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ORIGINAL ARTICLE

Potential Hepatoprotective Effects of Irbesartan, an Accessible Angiotensin II Receptor Blocker, Against Cisplatin-Induced Liver Injury in a Rat Model

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ABSTRACT

Objectives: Drug-induced liver injury is a common adverse reaction that frequently occurs with chemotherapeutic agents, such as cisplatin (CIS). This study seeks to enhance our understanding of drug actions and their associated adverse effects by examining the toxicity of CIS on rat liver tissue. We aimed to investigate the potential hepatoprotective effects of irbesartan (IRB), an easily accessible angiotensin II receptor blocker, in mitigating CIS-induced hepatotoxicity.

Materials and Methods: Wistar albino rats were divided into four groups. These groups included a control group [saline, *per* oral (*p.*o.)] for seven days, and 1 mL saline intraperitoneal [(i.p.) on the fourth day]; a CIS group (1 mL saline for seven days and 7.5 mg/kg CIS *i.p.* on the fourth day); a CIS + IRB group (IRB: 50 mg/kg *p.o.* for seven days and 7.5 mg/kg CIS *i.p.* on the fourth day), and an IRB group (50 mg/kg IRB *p.o.* for seven days). The effect of IRB on interleukin-1 beta (IL-1β) and caspase 3 levels was evaluated by immunohistochemical analysis, and its effects on mRNA expression levels of CCAAT/enhancer-binding protein homologous protein (CHOP) and immunoglobulin-heavy-chain-binding protein (BiP) were tested by quantitative real-time polymerase chain reaction.

Results: IRB administration mitigated CIS-induced liver toxicity by inhibiting endoplasmic reticulum (ER) stress. Specifically, this drug reduced the mRNA expression of ER stress markers, including CHOP and BiP. In addition, IRB treatment decreased oxidative stress, inflammatory responses, and apoptotic markers.

Conclusion: These findings suggest that IRB is a promising therapeutic option for preventing CIS-induced liver injury, potentially by modulating ER stress-related pathways.

Keywords: Cisplatin, ER-stress, irbesartan, liver toxicity.

INTRODUCTION

Cisplatin (CIS) (*cis*-diamminedichloroplatinum) is one of the most commonly preferred chemotherapeutic agents for treating malignancies. Several adverse effects can be observed with CIS treatment, such as nephrotoxicity, gastrointestinal disorders, neurotoxicity, ototoxicity, hepatotoxicity, and cardiotoxicity.[1,2] CIS-induced hepatotoxicity is the most frequently encountered adverse effect that reduces therapeutic efficacy and limits the usage of this drug in cancer therapy.[3] Studies have shown that CIS-induced toxicity might result from mitochondrial dysfunction, excessive reactive oxygen species (ROS) production, increased tumor necrosis factor-alpha (TNF-α) levels, and induction of endoplasmic reticulum (ER) stress. Although there are predictable links between ER stress and anticancer drug-induced liver injury, the underlying mechanism remains unclear.[4,5]

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ER is a multifunctional organelle found in eukaryotic cells and consists of sac-like structures and branched tubules. It regulates numerous pivotal functions, including protein biosynthesis, folding, trafficking, calcium storage, and lipogenesis.[6,7] Altering physiological conditions affect ER homeostasis for various reasons, such as genetic mutations, heat shock, oxidative stress, and multiple pathophysiologies. Furthermore, increased protein synthesis requirement, glucose deprivation, or imbalance in ER calcium stock levels can cause impaired functionality of the ER, termed ER stress.[8]

Unfolded protein response (UPR) signaling is responsible for re-establishing cellular homeostasis against ER stress, and it is regulated by three ER-membrane-localized transmembrane proteins: inositol-requiring kinase 1 alpha (IRE1α), protein kinase R-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6). It coordinates the ER protein folding capacity, proteostasis, and programmed cell death under prolonged UPR activation. Additionally, UPR has been associated with drug resistance in numerous pathologies.[7,9]

Protein aggregation in the ER lumen or insufficient ER capacity causes the release of glucose-regulated protein 78, also regarded as immunoglobulin-heavy-chain-binding protein (BiP), from the UPR sensors to which it is attached. BiP is a member of the heat shock protein family and is a key player that manages ER stress responses.[10] Thus, BiP levels in the ER pool play a critical role in managing ER stress. Furthermore, progesterone receptor regulates the expression level of various cell death-associated inducers, such as CCAAT/enhancer-binding protein homologous protein (CHOP), also known as GADD153.[11] CHOP is a pro-apoptotic transcription factor induced by ER stress and mediates apoptosis.[12] Because of the critical roles of BiP and CHOP proteins, changes in their levels are frequently evaluated in investigating ER stress at the cellular level.

