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Brain Injuries Induced by Experimental Renal Ischemia/Reperfusion in rat

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ABSTRACT

Renal ischemia reperfusion (IR) occurs in a variety of clinical settings including shock, sepsis and vascular surgery. This study was conducted to investigate the renal IR induced brain injuries. rats were divided into two groups; IR group (bilateral occlusion of renal pedicles for 45 min followed by reperfusion) and sham group. Samples were taken 1 and 3 days after ischemia. serum was prepared for biochemical analysis and brain was divided into two parts for histological and immunehistochemical analysis. Our results revealed that serum concentrations of creatinine and BUN were significantly increased in IR group. MDA level was increased while, (SOD, CAT, GSH-Px, GST & GSH) levels were decreased in brain tissue of renal ischemic rats. Renal IR induced various degenerative changes in neuronal cells and apoptosis; shown by reduced expression of Bcl-2 and increased expressions of Bax and caspase-3. oxidative stress is thought to play the key role.

KEYWORDS : renal ischemia, brain, oxidative stress, apoptosis.

Introduction

Renal ischemia reperfusion (IR) occurs in a variety of clinical situations including shock, sepsis, vascular surgery and many other conditions (Laura and Heitham, 2012 and Seifi *et al*. 2014). Several studies have demonstrated that Renal IR injury may cause failure of different distant systems as lungs, heart, gut, brain, hematologic system and liver (Gulec, 2011, Hassoun *et al*. 2007 and Golab *et al*. 2009). Although, the pathogenesis of remote organ failure is multifactorial and thought to be due to humeral or cellular factors, but the formation of reactive oxygen species (ROS) is the usually blamed mechanism (Gulec, 2011 and Hoke *et al*. 2007). Previous studies have shown that Renal ischemia reperfusion give rise to the production of ROS that induces oxidative dysfunction in the local tissue and leading to damage of the organs remote from the site of IR (Serteser *et al*. 2002 and Marian *et al*. 2007).

ROS are extremely unstable oxygen molecules able to produce cellular damage through lipids peroxidation in the membranes of the cell and the mitochondria in addition to DNA degradation (Gulec, 2011). It is also reported that the release of ROS initiates apoptosis of the cells at the late stage following IR (Marian *et al*. 2007). It is well known that excess production of oxygen free radicals negatively affects the endogenous antioxidants scavenging capacity, leading to cell injury owing to peroxidation of the lipids of mitochondrial and cellular membrane. The endogenous antioxidants; superoxide dismutase (SOD) and catalase (CAT) as well as glutathione (GSH) are playing an important role in the defense against ROS and attenuating ischemic and oxidative injuries to many tissues (Marian *et al*. 2007 and Gulec, 2011). The rat brain has shown a relatively high oxidative capacity with low antioxidant defense ability. Thus, the rat brain may be more susceptible to IR induced oxidative stress due to inefficient resistance (El-Sayed *et al*. 2007).

When the imbalance developed between the positive signals needed for cell survival and the negative signals resulting from increased cellular level of oxidants, apoptosis occurs (Hengartner and Bryant, 2000). The oxidative stress would initiate caspase-3 activation and apoptosis. Also, the balance of anti- and pro-apoptotic proteins (Bcl-2:Bax) in the mitochondria is considerably affected by ROS (Philchenkov *et al*. 2004 and Yousef *et al*. 2012).

This study was designed to investigate the possible remote effects of renal IR injury on rat brain and demonstrate the role of oxidative stress and mitochondrial mediated apoptosis in this injury.

Materials and methods

Animals

Twenty male Sprague-Dawley rats (200–250 g) were used. Rats were kept on a standard chow and water ad libitum. All experimental procedures were done according to guidelines of the Institutional Animal Care and Use Committee of the faculty of veterinary medicine, Suez Canal University.

Experimental design

Rats were randomly allocated into two groups (10 rats each).

Group I (Sham group): rats undergo the same surgical procedures as IR group with the exception of bilateral renal clamping.

Group II (IR) group: bilateral occlusion of renal pedicles was induced for 45 min as described (Hussein *et al*.2014) followed by reperfusion. Animals were sacrificed 1day and 3 days after ischemia (five animals in each group at each time of examination). Blood was collected from the heart. and serum was prepared for biochemical analysis. The brain was divided into two parts for histological and immunehistochemical analysis.

