladder, Its benefits are similar to anti-muscarinic medication such as solifenacin or tolterodine. It works by activating the β_3 adrenergic receptor in the bladder, resulting in its relaxation.

Mirabegron was approved for medical use in the United States and in the European Union in 2012 (Drug approval package., 2020 & Sacco, et al., 2014). In 2017, it was the 191st most commonly prescribed medication in the United States, with more than three million prescriptions (Betmiga., 2020).

Human cytochrome P450 enzymes and esterases involved in the metabolism of mirabegron, Mirabegron hydrolysis was catalyzed in human blood, plasma and butyrylcholinesterase (BChE) solution, but not in human liver micro-somes and intestinal microsomes. The inhibition profiles in human blood and plasma were also similar to those in BChE solution, suggesting that mirabegron hydrolysis is catalyzed by BChE (Takusagawa., 2012).

In preregistration clinical trials, serum aminotransferase elevations were uncommon and mild in patients treated with mirabegron and rates of serum enzyme elevations were similar to those with placebo treatment. Among several thousands of patients treated, there were no episodes of clinically apparent liver injury. Since its approval and more wide scale use, there have not been any published reports of hepatotoxicity attributed to mirabegron. The product label for mirabegron mentions occasional elevations in ALT and AST associated with treatment, but not clinically apparent hepatitis or hepatotoxicity (Hatanaka, et al., 2013).

Aim of the work

We aimed in this study to evaluate the possible effects of Mirabegron on rat liver in renal ischemia reperfusion injury.

Material and methods

Chemicals:

The β3 agonist, mirabegron (Sigma Aldrich, MO, USA) was suspended in 2% carboxymethyl cellulose (CMC) (Sigma Aldrich, MO, USA). Urethane was purchased from Sigma Aldrich (St. Louis, MO, USA. CMC was given orally by a dose of 0.5 ml/100 g.).

Animals:

The study was conducted at the laboratory of animals care unit, Faculty of Medicine, Assuit University in accordance with the guidelines for the care and use of laboratory animals established by the Animal Ethics Committee. The study included 50 male albino rats (4-8 weeks) weighting 200-300 gram.They maintained under controlled environmental conditions and provided with standard food for laboratory animals and water ad libitum.

Experimental design:

To evaluate the effect RIR on the liver and the role of the mirabegron in protecting the liver, rats were randomly divided into 5 groups, 10 rats each. Group I (control group) in which the rats received the vehicle; CMC without any surgical procedures.Group II (Sham group) in which the rats received the vehicle then undergone laboratomy without ligation of renal pedicle. Group III (Mirabegron group) where the rats received 0.3mg/kg mirabegron (Hatanaka, et al., 2013) without surgical procedures. Group IV; renal Ischemia Reperfusion group (RIR), rats administered the vehicle, after which ischemia induced

(30 min) followed by reperfusion (3 hours). Group V (Mirabegron + RIR group) in which the rats pretreated with 0.3 mg/kg mirabegron then RIR induced using the same procedures as that for the RIR group.

Surgical procedure

Rats were anesthetized using urethane by a dose of 1.4 mg/kg i.p. (Golder, et al., 2003). In a sterile condition, abdominal skin was shaved and laparotomy was conducted via a midline abdominal incision. The intestine was carefully evacuated from the abdominal cavity and the kidneys were localized, the vascular pedicles of both kidneys were exposed. To induce ischemia, clamping of both renal pedicles was performed using (a micro-vascular clamp) a traumatic mini-bulldogs (Aesculap, Tuttlingen, Germany) for 30 minutes. The intestine was returned into the abdominal cavity and to minimize the evaporation and cooling, it was covered with moistened compresses. After 30 minutes of ischemia induction, the clamps removed and both kidneys reperfused. Immediately after 3 hours of reperfusion, the liver dissected and prepared for histological examination.

Histopathological examination:

The liver tissues from all groups were fixed for 24 hours in 10% formalin, dehydrated by passing in ascending grades of ethyl alcohol, cleaned in xylene, and embedded in paraffin. Sections of 5 microns were prepared by using a microtome and stained with hematoxylin and eosin by a method described previously by Bancroft and Gamble (2008). The stained sections were examined and photographed by the light microscope (Olympus CX41 microscope, Olympus, Tokyo, Japan).