Metabolic dysregulation induced by excessive numbers of lipids, glucose, cytokines, neurotransmitters, or exposure to chemotherapeutic agents leads to ER stress. This disruption in normal cellular function can contribute to the exacerbation of inflammatory responses and other associated pathologies.[13] Recent studies suggest that ER stress management is a potential target for new therapeutic approaches to be developed against cancer or tissue injuries.[14,15]

Irbesartan (IRB) is an angiotensin II receptor blocker that is commonly used for treating hypertension. It acts on the renin-angiotensin system (RAS) and exerts protective effects on diverse organs in the body, such as the heart, kidney, and liver. In addition, IRB has anti-inflammatory, antioxidant, and antifibrotic effects.[16] Emerging research has demonstrated that various pharmaceutical agents used in cancer treatment can induce a robust ER stress response. This novel insight may offer new avenues for developing more effective therapeutic strategies.[17,18] In addition, the efficacy of drug or chemoresistance occurrence can be alleviated by modulation of ER stress. This approach can be an important factor that could alter the treatment of adverse responses.[18] However, the relationship between ER stress and the action of anticancer drugs remains unclear. In this study, we shed light on the potential protective mechanisms underlying IRB against CIS-induced hepatotoxicity. To achieve this, we evaluated the impact of IRB on ER stress, oxidative stress parameters, and anti-inflammatory cytokine expression. The present findings may offer valuable insights into the therapeutic potential of IRB in managing drug-induced liver injury.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats (n= 32) with an average weight of 250-300 g were obtained from the Animal Research Laboratory. They were group-housed (eight rats *per* cage) under a 12/12 hours light/dark cycle at room temperature (24 ± 1 ˚C) with a relative humidity of 50 ± 10% and access to food and water *ad libitum*. Animals were acclimatized for at least seven days before experimentation (Figure 1). All experimental procedures were permitted by the National Institutes of Health and the Committee on Animal Research according to the ethical rules (approval number: 09/03, date: 23.09.2021).

All rats were randomly separated into four groups as follows:

1) The control group was given mL saline [*per* oral (*p.o.*)] for seven days. On the fourth day, 1 mL of saline was given [intraperitoneal (*i.p.*)].

2) The CIS group was administered 1 mL saline *p.o.* for seven days, and 7.5 mg/kg *i.p.* CIS (CIS, Koçak Farma, Türkiye) was administered on the fourth day.[19]

3) The CIS + IRB group was administered 50 mg/kg IRB (Sandoz, Switzerland) *p.o.* for 7 days, and 7.5 mg/kg CIS *i.p.* was administered on the fourth day.[20]

4) The IRB group was administered 50 mg/kg IRB *p.o.* for seven days, and 1 mL saline *i.p.* was administered on the fourth day.

The sacrifice of animals was performed 6 hours after the last drug administration under ketamine (80-100 mg/kg) (Alfamin, Alfasan International Besloten Vennootschap) and 8-10 mg/kg xylazine bio 2% solution (Bioveta, Czech Republic) anesthesia. Liver tissue were then removed. One part of the tissues converged for total antioxidant status (TAS), total oxidant status (TOS), and immunoblotting assay. The remaining tissue was fixed in 10% buffered formaldehyde for histopathological examination and immunohistochemical (IHC) analysis.

Histopathological analysis

The liver was removed, fixed in 10% buffered formalin during necropsy, and taken for routine pathology processing after macroscopic sampling using an automatic tissue processor (Leica ASP300S, Wetzlar, Germany) and embedded in a paraffin block. 5 µ thick sections were taken from blocks using a microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Then, hematoxylin-eosin staining was used to stain the sections and monitored by a light microscope.

