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Original Article

Study of the Potential Use of Lithium in Treatment of Acute Kidney Injury in Rat Model

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Abstract

Background:

Management of acute kidney injury is still facing a big problem. It is only dependent up till now on supportive measures, like fluid resuscitation and renal replacement therapy. No current drug therapy has been approved for the treatment of acute kidney injury. Acute kidney injury situations in a lot of cases can be predicted so, finding a drug for AKI will really benefit many patients. The pathophysiology of AKI is complex and many signaling pathways are involved in it. The Glycogen synthase kinase 3B enzyme is an important member in some of these pathways. The effect of its inhibition by the FDA approved drug, lithium on AKI is still under study.

Material & Methods

The current study was conducted on28 Male Sprague–Dawley rats. We classified the rats into groups. We induced acute kidney injury to rats with cisplatin. We administered lithium chloride to treat AKI in comparison with saline treatment. We have done renal functions and histopathological examinations to all rats enrolled in our study.

Results

Single intraperitoneal injection of cisplatin (5 mg/kg) in rat induced acute kidney injury. The effect of lithium chloride treatment with dose (80 mg/kg) on serum creatinine and blood urea levels showed significant regression in the rising of serum creatinine and blood urea in lithium chloride treated rats in comparison to saline-treated rats. Pathological pictures and scores demonstrated an improvement in lithium chloride treated rats than saline-treated but results were not significant.

Conclusion

Administration of lithium may be a promising treatment for acute kidney injury.

Key words:

Acute kidney injury - Lithium

Introduction

Acute kidney injury (AKI) is defined "as a clinical syndrome characterized by a rapid decrease in renal function together with the accumulation of waste products such as urea"[1]. The incidence of AKI differs from community, hospital and ICU admissions. AKI incidence is 1-7% in hospital admitted patients and 1-25% in ICU admitted patients. AKI is a serious disease which increases the mortality rate ten to fifteen times in ICU admitted

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patients [2]. Drugs and various toxic substances are eliminated through the kidney. So, the kidney is one of the important organs to be affected by drugs. Unfortunately, the incidence of drug-induced kidney disease is not exactly confirmed. Some studies demonstrated that it is 6-80% in AKI that occurs during hospital admission [1].

Management of acute kidney injury is still facing a big problem. It is only dependent up till now on supportive measures, like fluid resuscitation and renal replacement therapy. No current drug therapy has been approved for the treatment of acute kidney injury [3]. AKI is now being considered an important cause of chronic kidney disease. Failure of complete recovery from AKI transfers acute kidney injury patient to be CKD patient in 90 days [4]. The pathophysiology of AKI is complex and many signals' pathways are involved in it. The Glycogen synthase kinase 3B enzyme is an important member in some of these pathways. GSK3B enzyme is serine/threonine protein kinase responsible for regulation of the metabolism of glucose [5] the effect of its inhibition by the FDA approved drug, lithium on AKI is still under study. [6]. Many factors affect recovery from AKI from them, the condition of the kidney before the development of AKI, the general condition of the patient, the presence or absence of chronic disease in the heart, chest or liver and the age of the patient is very important factor [7].

Materials and Methods

This study was conducted on 28 Male Sprague–Dawley rats between 1stof April 2017 to 20th of May 2017. All the rats were at the age of 6-8 weeks old weighting between 150 and 250 gm at the beginning of the study. The experimental protocols were approved by the Ethics Committee of the University of Menoufia. At the start of our work rats were classified into 4 groups. First group rats were subjected to induction of AKI by cisplatin (5mg/kg) intraperitoneal injection (IP). Second group, rats were given saline same dose like cisplatin IP. The previous 2groups' blood samples were obtained and tissue samples were taken from the left kidney at day 3 from the start. Third group, rats were given lithium chloride (80mg/kg) IP at day 3. Fourth group, rats were given saline IP the same dose like lithium chloride dose at day 3. The previous 2 groups, blood samples were obtained and tissue samples were taken from the left kidney at day 5from the start. Tissue samples from the left kidney were kept in buffered 10% formalin for histopathological examination. Blood samples were obtained from the retro-orbital vein of the rat for renal functions assessment. Formalin-fixed left kidney was embedded in paraffin and was prepared in 3-micrometer–thick sections. Hematoxylin and eosin were used to stain sections to estimate gross histologic kidney injury of Tubulo interstitial injury and tubular dilation/sloughing severity was semi quantitatively scored on a scale from 0 to 5 and reported as the mean of 20 random high-power (3200) fields per hematoxylin and eosin–stained section as the following **:(5)** '0: none, 1: 10%, 2: 11%–25%, 3: 26%–45%, 4: 46%–75%, 5: 76%'. Collection, tabulation and statistical analysis of collected results were done. Analysis by an IBM compatible personal computer with SPSS statistical package version 20 (SPSS Inc. Released 2011 was done. (IBM SPSS statistics for windows, version 23.0, Armonk, NY: IBM Corp.). Two types of statistical analysis were used: Descriptive statistics and Analytic statistics.