Immunohistochemistry:

Sections of 4 μm thickness prepared from rats of all groups were deparaffinized and rehydrated and endogenous peroxidase activity was blocked using 3% H_2O_2 in methanol. Sections were pretreated in citrate buffer (pH 6.0) in a microwave and then incubated at room temperature with rabbit polyclonal antibodies specific for the rat targets. The antibodies used were anti-inducible nitric oxide synthase (iNOS) antibodies (Thermo Scientific, USA, dilution 1:1000). After that, the sections were rinsed with TBS containing 0.05% Tween 20 twice and incubated with 2ry antibody; goat anti-rabbit IgG-HRP conjugate (Vivantis Technologies, Malaysia) at a dilution of 1:5000, for 1 h at 4°C. After another wash with TBS containing 0.05% Tween 20, the immunereactivity was developed with 0.05% diaminobenzidine (DAB) and 0.01% H_2O_2 for 1– 3 min and the tissue sections were observed for brown color formation using a light microscope. Slides were counterstained with hematoxylin. Sections were photographed using a light microscope (Olympus BX50, Tokyo, Japan) and figures were made using CorelDraw software.

Results

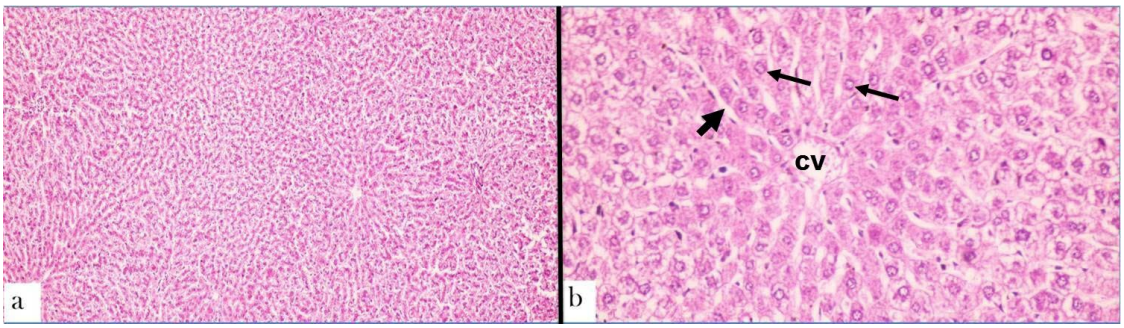

Fig 1: Photomicrographs of liver sections from control group stained with hematoxylin and eosin. (a) Low power section shows normally looked hepatic lobules formed of hepatocytes cords radiating from the central vein and separated by hepatic sinusoids (X100). (b) Higher magnification of the previous photomicrograph shows central vein (CV) and hepatocytes with rounded vesicular nuclei (thin arrow) forming the hepatic cords that are separated by hepatic sinusoids (short arrow) (X400).