Biochemical analysis

Renal function indices (serum creatinine and BUN) were measured by commercial kits (Fortress diagnostics, UK) at days 1 and 3 after ischemia.

Measurement of oxidative stress markers in brain tissue

Tissue samples were minced then homogenized (10% w/v) in ice-cold homogenization buffer (Abdel-Wahab, 2005). The homogenate was then used for determination of lipid peroxidation as well as evaluation of the brain contents of enzymatic and non enzymatic antioxidants. Lipid peroxidation was colorimetrically estimated by determining the tissue malondialdehyde (MDA) according to Uchiyama and Mihara (1978). superoxide dismutase (SOD) activity was evaluated according to (Marklund, 1985). The activity of catalase (CAT) was estimated by the method of Clairborne (1985). glutathione-S-transferase (GST) activity was determined by the method of Habig *et al*. (1974). Glutathione peroxidase (GSH-Px) activity was assessed by the method of Paglia and Valentine (1967). Brain content of reduced glutathione (GSH) was determined spectrophotometrically according to Sedlak and Lindsay (1968). Protein content was evaluated using bovine serum albumin as a standard (Lowry *et al*. 1951).

Histopathological examination

Tissue sections (5 mm) were fixed in 10% neutral formalin and embedded in paraffin. Paraffin blocks were cut into 3–5 µm sections and stained with hematoxylin and eosin (H&E) then examined.

Immunohistochemical analysis

The expressions of the apoptosis related genes including Bax (pro-apoptotic gene), Bcl-2(anti-apoptotic gene); and caspase-3 which is the apoptosis coordination enzyme were examined by immunohistochemistry for the brain tissues in the study groups at day 3; based on a streptavidin biotin peroxidase technique (Biogenex, San Ramon, CA, USA) as described before (Salakou *et al*., 2001 and Scopa *et al*., 2001). The integral optical density (IOD) of immunohistochemical intensity was then estimated using Image-Pro Plus 6.0 software (Salakou *et al*.,2007).

Statistical analysis
All data were presented as means ± standard errors mean (SEM) and were analyzed using the Statistical Package of Social Sciences (SPSS program, version 17, SPSS Inc., Chicago, IL, USA). Differences were considered significant at p≤ 0.05.

Results
Renal function parameters:
Table (1) indicated that serum creatinine and BUN were significantly high in IR groups at day 1 and day 3 after ischemia compared with sham groups (p ≤ 0.05).

Table 1: Renal function parameters in sham and IR groups

Groups		Creatinine (mg/dL)	BUN (mg/dL)
1 day	Sham	0.59±0.04	21.80±0.73
	IR	0.79±0.05*	26.76±1.52*
3day	Sham	0.76±0.05	23.54±1.15
	IR	1.03±0.09*	32.18±1.77*

Data are expressed as means ± SEM (n=5). Blood urea nitrogen (BUN)

* Significantly different from sham group using Student's t-test at P ≤ 0.05.

Oxidative stress markers in brain tissues:
Table (2) revealed that Renal IR resulted in a significant elevation in brain lipid peroxides represented as MDA rising along with a marked reduction in brain GSH content at day1 and day 3 as compared with sham groups.

Table 2: MDA and GSH in rat brain in sham and IR groups

Groups		MDA (nmol/mg protein)	GSH (µg/mg protein)
1 day	Sham	1.74± 0.13	5.50± 0.22
	IR	3.68±0.14* (+ 111.5 %)	2.22± 0.18* (- 59.6 %)
3day	Sham	1.62±0.12	5.86±0.31
	IR	3.48±0.25 (+ 114.8%)	2.44±0.20 (-58.4%)

Data are expressed as means ± SEM (n=5). malondialdehyde (MDA), reduced glutathione (GSH)

* Significantly different from sham group using Student's t-test at P ≤ 0.05.

Furthermore, the enzymatic antioxidant parameters in the brain; SOD, CAT, GSH-Px and GST were significantly decreased in IR groups at day 1 and day 3 with respect to the sham groups (table 3).

Table 3: Enzymatic antioxidants in rat brain in sham and IR groups

Groups		SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (nmol/ min/mg protein)	GST (nmol/ min/mg protein)
1 day	Sham	6.96± 0.21	4.26± 0.16	2.44± 0.17	5.48± 0.17
	IR	4.12± 0.19* (-40.8 %)	1.62± 0.12* (- 62.0 %)	1.24± 0.10* (- 49.2 %)	2.96± 0.18* (- 46.0 %)
3day	Sham	7.24±0.25	4.62±0.22	2.74±0.29	5.60±0.22
	IR	4.32±0.24* (-40.3%)	2.02±0.15* (-56.3%)	1.38±0.12* (-49.6)	3.04±0.20* (-45.7)

Data are expressed as means ± SEM (n=5). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST)

* Significantly different from sham group using Student's t-test at P ≤ 0.05.