IHC analysis

Two sections were taken from all groups of the liver samples and placed in poly-L-lysine coated slides. Then, IHC staining

of slices with anti-caspase-3 [sc-7272, 1:100, Santa Cruz (Texas, USA) and anti-interleukin-1 beta (IL-1β) [sc-52012, 1:100 dilution, Santa Cruz (Texas, USA)] was performed using the streptavidin-biotin technique consistent with the manufacturer's protocol. The incubation of sections with primary antibodies for 60 min was performed, and then immunohistochemistry using biotinylated secondary antibody and streptavidin-alkaline phosphate conjugate was performed. The secondary antibodies of the EXPOSE mouse and rabbit specific horseradish peroxidase/diaminobenzidine (HRP/DAB) Detection IHC kit (ab80436) (Abcam, Cambridge, UK) were used. DAB was used as the chromogen. Antigen dilution solution was used as a negative control. Blinding was performed in the examination of samples. Semiquantitative analysis was performed to quantify the intensity of the IHC markers using a grading score ranging from (0) to (3) as follows: (0) = negative, (1) = weak focal staining, and ≤ 10%, (2) = diffuse weak staining and ≥ 10%, and (3) = intense diffuse staining and ≥ 10%.[21] Independent ten different areas of each section were analyzed under 40X objective magnification by an experienced pathologist. The Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan) was used for morphometric analysis and microphotography.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from rat tissues using a column-type minipress kit (Bio-Rad, Hercules, CA, USA) following the manufacturer's protocol. Complementary DNA was then synthesized with a cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA). cDNA was amplified using the iTaq Universal SYBR Green Supermix in a CFX96 instrument (Bio-Rad Laboratories, Hercules, CA). In the polymerase chain reaction (PCR) processes, pre-denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 seconds, and at 60 °C for 30 seconds were followed. Melting curve analysis was performed to confirm the specificity of the PCR amplicons. Specific primers were designed to amplify BiP (Forward 5'-*TGT GAC TGT ACC AGC TTA CTT C-3'*, Reverse 5'-*TCT TCT CTC CCT CTC TCT TAT CC-3'*), CHOP (Forward 5'-*GGA GGC TAC ACT CTA CAA AGA AC-3'*, Reverse 5'-*CCT TCT AAC GCT TCC CAA AGA-3'*), and GAPDH (forward 5'-*CAA GGT CAT CCC AGA GCT GAA-3'*, Reverse 5'-*CAT GTA GGC CAT GAG GTC CAC-3'*). The housekeeping gene GAPDH was used to normalize relative gene expression. Relative mRNA expression levels were analyzed using the Livak method.[22]

Detection of oxidative stress markers

Biochemical analyses included measurements of TAS, TOS, and Open Systems Interconnection (OSI) levels. For oxidant-antioxidant analysis, liver tissue samples were homogenized. The spectrophotometric measurement of the TAS and TOS was performed using commercial kits consistent with the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Türkiye), and the TOS/TAS ratio was noticed as OSI value.[23,24]

Liver function parameter measurement

Serum samples were obtained from the blood of rats by centrifugation at 3000 rpm for 10 min. The levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) in serum were determined using the spectrophotometric technique on an autoanalyzer (Beckman Coulter, USA) with the instrument's kit.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software (San Diego, California, USA). Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test was used to compare the groups. $p < 0.05$ were considered significant.

RESULTS

Histopathological finding

In hematoxylin-eosin staining, ten fields at 40X magnification were evaluated in the same way IHC expressions were assessed. The liver is typically organized into hexagonal lobules containing the central vein at the center and the portal vein, hepatic artery, and bile duct at the corners. Hepatic sinusoids connect the central vein to the portal vein (as depicted in Figure 2E). Following four days of CIS treatment, rats exhibited liver congestion, increased Kupffer cells, and reactive changes such as nucleolar prominence, as observed in liver sections (Figures 2A, 2B). However, in the group treated with both CIS and IRB, congestion in the hepatic sinus was reduced, as demonstrated by the H&E slides (Figure 2C). Interestingly, in the IRB-treated group, there was an increase in cytoplasmic eosinophilia (Figure 2D).

IHC findings

IHC evaluation showed that CIS administration caused a significant increase in caspase 3 and IL-1β expressions in the liver tissues of rats ($p < 0.001$). IRB treatment reversed the CIS-induced increment in caspase -3 and IL-1β expressions ($p < 0.01$, $p < 0.001$, respectively) (Figures 3, 4).