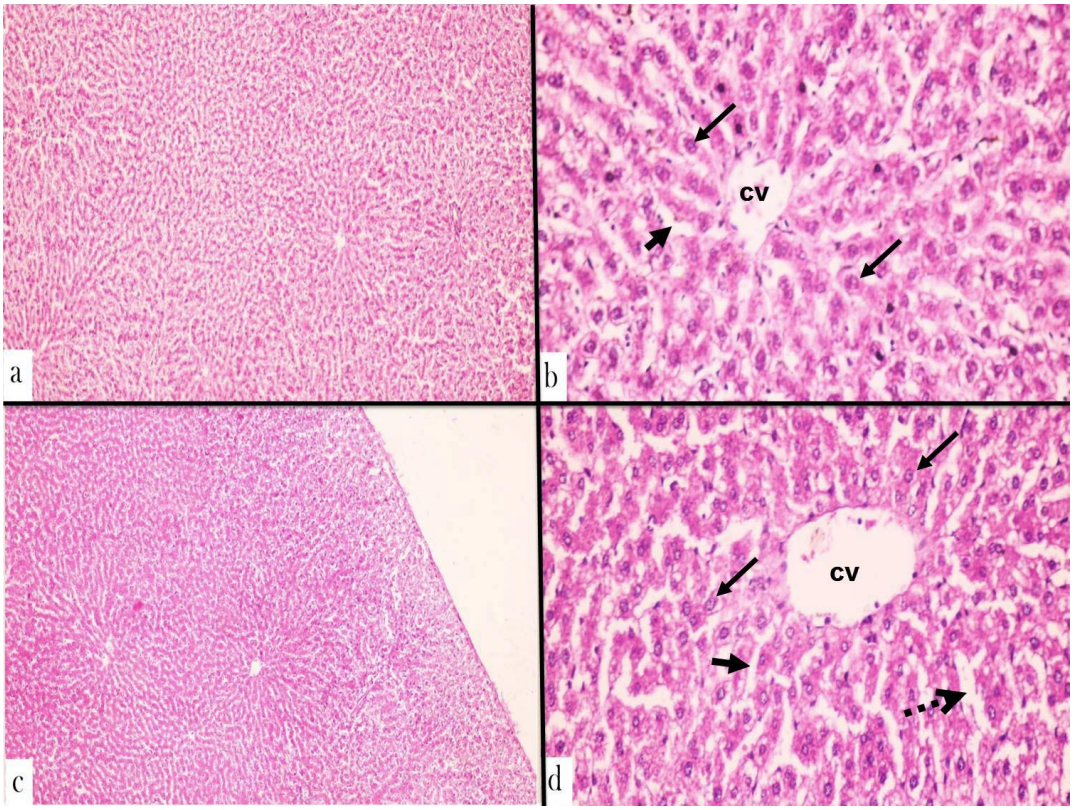

Fig 2: Photomicrographs of liver sections stained with hematoxylin and eosin. **(a)** Representive section from sham group shows normally looked hepatic lobules formed of hepatocytes cords radiating from the central vein and separated by hepatic sinusoids (X100). **(b)** Higher magnification from sham group showing hepatocytes with rounded vesicular nuclei (thin arrow), hepatic sinusoids (short arrow) and central vein (CV) (X400). **(c)** Representive section from Mirabegron group shows normally appeared hepatic lobules formed of hepatocytes cords radiating from the central vein and separated by hepatic sinusoids (X100). **(d)** Higher magnification of the previous photomicrograph showing central vein (CV), hepatocytes with rounded nuclei (thin arrow) and hepatic sinusoids (short arrow). Many hepatic sinusoids look wide (dotted arrow) (X400).