Histopathological examination and Immunostaining:
Histopathological examination of brain tissue of the control rats displayed normal histological structure of neuronal cells with no microgliosis while, rats in the IR groups showed pyknotic and degenerated neuronal cells of the brain with increased cellularity due to increased number of microglia cells and inflammatory cells (Figure 1).

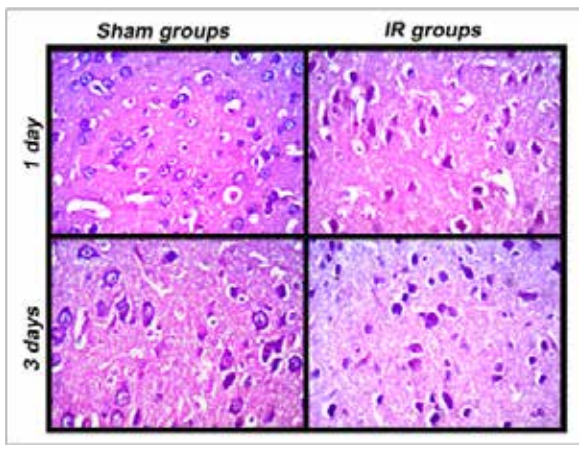

Figure 1: H&E staining of brain tissue of sham and ischemic rats at day1 and day3 of the experiment (400x).

The expressions of Bcl-2 in IR groups were significantly reduced, whereas the expressions of Bax and caspase-3 in the IR groups were significantly increased in comparison to the sham group. These results indicated that renal ischemia initiate the apoptosis process in the brain tissue (Figure 2)

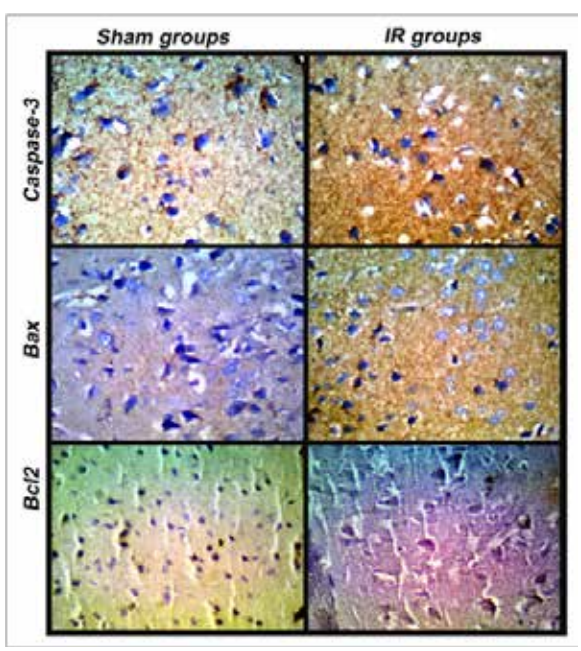

FIGURE 2 (A): IMMUNOSTAINING FOR CASPASE-3, BAX AND BCL-2 IN THE SHAM AND IR GROUPS AT DAY3 OF THE EXPERIMENT (400X).

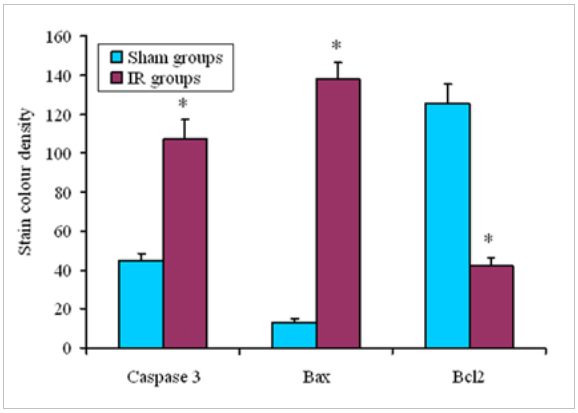

FIGURE 2 (B): THE IOD OF CASPASE-3, BAX AND BCL-2. DATA ARE EXPRESSED AS MEANS ± SEM (N=5). * SIGNIFICANTLY DIFFERENT FROM SHAM GROUP USING STUDENT'S T-TEST AT P ≤ 0.05.