Results

The results of our study revealed that induction of AKI was achieved by a single intraperitoneal injection of cisplatin (5 mg/kg) in rat. (Table 1) cisplatin injury

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resulted in an elevation of serum creatinine and blood urea levels by the third day. At day 3, cisplatin induced a typical pattern of (ATN), which was characterized by epithelial simplification, vacuolization of proximal tubular epithelium, epithelial necrosis, and luminalectasia, loss of brush border and slough of tubular cell into lumen (Table 2) (Figure 1).

Saline injection same dose like cisplatin did not cause any elevation in serum creatinine and blood urea levels at day 3. Histopathology of kidneys from rats treated alone with saline remained normal (Figure2).

After day 3, serum creatinine and blood urea levels in cisplatin-injured rats continued to rise. The effect of lithium chloride treatment on serum creatinine and blood urea levels showed significant regression in the rising of serum creatinine and blood urea in lithium chloride treated rats in comparison to saline-treated rats.

Serum creatinine (6.06 ± 0.72) in saline group versus (3.15 ± 1.87) in lithium chloride low dose group and 4.06± 1.99 in lithium chloride high dose group. Blood urea (407.84 ± 171.59) in saline group and (128.88 ± 76.06) in lithium chloride group which signifies an increased renal recovery from AKI with the injection of lithium chloride.

The lithium-enhanced recovery in renal function was accompanied by improved histopathologic picture and pathology scores. Pathological pictures and scores demonstrated an improvement in lithium chloride treated rats than saline-treated but results were not significant. (Figure3) (Figure4) (Table3) (Table4).

Collectively, these data suggest that lithium chloride has an obvious improving effect that enhances renal function recovery and kidney repair after Acute Kidney Injury.

Note of, the dose of lithium used in our study (80 mg/kg) is lower than the dose of lithium (120 mg/kg) that has been safely and routinely used for studies in neurobiology in rodents [8].

The figures were taken during histopathological examination of rat's kidney

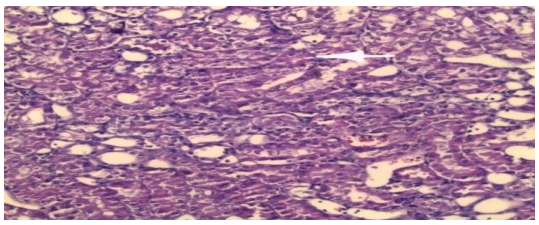

Figure (1): *Cisplatin-induced AKI day3. rat n: 7(Necrosis)*

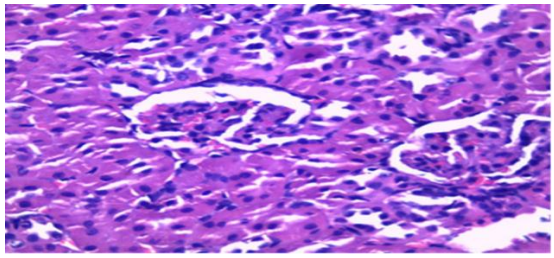

Figure (2): *Saline kidney. Day 3 Rat n: 6(no changes)*

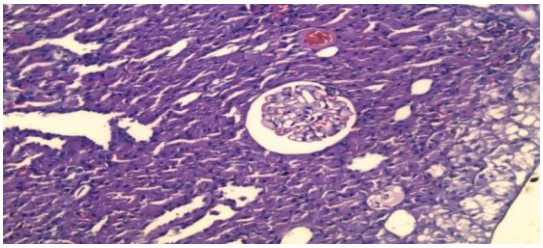

Figure (3): *Lithium chloride treated rats. Day 5: rat n: 5(Cellular vacuolization & foal loss of brush border)*

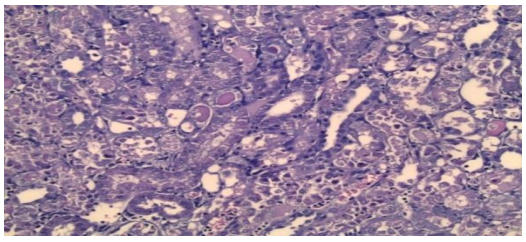

Figure (4)*: Saline treated rats. Day 5: rat n: 7(loss of brush border, sloughing, Focal vacuolization and epithelial death)*

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Table1: comparison between day3 renal functions of rats with cisplatin-induced AKI (GroupI) and saline-injected rats (GroupII)

	Group I (n = 7) Mean ± SD	**Group II (n = 7) Mean ± SD**	**Mann Whitney test**	**P value**
Blood Urea (mg/dl)	132.63 ± 44.73	64.97 ± 7.99	3.13	0.002
Serum Creatinine (mg/dl)	0.70 ± 0.26	0.41 ± 0.06	2.82	0.005

This table demonstrates that Blood urea and serum creatinine were significantly higher in group I than group II.This means that induction of AKI was achieved by a single intraperitoneal injection of cisplatin (5 mg/kg) in rat.
SD: standard deviation

Table2: comparison between day3 pathology scores of rats with cisplatin-induced AKI (GroupI) and saline-injected rats (GroupII)