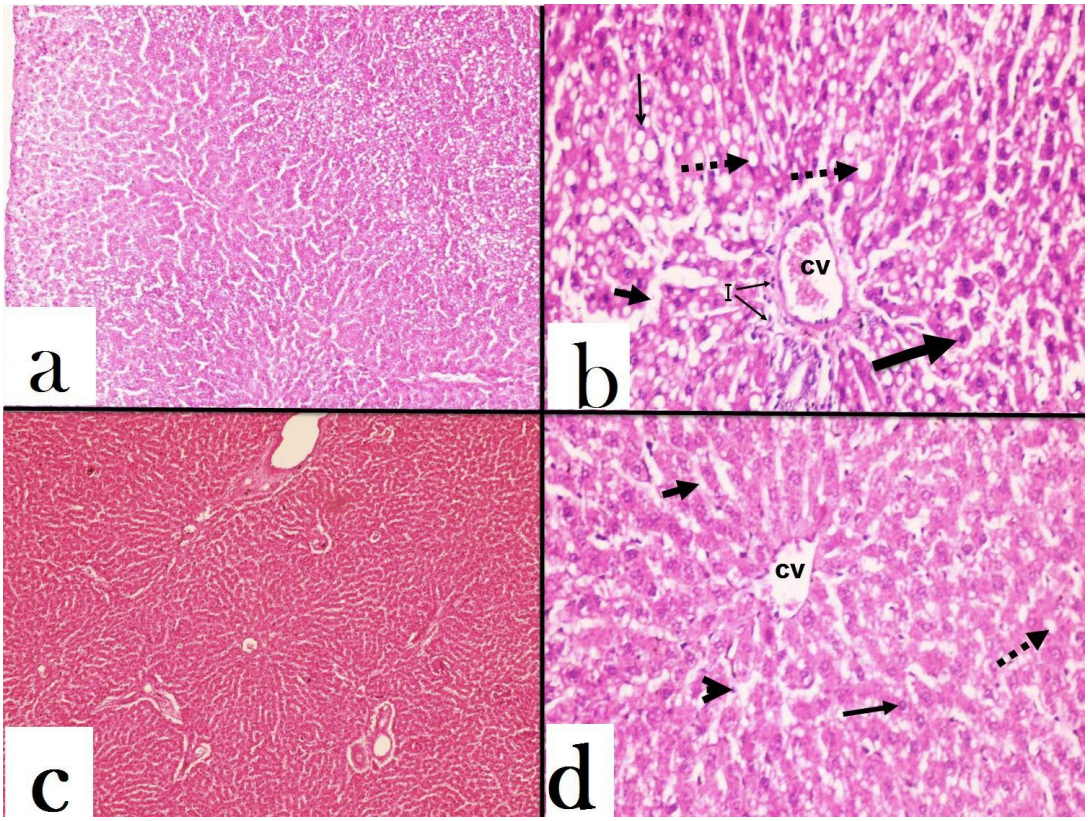

Fig 3: Photomicrographs of liver sections stained with hematoxylin and eosin. **(a)** Representive section from RIR group showing loss of normal architecture of hepatic lobules (X100). **(b)** Higher magnification of the previous photomicrograph showing congested central vein (CV) and surrounding inflammatory cell infiltration (I). Most of hepatocytes look vacuolated (dotted arrow) with eccentric nuclei (thin arrow) and others have pyknotic dense nuclei (thick arrow). The hepatic sinusoids look dilated (short arrow) (X400). **(c)** Representive section from Mirabegron + RIR group showing restoration of the normal architecture of the hepatic lobules (X100). **(d)** In highly magnified photomicrograph from Mirabegron + RIR group; the central vein (CV) looks less congested and the hepatocytes have rounded nuclei (thin arrow), but still many of them are vacuolated (dotted arrow). The hepatic sinusoids look normal (short arrow) and few are dilated (arrowhead) (X400).

