Possible Protective Effect of Thyroid Hormone on Hepatic Ischemia Reperfusion Injury in Rats

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Abstract

Background: Ischemia Reperfusion Injury (IRI) is one of the major complications affecting the outcomes of liver surgical interventions. This study aimed at evaluating the possible protective effects of Thyroid Hormone (TH) on IRI in the liver of adult male albino rats.

Methods: Forty male rats were included and divided equally into 4 groups: Group I (sham operated control group); Group II: Received TH 48 hours before sham operation; Group III: Induction of hepatic IRI as 45min. of ischemia and 4 hours of reperfusion; Group IV: Received TH 48 hours before IRI. Before sacrifice, serum AST and ALT levels were measured. Liver sections were stained with H & E, and immunohistochemical stain for anti-CD68.

Results: In TH group, there was no significant difference in AST and ALT as compared to the control. Also, the histological architecture of the liver appeared as in control rats. The mean area % of anti-CD68 immunoreactivity showed no significant difference as compared to the control. IRI group showed significant increase in AST and ALT when compared to other groups. Liver sections revealed disorganization, degeneration and necrosis of the hepatocytes with significant increase in the mean area % of anti-CD68 immunoreactivity compared to control. In TH & IRI group, there was significant decrease in AST & ALT when compared to IRI group. Liver sections showed decrease in the histological alterations caused by IR. There was significant decrease in the mean area anti-CD68 immunoreactivity when compared to Group III.

Conclusion: TH has a protective effect against hepatic IRI.

Key Words: Ischemia reperfusion injury – Liver enzymes – Kupffer cells – CD68 – Thyroid hormone.

Introduction

ISCHEMIA Reperfusion Injury (IRI) is a phenomenon in which cellular damage, due to hypoxia, is exacerbated following restoration of O2 and nutrient supply. These complications are associated with liver resection, transplantation or trauma [1]. The mechanism of hepatic IRI is a cascade of inflammatory events involving multiple interconnected factors, as hepatic and sinusoidal endothelial cells injury, disturbances of microvascular circulation, activation of Kupffer cells with production and release of Reactive Oxygen Species (ROS) and extravasation of inflammatory cells [2].

Thyroid hormone is important for the normal function of most tissues. In the liver, thyroid hormone leads to induction of transient and mild pro-oxidant state, which elicits the redox up-regulation of the expression of proteins affording cell protection, as an adaptive and a preconditioning strategy against liver IRI [3].

The aim of this work was to study the possible protective effects of thyroid hormone on ischemia reperfusion injury in the liver of adult male albino rats using histological and immunohistochemical methods.

Material and Methods

Thyroid hormone drug:

Thyroid hormone tablets (each tablet containing 100 micrograms of thyroxin sodium) were used. Drug was purchased from Medical Union Pharmaceuticals Co., Ismailia, Egypt. The thyroxin tablets were crushed and dissolved in saline to be used as single dose of 0.1mg/kg injected intraperitoneally [3].

Induction of hepatic IRI in rats:

Hepatic ischemia was induced by occlusion of the hepatic blood flow by micro vascular clamps. The duration of hepatic ischemia was 45 minutes.
Reperfusion was performed by gentle removal of the clamps and allowing the hepatic blood flow for 4 hours [3].

All surgical procedures were done under aseptic condition. Ischemic reperfusion was induced by segmental occlusion of the hepatic blood flow. Rats were anesthetized by intraperitoneal thiopental sodium (40mg/kg), and then rats were placed on supine position on a heating pad and covered with sterile towels to maintain body temperature and also warmed with a heat lamp. Rat limbs were tied to the edges of the heating pad to avoid the involuntary movements during surgery and their tongues were pulled out to avoid tongue falling backward and suffocation [4].