Quantitative reverse transcriptase polymerase chain reaction results

Elevated mRNA expression levels of CHOP and BiP were detected in the CIS group compared with the control ($p < 0.05$, $p < 0.001$; respectively). The combined treatment of CIS and

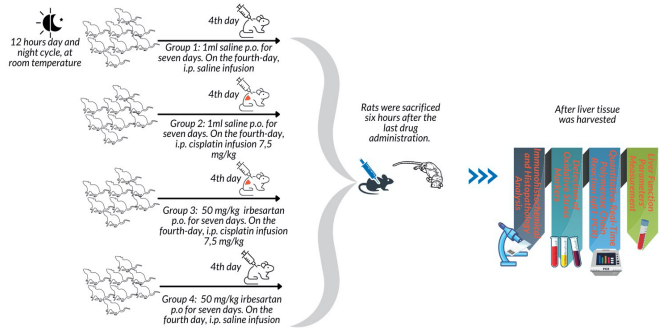

Figure 1. Experimental procedures are depicted as in the drawing

IRB significantly decreased the expression levels of CHOP and BiP compared with the CIS group ($p < 0.01$). Only IRB treatment significantly reduced CHOP and BiP levels compared with the CIS group ($p < 0.001$, $p < 0.01$, respectively) (Figure 5).

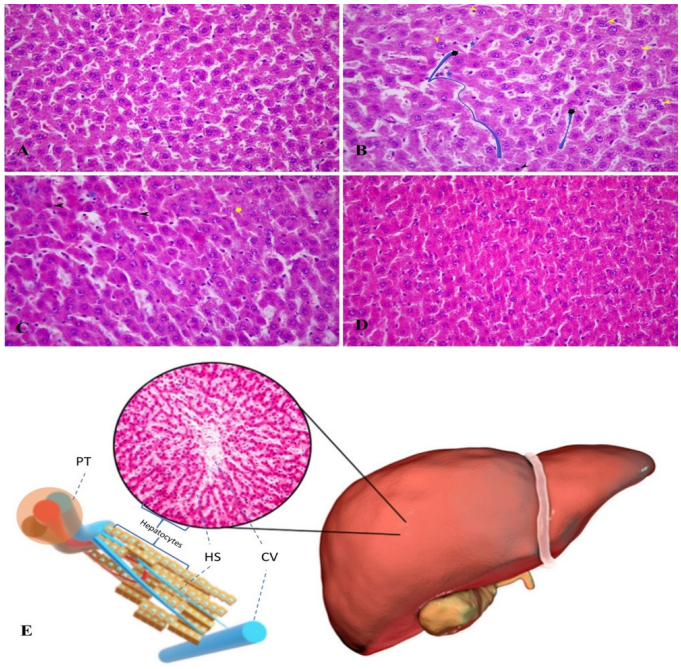

Figure 2. Histopathological findings in the liver tissue. Normal parenchymal hepatocytes in control mouse (A), CIS-treated group; sinusoidal spacing (shown in 3D line), sinusoidal Kupffer cells (blue star), reactive cellular alterations and nucleolar prominence (yellow arrows), and occasional apoptosis (black arrows) (B), CIS + IRB group; congestion, and sinusoidal spaces, increased eosinophilic cytoplasm (yellow mark), and sinusoidal Kupffer cells (blue star) and sporadic apoptotic hepatocytes (black arrow) (C), IRB group; eosinophilic cytoplasm (D), hepatic lobule schema (E) (all H&E slides were captured by the microscope Nikon Ni-U at 200x magnification)

HS: Hepatic sinus, CV: Central vein, PT: Portal triad, CIS: Cisplatin, IRB: Irbesartan

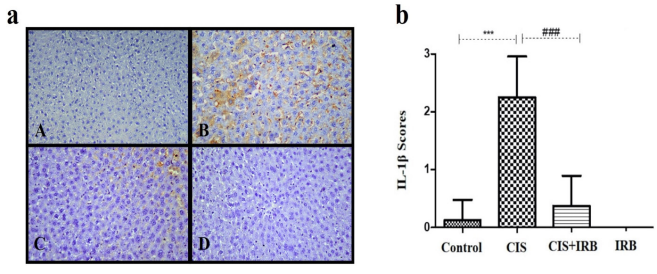

Figure 3. A) IHC evaluation of IL-1β levels. Negative expression in the control group (A), marked increase in expression in the CIS group (B), decreased expression in the CIS + IRB group (C), and lack of expression in the IRB group (D). B) Statistical analysis of IL-1β levels. In the CIS group IL-1β scores were significantly higher than those in the control group ($p < 0.001$). In the CIS + IRB group, IL-1β scores significantly decreased compared with those in the CIS group ($p < 0.001$). In the IRB group, IL-1β scores were significantly lower than those in the CIS group ($p < 0.001$). Comparison between groups was assessed by One-Way ANOVA test followed by *post-hoc* Bonferroni multiple comparison test.