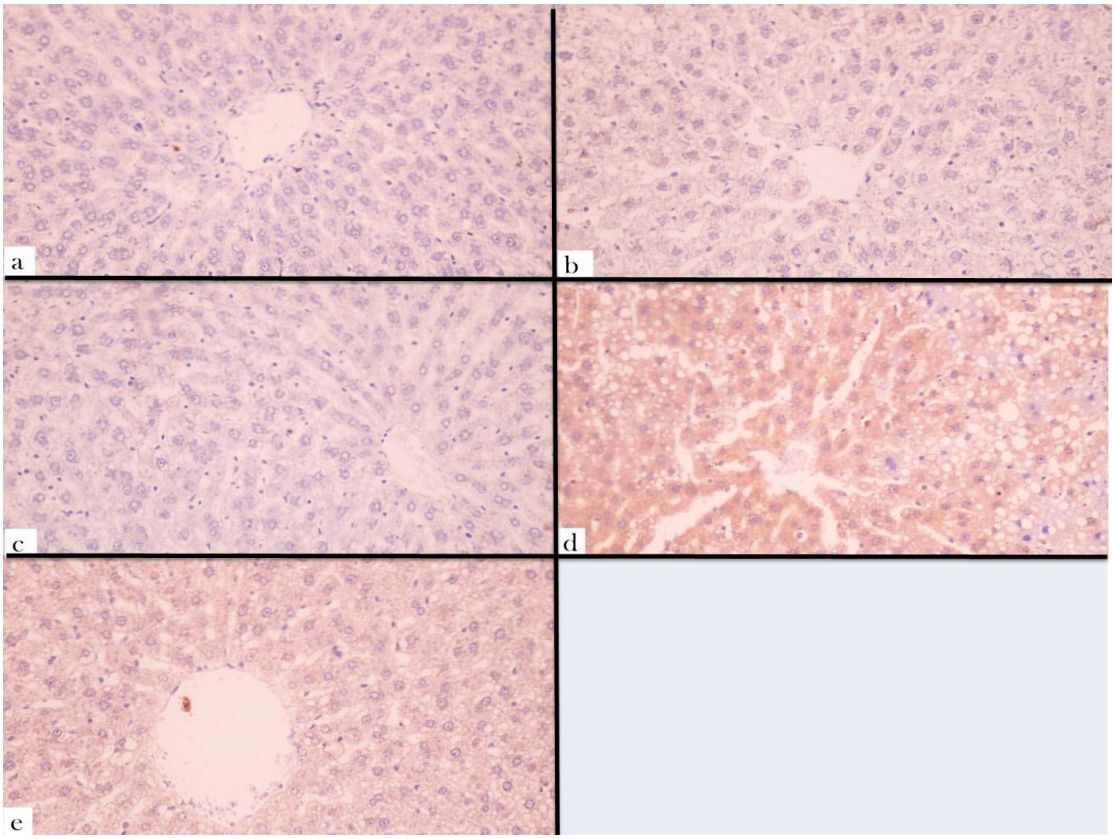

Fig 4: Immunohistochemical localization of iNOS in liver tissues is examined and the positive expression is represented by the brown color. Representative sections from control **(a),** sham (b), Mirabegron **(c)** RIR **(d)** and Mirabegron + RIR rats **(e).**
Negative expression of iNOS in hepatocytes from control **(a),** sham **(b)** and Mirabegron rats **(c)** is noticed. **(d)** Shows positive iNOS immunostaining in hepatocytes of RIR rats. Mirabegron pretreatment reversed the increase in iNOS expression caused by RIR induction **(e).**

Results

Effect of Mirabegron on Histopathological changes in liver tissues induced by RIR

Examination of liver sections from control rats (Fig 1a) reported that it is formed of hepatic lobules that composed of hepatocytes cords radiating from the central vein and separated by hepatic sinusoids. Higher magnification of the previous photomicrograph showed (Fig 1b) hepatocytes with rounded vesicular nuclei forming the hepatic cords that are separated by hepatic sinusoids.

Examination of liver sections rats from sham (Fig 2a, b) and Mirabegron (Fig 2c, d) groups showed the same results as control group except for few dilated hepatic sinusoids in Mirabegron group (Fig 2d).

In contrast, liver sections from RIR rats (Fig 3a) showed loss of normal architecture of hepatic lobules and in highly magnified pictures (Fig 3b), we reported congested central vein, restricted inflammatory cell infiltration and dilated hepatic sinusoids. Most of hepatocytes looked vacuolated with eccentric nuclei and others showed pyknotic dense nuclei. In comparison, pretreating the RIR rats with Mirabegron showed restoration of the normal architecture of the hepatic lobules (Fig 3c). At high magnification (Fig 3d), the central vein appeared less congested and the hepatocytes had e rounded nuclei, but still many of them were vacuolated. The hepatic sinusoids were normal and few were dilated. No inflammatory cell infiltration was noticed

Effect of Mirabegron on RIR-induced expression of iNOS in hepatic tissue Negative expression of iNOS in hepatocytes from control **(Fig. 4a),** sham **(Fig. 4b)** and mirabegron rats **(Fig. 4c)** is noticed. **(Fig. 4d)** Shows positive iNOS immunostaining in hepatocytes of RIR rats. Mirabegron pretreatment reversed the increase in iNOS expression caused by RIR induction **(Fig. 4e).**

Discussion

As regard, the effects of Mirabegron on histopathological changes in liver tissues induced by RIR. Examination of liver sections from control rats showed hepatic lobules that composed of hepatocytes cords radiating from the central vein and separated by hepatic sinusoids. Hepatocytes with rounded vesicular nuclei forming the hepatic cords that are separated by hepatic sinusoids.

Examination of liver sections of rats from sham and Mirabegron groups showed the same results as control group. This can be explained by the fact that mirabegron is a new oral β3-adrenergic receptor agonist for the treatment of overactive bladder (OAB). (Bridgeman, et al., 2013).