First, abdominal wall was clipped of hair and cleaned with betadine. Abdominal laparotomy was done by a midline incision; liver was exposed, followed by isolation of the hepatoduodenal ligament to reach the portahepatis. The structures of the portal triad were overviewed (hepatic artery, portal vein and common bile duct). Non lethal partial (70%) segmental hepatic ischemia was performed. The left and the median lobes of the liver represent 70% of the liver mass. Afferent vessels to these two lobes were reached by everting the hepatic lobes upwards and were occluded by micro vascular atraumatic Bull Dog clamps for 45 minutes. Ischemia was confirmed by the pale blanching of ischemic lobes [5].

Incomplete partial liver ischemia was used to prevent mesenteric congestion by portal venous shunting through the right lobe and caudate lobe which are called "shunted lobes". Reperfusion is performed by gentle removal of the clamps and allowing the hepatic blood flow for 4 hours. Immediate color change was observed [4].

Animals:

Forty adult male albino rats were used in this study. Their body weight ranged from 200-250 grams. Animal care was provided by Laboratory Animal House Unit of Kasr Al-Ainy Faculty of Medicine, Cairo University. The animals were housed in standard stainless-steel cages at a 12h cycle of light and dark and received standard rat chow diet and water ad libitum. Room temperature was kept at 24±2°C.

Experimental design:

This study was done over a period from 2014 to 2015. All animal experiments were carried out in strict compliance with the guidelines of the Institutional Animal Ethics Committee on the care and use of laboratory animals. The forty rats were divided equally into four groups as follows:

Group (GI): (Sham operated control group): Rats were subjected to Sham operation.

Group (GII): (Sham operated-Thyroid Hormone group) (TH): Rats received thyroid hormone 48 hours before sham operation.

Group (GIII): (Ischemia Reperfusion Injury group) (IRI): Rats were subjected to hepatic IRI as 45min. of ischemia and 4 hours of reperfusion.

Group (GVI): (IRI-Thyroid Hormone group) (TH & IRI): Rats received thyroid hormone 48 hours before induction of IRI.

Samples collection:

Blood samples:

Immediately before scarification, blood samples were collected in fine heparinized capillary tubes by retro-orbital punctures and used for the assessment of liver function tests; serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST). The blood analysis was done at the Laboratory Unit of the Biochemistry Department of Kasr Al-Ainy Faculty of Medicine, Cairo University.

Liver specimens:

The animals were scarified by over dose of intravenous injection of phenobarbital 100mg/kg [6]. Liver specimens were fixed in 10% formal saline. Paraffin-embedded sections were cut at 5-7 µm thickness and were subjected to:

A- Hematoxylin & Eosin (H & E) [7].

B- Immunohistochemical staining for anti-CD68: A mouse monoclonal antibody (Novocastra Lyophilized Corporation laboratories, UK). Anti-CD68 immunopositive staining appeared as cytoplasmic brown deposits in Kupffer cells [2].

Morphometric studies:

Using "Leica Qwin 500 C" image analyzer computer system Ltd, one liver section for each animal in each group was examined under the light microscope; in each section ten serial non overlapping fields were examined for measurement of area percent of iNOS immunopositive cells.

Statistical analysis:

Data were expressed as group mean ± SD. The statistical analysis was carried out using student t-test and ANOVA test, with SPSS version 19 (SPSS, Chicago, Illinois, USA). p-value of 0.05 or less was considered as statistically significant.
Results

Histological results:
Hematoxylin and Eosin:

Rat liver sections in the control group revealed normal liver parenchyma with preserved architecture of hepatic lobules containing hepatocyte cords radiating from central veins. The hepatocytes displayed eosinophilic cytoplasm; nuclei were central, large and vesicular with prominent nucleoli Fig. (1). Also, rat liver sections from TH group revealed picture similar to that of control group Fig. (2). In IRI the sections revealed disrupted hepatic plates and irregular sinusoidal architecture. The hepatocytes had pyknotic nuclei. While some hepatocytes showed marked nuclear fragmentation, others were devoid of nuclei and there were areas of necrosis Fig. (3). Sections of TH and IRI group showed apparently protected liver structure. The hepatic lobular architecture was preserved with apparently normal blood sinusoids. Most of hepatocytes displayed normal arrangement with acidophilic cytoplasm and vesicular nuclei Fig. (4).