IHC: Immunohistochemical, IL-1β: Interleukin-1 beta, CIS: Cisplatin, IRB: Irbesartan, ANOVA: Analysis of variance

Biochemical results

Oxidative stress parameters

A significant decrement in TAS levels was determined in the CIS group compared with the controlgroup ($p < 0.01$, Figure 6). In the CIS + IRB and IRB groups, TAS levels increased compared with the CIS group ($p < 0.001$ for both). OSI levels were elevated in the CIS group compared with the control group ($p < 0.01$). In the CIS + IRB group, OSI level was attenuated compared with the CIS group ($p < 0.01$) (Figure 6).

Liver function parameters

The ALT and AST levels of the CIS group were significantly higher than the control ($p < 0.001$, $p < 0.01$, respectively). In the CIS + IRB group, ALT and AST levels were attenuated compared with the CIS group ($p < 0.001$, $p < 0.01$, respectively). In contrast, ALT levels were attenuated in the IRB group compared with the CIS + IRB group ($p < 0.05$) (Figure 7).

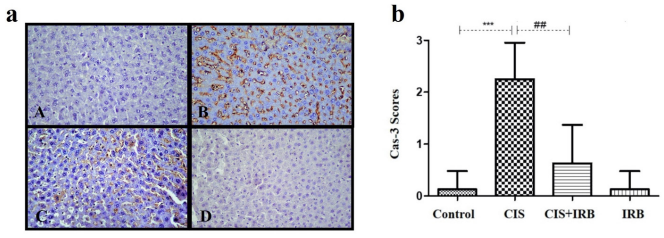

Figure 4. a) IHC evaluation of caspase-3 levels. Negative expression in the control group (A), marked increase in expression in the CIS group (B), decreased expression in the CIS + IRB group (C), and lack of expression in the IRB group (D). b) Statistical analysis of caspase-3 levels. In the CIS group, caspase-3 scores were significantly higher than those in the control group ($p < 0.001$). In the CIS + IRB group, caspase-3 scores significantly decreased compared with those in the CIS group ($p < 0.01$). Comparison between groups was assessed by One-Way ANOVA followed by *post-hoc* Bonferroni multiple comparison test

IHC: Immunohistochemical, IL-1β: Interleukin-1 beta, CIS: Cisplatin, IRB: Irbesartan, ANOVA: Analysis of variance

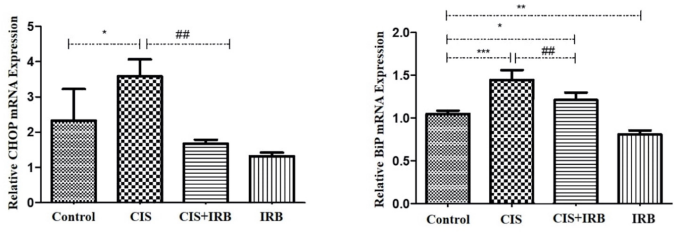

Figure 5. Evaluation of the relative mRNA expression levels of CHOP and BiP. mRNA expression levels of CHOP and BiP were analyzed by qRT-PCR. Relative mRNA expression values were calculated using $2^{-\Delta\Delta CT}$ and GAPDH was used as a housekeeping marker. Data represent the mean of four independent biological replicates in triplicates, and error bars represent SD. Comparison between groups was assessed by One-Way ANOVA followed by *post-hoc* Bonferroni multiple comparison test. Values are represented as means ± SD.