Mirabegron, administered as a solution, is rapidly absorbed after oral administration, circulates in plasma as the unchanged form and metabolites, and is recovered in urine and feces mainly as the unchanged form (Takusagawa, et al., 2012).

It is eliminated by renal and metabolic routes. Pharmacokinetic changes of mirabegron observed in subjects with mild or moderate renal impairment or mild hepatic impairment are of small magnitude and likely to be without clinical importance (Dickinson, et al., 2013).

There have not been any published reports of hepatotoxicity attributed to mirabegron. The product label for mirabegron mentions occasional elevations in ALT and AST associated with treatment, but not clinically apparent hepatitis or hepatotoxicity. Mirabegron has not been implycated in causing liver enzyme elevations or clinically apparent acute liver injury.

Mirabegron is extensively metabolized by Cytochrome 2D6 (CYP 2D6) an enzyme that in humans is encoded by the CYP2D6 gene. CYP2D6 is primarily expressed in the liver. CYP 2D6 and inhibitors of its activity making it susceptible to drug-drug interactions with agents that are also metabolized by this enzyme.

The presence of few dilated hepatic sinusoids in mirabegron group might result from its metabolism to a toxic or immunogenic inter-mediate but the possible cause of liver injury due to mirabegron is not known (Bethesda, 2012).

In contrast, liver sections from RIR rats showed loss of normal architecture of hepatic lobules, congested central vein, restricted inflammatory cell infiltration and dilated hepatic sinusoids. Most of hepatocytes looked vacuolated with eccentric nuclei and others showed pyknotic dense nuclei.

This can be explained by the target cell (endothelial cells, vascular smooth muscle, and parenchymal cells) responses to I/R injury. There is an evidence implicating leukocytes as mediators of I/R injury in several tissues, including heart, skeletal muscle, brain, intestine, and liver. (Granger and Korthuis, 1995). Other cells (e.g., mast cells) appear to contribute to I/R injury in a more indirect fashion, by releasing factors that promote the recruitment and activation of leukocytes. (Kanwar and Kubes, 1994).

The Kupffer cell (KC), the resident macrophage of the liver, appears to contribute to the modulation of acute inflammatory responses in that organ. The KC exert both immunologic and metabolic functions by presenting antigens on their surface and by producing/releasing chemical mediators such as interleukin (IL)-1, platelet-activating factor (PAF), tumor necrosis factor (TNF)-a, and different prostaglandins. (Decker. 1990) Many of the substances liberated by activated KCs are known to promote the adhesion of leukocytes to vascular endothelium.

These results coincide with Sara, et al., who found by histological, immunohistochemical, and electron microscopic studies liver injury especially Kupffer cells in renal ischemia/ reperfusion injury (Sara, 2019).

In comparison, pretreating the RIR rats with Mirabegron showed restoration of the normal architecture of the hepatic lobules. The central vein appeared less congested and the hepatocytes had rounded nuclei, but still many of them were vacuolated. The hepatic sinusoids were normal and few were dilated. No inflammatory cell infiltration was noticed. This can be explained by the protective effect of a β3-Adrenoceptor agonist (mirabegron) on chronic renal Ischemia (Sawada, et al., 2013).

As regard, the effects of Mirabegron on RIR-induced expression of iNOS in hepatic tissue. It was found by examination of immunehisto-chemical localization of iNOS in liver tissues the positive expression which is represented by the brown color. The positive iNOS immune-staining in hepatocytes of RIR rats can be explained by the fact that proinflammatory cytokines such as tumor necrosis factor alpha and interleukin (IL) 1beta stimulate the induction of inducible nitric oxide synthase (iNOS) in hepatocytes, followed by massive production of nitric oxide. I/R upre-gulated the susceptibility of hepatocytes to confer the induction of iNOS gene expression. I/R may augment hepatocyte susceptibility for the induction of iNOS gene expression through the enhancement of IL-1R1. Ischemia/R also increased the levels of iNOS protein and its messenger RNA. Furthermore, I/R enhanced the activation of transcription factor NF-kappaB and the transactivation of iNOS promoter (Yanagida, et al., 2006).