	Group I (n = 7) Mean ± SD	**Group II (n = 7) Mean ± SD**	**Mann Whitney test**	**P value**
Pathology score	3.14 ± 1.34	0.14 ± 0.37	3.28	0.001
	No. %	No.%	Z test	P value
Pathology score 0 1 2 3 5	0 0.0 0 0.0 3 42.9 2 28.6 2 28.6	6 85.7 1 14.3 0 0.0 0 0.0 0 0.0	2.7 0.00 1.30 0.76 0.76	0.006 0.99 0.19 0.44 0.44

This table demonstrates that the mean pathology score was significantly higher in group I (cisplatin-induced AKI group) than group II (saline-injected rats).
SD: standard deviation

Table3: comparison between day5 renal functions between cisplatin induced AKI rats that were treated with lithium chloride in day 3(GroupIII) and were treated with saline in day3 (GroupIV)

	Group III (n = 6) Mean ± SD	**Group IV (n = 6) Mean ± SD**	**Mann Whitney test**	**P value**
Blood Urea (mg/dl)	128.88 ± 76.06	407.84 ± 171.59	2.55	0.01
Serum Creatinine (mg/dl)	4.06 ± 1.99	6.06 ± 0.72	2.19	0.02

This table demonstrates that lithium chloride treated rats (GroupIII) shows significant reduction in renal functions than saline-treated rats (GroupIV)
SD: standard deviation

Table4: comparison between day5 pathology scores between cisplatin-induced AKI rats that were treated with lithium chloride in day 3(GroupIII) and rats that were treated with saline in day3 (GroupIV)

	GroupIII (n = 7) Mean ± SD	**GroupIV (n = 7) Mean ± SD**	**Mann Whitney test**	**P value**
Pathology score	2.42 ± 1.27	2.57 ± 0.78	0.33	0.73
	No. %	No.%	Z test	P value
Pathology score 1 2 3 4	2 28.6 2 28.6 1 14.3 2 28.6	0 0.0 4 57.1 2 28.6 1 14.3	0.76 0.54 0.00 0.00	0.44 0.59 0.99 0.99

This table demonstrates that lithium chloride treated rats (GroupIII) shows improvement in pathology scores than saline-treated rats (Group IV)
SD: standard deviation

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Discussion

Lithium is one of the important inhibitors of the GSK3B enzyme. It has a regenerative effect on various body tissues. Involving tissues of the central nervous system and tissues in bone marrow forming hematologic system. Kidney tissues is emerging now to benefit from the regenerative effect of lithium but this is still under research [5].

In agreement with our results: Plotnikov and coworkers 2016 [9], in their preclinical animal study, founded that inhibition of GSK-3b activity through pharmacological treatment by lithium chloride resulted in significant decrease of AKI. Wangand Tong 2015 [10], in their preclinical animal study on rats, demonstrated that glycogen synthase kinase-3B inhibition by TDZD-8 provided a protection to the kidney. Bao and coworkers2014 [5], in their preclinical animal study examined the effect of administration of lithium on cisplatin and ischemia/reperfusion-induced AKI. Their results are in consistent with our study results.

Plotnikov and coworkers 2013[11], in their preclinical animal study demonstrated that LiCl treatment reduced renal tubular cell death and reduced kidney injury caused by gentamicin. Bao and coworkers 2012 [12], in their preclinical animal study, suggested that the inhibition of GSK3β by new selective small molecule inhibitors or existing FDA approved drugs with GSK3β inhibitory function, like lithium and valproate, represents a new therapeutic strategy for AKI treatment, especially for NSAID-induced AKI. The results of the previous study are in consistent with our study results.

Howard and coworkers 2012[13] in their preclinical animal study on mice clearly demonstrates the essential role of GSK-3β in AKI development so, its inhibition according to these results really is beneficial in treating AKI. Wang and coworkers 2010 [14] in their study demonstrated that activation of GSK3β-mediated Bax activation induced apoptosis and tubular damage. Therefore, its inhibition will improve AKI through this pathway.

Our study was carried out on 42 Male Sprague–Dawley rats aged 6-8 weeks old weighted between 150 and 250 gm. Rats were classified into 4 groups. Group I: rats were subjected to induction of AKI by cisplatin (5mg/kg) intraperitoneal injection(IP). Group II: rats were given saline same dose like cisplatin IP. The previous 2 groups' blood samples were obtained and tissue samples were taken from the left kidney at day 3 from the start. Group III: rats were given lithium chloride (80mg/kg) IP at day 3. Group IV: rats were given saline IP the same dose like lithium chloride dose at day 3. The previous 2 groups, blood samples were obtained and tissue samples were taken from the left kidney at day 5 from the start. Our study results revealed that cisplatin (5mg/kg) induce AKI. Lithium chloride promotes renal recovery after its administration for treatment of acute kidney injury.

Conclusion

Administration of lithium, the FDA approved drug as one of the glycogen synthase kinase 3B inhibitors may be a promising treatment for acute kidney injury.

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