Discussion

This study was conducted to determine the effects of renal IR on the brain tissue. Biochemically, the serum concentrations of creatinine and BUN were significantly increased in IR groups at day 1 and day 3 after ischemia compared to sham group. These results were matching to other studies (Tuğtepe *et al*. 2007 , Ardalan *et al*. 2013, Chen *et al*. 2013, Collino *et al*. 2013 and Wang *et al*. 2013) . these biochemical alterations may be owing to the effect of short-term ischemia and reperfusion method which generate active oxygen species that injure the endothelium and impair the kidney functions. Our findings demonstrated that renal IR leads to the damage of the brain as a remote organ. various mechanisms are suggested to be involved in remote organ failure, but their exact pathophysiological roles are not completely understood, oxidative stress and the excessive production of reactive oxygen species, as a result of renal reperfusion injury, are thought to play a key role in generating the local and remote tissue damage (Di-Pietro *et al*. 2008 and Seifi *et al*. 2014). Our results revealed that levels of lipid peroxidation (represented by increased MDA) were found to be increased in rat brain at different periods after renal ischemia. Also, both of enzymatic (SOD, CAT, GSH-Px & GST) and non-enzymatic (GSH) antioxidant defense systems were impaired in renal ischemic rats compared with rats in the sham group. GSH is an important endogenous free radical scavenger which contributes in enzymatic reduction of membrane hydroperoxy-phospholipids and inhibit the formation of secondary alkoxyl radicals. Reduction of GSH in cells enhances their susceptibility to oxidative damage (Reed, 1990; Sen and Hanninen, 1994 and El-Sayed *et al*. 2007). Both GSH-Px and GST are glutathione-dependent intracellular enzymatic antioxidants. GSH-Px is concerned with the removal of ROS, such as peroxides, while GST is important for conjugation (El-Sayed *et al*. 2007). Superoxide dismutase (SOD) and catalase are amongst the essential enzymatic antioxidant systems in the body. SOD is the first line of defense against reactive oxygen metabolites; it is responsible for transforming superoxide ion to H2O2 which is a less reactive molecule (Kadkhodaee *et al*. 2007). Reactive oxygen species oxidize polyunsaturated fatty acids resulting in formation of the cytotoxic reactive aldehyde (MDA) which considered as a biomarker of the oxidative stress (Nafar *et al*. 2011). Previous studies suggested that ischemia/reperfusion (IR) induced marked oxidative stress mediated by the generation of reactive oxygen species (Walker *et al*. 2001, Aragno *et al*. 2003, . Muñoz-Casares *et al*. 2006, Valko *et al*. 2007 and Yildirim *et al*. 2009). Different experimental models have shown that IR provokes elevation in pro-oxidant levels and loss of antioxidant systems in different organs such as, kidney, liver, lung and heart leading to oxidative damage and dysfunction (Serteser *et al*. 2002, Tuğtepe *et al*. 2007, Wang *et al*. 2013, Seifi *et al*. 2013, Chen *et al*. 2013 and Ardalan *et al*. 2013). Meanwhile, to our knowledge, there is no studies indicated the oxidative status in the brain tissue after renal ischemia.

The histopathological findings revealed that renal ischemia followed by reperfusion induced various degenerative changes in neuronal cells which confirmed the biochemical evidence of oxidative stress.

Additionally, renal IR induced apoptosis; shown by reduced expression of Bcl-2 and increased expressions of Bax and caspase-3 compared with the sham group. These results are in agreement with the results of (Zhang *et al*. 2014) in which, renal ischemia-reperfusion induced brain tissues apoptosis through decreased Bcl-2 expression and increased Bax expression. Oxidative stress would activate caspase-3 and apoptosis. Also, the balance of anti- and pro- apoptotic proteins (Bcl-2: Bax) in the mitochondria is radically affected by ROS (Cem Koçkar *et al*. 2012). Bcl-2 is an inhibitor of the cell apoptosis induced by free radicals and lipid peroxidation through inhibiting the formation of active oxygen and preserving mitochondrial oxidative function (Chen *et al*. 2004).

In summary, this study demonstrates that renal IR induced brain damage might be related to oxidative stress and that the excessive production of ROS during IR play a role in the tissue injury and apoptosis.

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