Anti-CD68 immunoreactivity:

Liver sections of the control group revealed mild immunoreaction for anti-CD68 appearing as cytoplasmic brown coloration of Kupffer cells Fig. (5). In TH group there was mild increase in immunoreactivity for anti-CD68 Fig. (6), while sections of IRI group displayed strong anti-CD68 immunoreactivity Fig. (7). In TH-IRI group, sections showed mild to moderate anti-CD68 positive immunoreaction in Kupffer cells, similar to control Fig. (8).

Morphometric results:

The mean serum levels of ALT and AST:

There was no significant difference between the control and TH group. As for IRI group, it showed significant increase when compared to other groups. In TH & IRI group, there was significant decrease when compared to IRI group (Table 1) & Histogram (1).

The mean area % of anti-CD68:

The mean area % of anti-CD68 immunopositive cells showed significant increase in the studied groups as compared to the control. IRI group showed significant increase in the mean area % of the anti-CD68 immunoreactivity as compared to both TH group and TH & IRI group. In addition, TH & IRI group showed significant decrease in the mean area % of the anti-CD68 immunoreactivity as compared to IRI group (Table 1) & Histogram (2).

Table (1): Mean levels of serum AST and ALT and the mean area % of anti-CD68 immunoreactions (± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>AST level</th>
<th>ALT level</th>
<th>Anti-CD68 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60.150±3.53</td>
<td>21.94±1.89</td>
<td>1.79±0.85</td>
</tr>
<tr>
<td>II</td>
<td>62±7.20</td>
<td>23.04±3.61</td>
<td>4.14±1.19</td>
</tr>
<tr>
<td>III</td>
<td>166.2±31.47*+</td>
<td>96.88±19.98*+</td>
<td>15.70±3.94*+</td>
</tr>
<tr>
<td>IV</td>
<td>84±10.63*+#</td>
<td>40.84±9.14*+#</td>
<td>7.96±1.54*+#</td>
</tr>
</tbody>
</table>

*: Significant as compared to Group I (p<0.05).
+: Significant as compared to Group II (p<0.05).
#: Significant as compared to Group III (p<0.05).
Fig. (1): A photomicrograph of a section in the liver of a rat from control Group (GI) showing plates of hepatocytes radiating from the central vein (arrows). The blood sinusoids are lined by endothelium (curved arrow). (H & E X400).

Fig. (2): A photomicrograph of a section in the liver of a rat from TH Group (GII) showing plates of hepatocytes radiating from the Central Vein (CV). The hepatocytes are polygonal and displaying eosinophilic cytoplasm with central large vesicular nuclei and prominent nucleoli (arrows). (H & E X400).

Fig. (3): A photomicrograph of a section in the liver of a rat from IRI Group (GIII) showing severe disrupted hepatic plates at zone III with irregular sinusoidal architecture (curved arrow). The hepatocytes showing increased acidophilia (black arrow) with cytoplasmic blebs and apoptotic bodies (blue arrows). Others hepatocytes devoid of nuclei (asterix).

Fig. (4): A photomicrograph of a section in the liver of a rat from TH & IRI Group (GIV) showing mild vacuolatin of hepatocytes with preservation of their arrangement and radiation around CV. Most liver cells have pale nuclei (blue arrows) while few others have pyknotic nuclei (black arrow). Mildly dilated blood sinusoids can detected (curved arrow).

Fig. (5): A photomicrograph of a section in the liver of a rat from GI showing few immuno-stained Kupffer cells (black arrows) observed inbetween hepatocytes radiating from CV. (Anti-CD68 X400).