(n=3), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, * represents comparison with control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, # represents comparison with CIS group, CIS: Cisplatin, IRB: Irbesartan, CHOP: CCAAT/enhancer-binding protein homologous protein, BiP: Immunoglobulin-heavy-chain-binding protein, qRT-PCR: Quantitative reverse transcriptase polymerase chain reaction, SD: Standard deviation, ANOVA: Analysis of variance

DISCUSSION

IRB is a commonly used angiotensin-converting enzyme-II blocker and is effective in treating hypertension-related cardiovascular diseases.[25] The RAS plays a pivotal role in the physiological system, especially in blood pressure regulation and its components, including angiotensin II, which is highly expressed in various tissues, such as the kidney, adipose, and liver.[26-28] Moreover, inhibition of RAS by angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor antagonists has been presented as a therapeutic approach for liver fibrosis.[29] It has been shown that the effects of these drugs are not limited to angiotensin blockage; they also have ameliorative activity on oxidative stress, glutathione depletion, and lipid peroxidation.[30-32] *In vivo* and *in vitro* studies performed on cardiomyocytes have shown that angiotensin II induces ER stress.[33] A study conducted on human pancreatic islet cells demonstrated a protective effect of Losartan, another ACE inhibitor, against glucose-induced ER stress responses.[34] Although detailed examinations of the anti-inflammatory and antioxidant effects of IRB have been conducted in various studies, the ER stress-related outcomes of IRB still need to be clearly understood.[25] Kabel et al.[16] demonstrated that IRB exhibited hepatoprotective effects by inhibiting apoptosis in hepatic tissues.[16] Thus, we investigated the underlying mechanism of IRB in CIS-induced hepatotoxicity by evaluating ER stress-related responses.

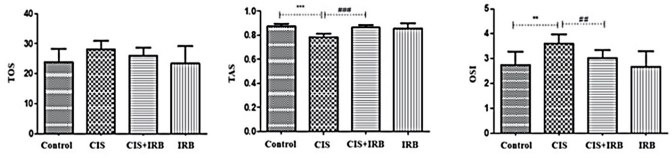

Figure 6. Oxidative stress parameters of the liver tissue. Values are presented as means ± SD.

Comparison between groups and results of oxidative stress markers were assessed by One-Way ANOVA test followed by *post-hoc* Bonferroni multiple comparison test

**p* < 0.05, ***p* < 0.01, ****p* < 0.001,* represents comparison with control group, #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001, # represents comparison with CIS group, CIS: Cisplatin, IRB: Irbesartan, SD: Standard deviation, ANOVA: Analysis of variance, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Open Systems Interconnection

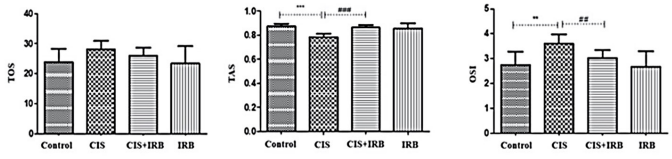

Figure 7. Evaluation of liver function parameters. Values are presented as means ± SD. Comparison between groups and results of oxidative stress markers were assessed by One-Way ANOVA test followed by *post-hoc* Bonferroni multiple comparison test

**p* < 0.05, ***p* < 0.01, ****p* < 0.001, * represents comparison with control group, #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001, # represents comparison with CIS group, CIS: Cisplatin, IRB: Irbesartan, SD: Standard deviation, ANOVA: Analysis of variance, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

CIS is a chemotherapeutic drug that shows efficiency against various cancer types such as testicular, gastric, ovarian, lung, and breast.[4] However, almost 30% of patients may face the adverse effects of CIS, which limits drug usage in therapy.[3] There are numerous side effects of CIS, but hepatotoxicity is still the most common. To date, the proven mechanisms of CIS-induced hepatotoxicity are mitochondrial dysfunction, oxidative stress, inflammatory apoptosis, and disrupted Ca^{+2} homeostasis.[35]

Recent studies have shown that possible ER stress-mediated mechanisms may have a critical role in hepatotoxicity progression.[36] ER membrane-localized sensor proteins (IRE1α, PERK, and ATF6) fine-tune the ER stress responses for the cells to adapt to changing physiological conditions.[11] In particular, releasing BiP protein from ER stress sensors activates UPR signaling. When the cells cannot overcome prolonged ER stress, the expression levels of CHOP, which is a downstream effector of the PERK branch of UPR, increase, and programed cell death is triggered through CHOP-controlled apoptotic proteins.[37] Thus, cell survival or programmed cell death decisions are made under ER stress. Herein, we evaluated the mRNA expression levels of CHOP, a pro-apoptotic factor, and found that its high expression levels lead cells to mediate programed cell death. In addition, we tested the mRNA expression levels of BiP, which is an ER chaperone and is frequently used as a marker to monitor ER stress.[38]

In studies that investigated the protective effects of IRB against CIS-induced hepatotoxicity, our findings indicated that IRB administration significantly restores CIS-induced tissue damage by decreasing ER stress induction (Figure 6). We determined that IRB significantly reduced the CIS-induced mRNA expression levels of BiP and CHOP. These results suggest that IRB plays a protective role in CIS-induced hepatotoxicity by reducing CIS-mediated elevation of BiP and CHOP levels.