The mirabegron pretreatment reversed the increase in iNOS expression caused by RIR induction which can be explained by absence of proinflammatory cytokines such as tumor necrosis factor alpha and interleukin (IL) 1beta which stimulate the induction of inducible nitric oxide synthase (iNOS) in hepatocytes.

Conclusion

If the results are valid for humans, they support β3-AR agonism (mirabegron) as a potential treatment of chronic renal ischemia.

References

1. Park SW, Chen SW, Kim M, Brown KM, Kolls JK, et al., (2011): Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. Lab Invest (91): 63-84.
2. Thakar CV, Christianson A, Freyberg R (2009): Incidence and outcomes of acute kidney injury in intensive care units: A veteran's administration study. Crit Care Med (37): 2552-2558.
3. Malek M, Nematbakhsh M (2015): Renal ischemia/reperfusion injury from pathophy-siology to treatment. J Renal Injury Prevention (4): 20-27.
4. Zhu XY, Lerman A, Lerman LO (2013): Concise Review: Mesenchymal Stem Cell Treatment for Ischemic Kidney Disease. Stem Cells 31: 1731-1736
5. Serteser M, Koken T, Kahraman A, Yilmaz K, Akbulut G, et al., (2002): Changes in hepatic TNF-alpha levels, antioxidant status, and oxidation products after renal isch-emia/reperfusion injury in mice. J Surg Res (107): 234-240.
6. Kaçmaz A, User EY, Sehirli AO, Tilki M, Ozkan S, et al., (2005): Protective effect of melatonin against ischemia/reperfusion-induced oxidative remote organ injury in the rat. Surg Today(35): 744-750.
7. Kadkhodaee M, Golab F, Zahmatkesh M, Ghaznavi R, Hedayati M (2009) :Effects of different periods of renal ischemia on liver as a remote organ. World J Gastroenterol (15): 1113-1118
8. Lameire NH, Bagga A, Cruz D, De Maeseneer J, Endre Z, et al., (2013): Acute kidney injury: an increasing global concern. Lancet (382): 170-179.
9. Kim M, Park S, Kim M, D'Agati V, Thomas H. (2011): Isoflurane activates intestinal sphingosine kinase to protect against renal ischemia-reperfusion induced liver and inte-stine injury. Anesthesiology (114):363-373.
10. Nashwa Fathy Gamal El-Tahawy and Abdel Hamid Sayed AboBakr Ali (2017): Possible Protective Effect of Bone Marrow-Mesenchymal Stem Cells (BM-MSCs) Against the Remote Liver Injury Induced by Renal Ischemia Reperfusion in Male Albino Rats. J Cytol Histol 2017, 8:5.
11. "Drug Approval Package: Myrbetriq (mirabegron) Extended Release Tablets NDA #202611". U.S. Food and Drug Admi-

nistration (FDA), 10th August 2012. Retrieved 28 April 2020.
12. Sacco E, Bientinesi R, Tienforti D, Racioppi M, Gulino G, D'Agostino D, et al., (2014): "Discovery history and clinical development of mirabegron for the treatment of overactive bladder and urinary incontinence". Expert Opinion on Drug Discovery. 9(4): 433 48.
13. "Betmiga EPAR". European Medicines Agency. Retrieved 28 April 2020: This article incorporates text from this source, which is in the public domain. "The Top 300 of 2020". ClinCalc. Retrieved 11 April 2020.
14. Takusagawa, S., Yajima, K., Aiji Miyashita, Shotaro Uehara, Takafumi Iwatsubo & Takashi Usui (2012): Identification of human cytochrome P450 isoforms and esterases involved in the metabolism of mirabegron, a potent and selective β_3-adrenoceptor agonist. Journal Xenobiotica. Pages 957-967 | .Received 02 Feb 2012, Accepted 08 Mar 2012, Published online: 18 Apr 2012 Volume 42, 2012 - Issue 10.
15. Hatanaka, T., M. Ukai, M. et al., (2013): In vitro and in vivo pharmacological profile of the selective β 3-adrenoceptor agonist mirabegron in rats. [Volume Naunyn-Schmiedeberg's archives of pharmacology 386(3), pp. 247-253.