Fig. (6): A photomicrograph of a section in the liver of a rat from GII showing mild immunoreactivity in kupffer cells (black arrows) inbetween hepatocytes radiating from Central Vein (CV). (Anti-CD68 X400).
Discussion

Ischemia Reperfusion Injury (IRI) is an important cause of liver damage occurring during surgical procedures and is a main cause of graft dysfunction post-transplantation. Cellular and biochemical processes occurring during hepatic IRI are diverse and complex and a significant part of these processes are still unknown or unclear [8].

In the past years, several therapeutic strategies have been developed to attenuate or prevent liver IRI such as gene therapy, surgical strategies, and pharmacological approaches. One of the most promising protective strategies against IRI is Thyroid Hormone (TH) preconditioning, which appears capable of increasing the resistance of liver cells. This is based on the fact that TH is a natural occurring molecule, widely used and well-tolerated therapeutic agent whose side effects can be readily controlled [9]. In this study, an attempt was done to examine this protective effect.

The mean serum levels of AST and ALT in the control group were normal (21.94 and 60.15IU/L, respectively). This is in accordance with previous researcher who mentioned that the normal serum levels of AST and ALT levels in rats was 17.5-30.2 and 45.7-80.8IU/L, respectively [10].

In TH group, there was no significant difference in the mean serum levels of AST and ALT as compared to the control. In IRI group, the mean serum levels of AST and ALT were significantly increased compared to all other groups. While treatment with TH before IRI resulted in significant decrease in serum levels of ALT and AST when compared to IRI group, levels were still significantly higher than the control. The alterations in the activities of the liver enzymes induced by IRI might be due to the leakage of these enzymes from the hepatocytes as a result of hepatocellular damage caused by IR [11]. In addition, previous work detected significant decrease in the levels of serum transaminases in the TH treated animals and attributed these results to the protective effects of TH against IRI [12].

In the present work, liver sections from TH group showed similar histological architecture of the hepatic lobules as in control. Also, there was no significant difference in the mean area % of anti-CD68 when compared to the control. These findings are in agreement with [13] who found that rats given TH showed normal liver architecture with absence of necrosis. They also detected that TH did not affect hepatic cell functions.

It was also found that ROS affected the mitochondrial permeability through affecting unsaturated fatty acids in the mitochondrial membrane. The mitochondrial membrane is a key for the balances between the survival and apoptosis. Increased mitochondrial permeability releases cytochrome-c and forms apoptosome that activates the procaspase 9. Caspase 9 is an initiator protease that activates caspase 3 and downstream caspases to initiate cellular destruction [14].

Proliferation of the Kupffer cells in IRI group was proved by the significant increase in the mean area % of CD68 immunoreactivity when compared to the control. Some studies detected that Kupffer cell may be a contributor to hepatic oxidative stress that could be correlated with the amount of IRI induced to hepatic tissue, where this hyperplasia represents a defensive mechanism [2]. This is supported by recent reports which proved that hepatic
CD68 +ve cells exhibited phagocytic and cytotoxic activity via production of ROS and superoxide [15].

Activated Kupffer cells promote both local and systemic inflammation after liver IRI by producing an array of inflammatory mediators, including cytokines, chemokines, ROS, NO, and bioactive lipids. These agents activate the endothelium and increase vascular permeability, activate neutrophils, activate lymphocytes, and result in the paracrine activation of protective or apoptotic signaling pathways of hepatocytes [16].

Liver sections from rats in TH and IRI group were mostly normal while some focal areas showed slight disorganization. There was significant decrease in the mean area % of anti-CD68 immunoreactivity when compared to IRI group indicating the protective effect of the TH against IR induced liver injury. This was in agreement with previous study [13]. TH triggered the hepatic production of ROS and the activation of nuclear transcription factor erythroid-related factor 2 (Nrf2). The enhancement of liver Nrf2-DNA binding is associated with upregulation of the expression of detoxification and drug transport proteins. These changes, in addition to antioxidant protein induction represent cytoprotective mechanisms underlying TH preconditioning [17].