The CHOP protein is also associated with one of the mechanisms that leads to ROS production linked to ER stress.[39] For this reason, to better understand the protective role of IRB against CIS-induced tissue damage, we evaluated the TAS, TOS, and OSI levels. As expected, CIS treatment caused a decrease in the TAS level and an increase in the OSI level (Figure 7).

The feedback between inflammatory cell responses and the ER stress response might trigger apoptosis in several pathological conditions.[40] ER stress may lead to activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) phosphorylation and increment in the proinflammatory and apoptotic cytokine synthesis and release from the cell, contributing to the inflammatory and apoptotic cycle progression.[41] As known, IL-1β and Cas-3 are common inflammatory and apoptotic mediators, respectively. In addition, they cross-talk with each other through the NF-κB pathway.[42] Previous studies have indicated that IRB could prevent inflammation by inhibiting the NF-κB pathway.[25]

Our findings suggested that CIS administration strongly increased CHOP and BiP mRNA expressions, resulting in increased IL-1 and Cas-3 levels; in contrast, IRB administration

significantly decreased IL-1 and Cas-3 levels, as seen in the results of immunohistochemistry. In addition, the present histopathologic findings overlapped with the literature.[25,43] Consistent with immunostaining results, our histological findings support the protective role of IRB against CIS-induced liver tissue damage. In the CIS group, sinusoidal dilatation and apoptotic bodies increased compared with the normal group. In addition, we detected a significant increase in eosinophilic cytoplasm in the IRB + CIS and IRB groups, which might result from cellular hypertrophy or peroxisomal and smooth ER or mitochondrial enhanced activity in the liver cells. Collectively, these results suggest that IRB effectively reverses CIS-induced hepatocellular tissue damage by decreasing ER stress and inflammatory responses triggered by CIS administration.

Moreover, we measured the impact of IRB on hepatic tissue-related biochemical parameters. ALT and AST are serological markers of pathologies in liver tissue. Biochemical detection of ALT is particularly susceptible to detecting liver injury.[44] In addition, AST is found in the mitochondria of hepatocytes, and when injury of hepatocytes occurs, it is released into the blood.[45] Our results showed that CIS administration elevated ALT and AST levels (Figure 7). These results suggest that IRB significantly reverses CIS-induced liver injury and inflammation in hepatocellular tissue. In addition, consistent with the literature, our findings indicate that IRB might protect the hepatocellular tissue against CIS-induced tissue damage by regulating ER stress and oxidative stress status.

Main points

- IRB reverses CIS-induced oxidative stress.
- IRB modulates CIS-induced liver dysfunction by reorganizing the main liver enzymes.
- IRB reduces CIS-induced ER stress.

Study limitations

The present study tested the effects of IRB on mRNA levels of CHOP and BiP. Therefore, further detailed analyses are required for a better understanding of the effect of IRB on ER stress modulation and its protective roles against CIS-induced liver injury.

CONCLUSION

Herein, we investigated the possible effects of IRB on CIS-induced hepatotoxicity. Our findings suggest that IRB may play protective roles against CIS-induced liver toxicity. However, further studies are needed to elucidate the mode of action of IRB in liver toxicity.

Ethics

Ethics Committee Approval: All experimental procedures were permitted by the National Institutes of Health and the Committee on Animal Research according to the ethical rules (approval number: 09/03, date: 23.09.2021).

Informed Consent: Not necessary.

Authorship Contributions

Surgical and Medical Practices: O.E., M.S., Concept: O.E., Y.E., M.S., Design: O.E., Y.E., M.S., Data Collection or Processing: O.E., Y.E., D.Ç., Ş.P., E.D.K., Analysis or Interpretation: O.E., Y.E., D.C., Literature Search: O.E., Y.E., D.C., Writing: O.E., Y.E., D.C.

Conflict of Interest: The authors declare no competing financial interests.

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