The protective effect of TH upon liver is based on the calorigenic action leading to stimulation of basal thermogenesis that is carried out through genomic and non-genomic mechanisms. The genomic pathway involves the formation of transcriptionally active complexes of TH with Thyroid hormone Receptors (TRs), which upon recognition by specific TH response elements in DNA triggers the expression of respiratory, metabolic, and uncoupling protein genes, with consequent enhancement in the rate of O$_2$ consumption of the liver cells the non-genomic pathway involves significant activation of cytochrome-c-oxidase, thus enhancing mitochondrial respiration. The pro-oxidant state induced in the liver by TH represented a mild, non-toxic redox alteration [18].

Sections from TH & IRI group revealed significant decrease in mean area percent of CD68 immunoreactivity as compared to IRI group. However, immunoreactivity was still significantly increased as compared to the control. The thyroid hormone-induced calorigenesis triggered liver oxidative stress with concomitant TNF-α production by Kupffer cells and up-regulation of its gene expression [19].

Other work described the molecular basis for TH liver preconditioning; it involved enhancement in the homeostatic potential including antioxidant, antiapoptotic, antiinflammatory and cell proliferation responses, besides its higher detoxification capabilities and energy supply through AMP-activated protein kinase upregulation [20].

Conclusion:

Thyroid hormone has protective effects against hepatic ischemia reperfusion injury in rats.

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الملخص العربي

الإصابة بنقص التروية واعادة ضخ الدم سبب رئيسي لوفيات في جراحات استئصال وزراعة الكبد.

يهدف هذا العمل إلى دراسة التأثير الفوقي المحتمل للدواء الغدة الدائرية في حماية وتنقية الإصابة الكبدية الناجمة عن نقص التروية وإعادة ضخ الدم. وقد أجريت هذه الدراسة على 120 جرذانًا من ذكور الحزم البيضاء، تم تقسيمهم إلى أربع مجموعات: المجموعة الضاغطة (جارحة زائدة)، المجموعة الجزئية الزائدة، المجموعة النادرة وإعادة ضخ الدم. تم تنقيح النتائج الناجمة عن نقص التروية وإعادة ضخ الدم في المجموعة الأولى والثانية النتيجة وניתين للإجابة. كما أظهرت مجموعات وزيادة الكبد إلى المجموعة الفرزة، وال보호ات التي نشرنها من طبيعية. أما النتائج الناجمة عن نقص التروية فإنه تم قياسها في مصروف المعالجة لعلاج الكبد. بينما أنماجها نتجت نتائج عديدة من المجموعة البدنية بنقص التروية وإعادة ضخ الدم. فعلى فئة بانتظام في مستوى الإصابات الكبد مع النشاط البدني الضاغط، ونسبة الكبد المحسوبة عن النشاط البدني الضاغط. واعتباره تعديل ونظام تقييم في موقع الكبد الكبدية واضحة إلا زيادة ذات إسباب كبدية عالية في الموقع الجسيم. وفي حق الاصابة في الأنسجة النواوية من النسيج الانقسام للغدة الدائرية في مستوى الفرزة في النشاط البدني الضاغط واعادة ضخ الدم. ونسبة الكبد المحسوبة بيضاكة بإنهزام في التغيرات النسيجية الناجمة عن نقص التروية وإعادة ضخ الدم ونسبة الكبد في النشاط البدني الضاغط واعادة ضخ الدم. ونسبة الكبد المحسوبة بيضاكة بإنهزام في التغيرات النسيجية الناجمة عن نقص التروية وإعادة ضخ الدم ونسبة الكبد في النشاط البدني الضاغط واعادة ضخ الدم. ونسبة الكبد المحسوبة بيضاكة بإنهزام في التغيرات النسيجية الناجمة عن نقص التروية وإعادة ضخ الدم ونسبة الكبد المحسوبة بيضاكة بإنهزام في التغيرات النسيجية الناجمة عن نقص التروية وإعادة ضخ الدم ونسبة الكبد المحسوبة بيضاكة إ