MJMR, Vol. 35, No. 1, 2024, pages (75-79).

Open Access
ISSN:2682-4558

Research Article

Impact of metformin onto ischemia/reperfusion damage of the kidney

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DOI: 10.21608/MJMR.2024.260928.1614

Abstract

Background: Autophagy means bulk degradation and recycling of cytoplasmic constituents to maintain cellular homeostasis. In response to stress, autophagy is induced and may contribute to serve as a cell survival mechanism. Very little is known about autophagy in renal pathophysiology. **Aim of the study**; the aim of the present study is to examine autophagy activator metformin and its role in renal cell injury using models of ischemia−reperfusion. **Methods**; Sham rats (control, n = 10); the rats in the sham group underwent laparotomy under anesthesia without any treatment, Group 2 (Ischemic-reperfusion injury, I/R, n =20); bilateral renal pedicles were clipped for 45 min, followed by perfusion for 24 h to establish I/R model, Group 3(IR + metformin 300 mg/kg, n = 20), Animals were pretreated with the metformin (Met) at 300 mg/kg 2 does 2h, 12h prior to 45 min of ischemia. **Results:** the effect of I/R of the kidney on the renal cells resulted in disturbed renal function as evidenced by the increase in kidney Function tests parameters and protective effect of metformin on renal cells that was evaluated by assessment of urea, creatinine, MDA. **conclusion**: The results suggested the protective effects of metformin on induced autophagy ameliorating the I/R injury of the kidney.

Key words: kidney, metformin, I/R damage, MDA.

Introduction

Rapid cellular damage and alternation of kidney functions are symptoms of renal ischemia-reperfusion (I/R) which consider form of acute kidney damage that cause high morbidity and mortality. Urological intervention and renal transplant that include revascularization of renal artery are common causes of I/R injury [1]. It is important to differentiate two stage of cellular damage: damage that occurs during ischemia and damage occurs after the reperfusion [2]. Generation of oxygen free-radicals and creation of pro-inflammatory mediators may be associated with Ischemia-reperfusion injury[3]. Autophagy is a basic catabolism process that involves the destruction of components of cells and is involved onto cellular growth, home-ostasis, survival, and death [4].

Both activated protein kinase (AMPK), and the mammalian target of rapamycin (mTOR) are important molecules that control autophagy[5]. One of the oral antidiabetic medications frequently used for Type 2 diabetes is metformin (MET) (T2DM)[6]. By inducing autophagy and AMP-activated kinase (AMPK), metformin has been established to help protection renal tubular cells from apoptosis, inflammation, reactive oxygen stress (ROS), and endoplasmic reticulum (ER) stress[7]. Research into AMPK-dependent autophagy may provide light on novel approaches based on improving energy metabolism[8]. Offering a workable approach to reduce renal IR damage and maybe offering novel therapeutic approaches for additional clinical intervention[9].

This research aim to examine the impact of metformin on ischemia-reperfusion injury related kidney changes.

Materials and methods

1- Animals

Fifty mature Wistar rats weighting 150-250g were used in this invistigation. Before the trial began, the animals were acclimated for a duration of 72 hours. The National Institutes of Health's Instructions for the use and care of Experimental Animals were followed in every animal care and procedure. Throughout the trial, the animals were kept in cages made of stainless steel and were allowed unlimited availability of food and water. The Minia University Faculty of Medicine's Medical Research Ethics Committee gave the project ethical permission.N0.339/05/2022.Date: May 2022

2- Chemicals

Metformin (1, 1-dimethylbiguanide hydrochloride); obtained from (Sigma- Aldrich) was dissolved in sterile saline.

3- Experimental design

* ***Group 1***; (control group, n = 10); the rats in the control group had laparotomy surgery while under anesthesia, but they were not given any medication (The abdomens of the rats in the control group were opened and both kidneys in the rats were exposed for 45 min, but renal pedicles were not clipped through surgical procedure, then closed).

* ***Group 2***; Ischemic-reperfusion injury (I/R, n =20); To create the I/R model, both renal pedicles were cut for 45 minutes, then perfusion was applied for 24 hours [10].

* ***Group 3;*** (IR + metformin 300 mg/kg, n = 20), 12 and 2 hours before the 45-minute ischemia, the animals received oral gavage dose of 300 mg/kg of metformin (Met) [11]. Because this dosage produced serum metformin concentrations that matched those observed in patients following maintenance metformin therapy, the concentration of 300 mg/kg body weight was selected [12].

Rats were received doses through oral gavage 12 and 2 hours before I/R. The dosage and oral gavage method refer to the previously reported papers [9] [11]

Establishment of renal ischemia-reperfusion model:

Before surgery, the rats were given a 12-hour fast. After the administration of xylazine (10 mg/kg bw) as well as ketamine (50 mg/kg bw) intraperitoneally for anesthesia, the patient's abdominal hair was extracted, and a medi-ventral line incision was done to expose the abdominal cavity. To keep the rat's rectal temperature constant (37°C) during the procedure, the anaesthetized animal was placed on a heating pad. To separate the kidneys, the renal capsule was sharply split.

For 45 minutes, vascular clamps were used to close off the renal pedicles. After 45 minutes, the clamp was withdrawn and the kidney was monitored for 4-5 minutes to check that reperfusion had been established effectively. The wounds were closed with stitches and reperfusion remained for 24 hours [13].

The next variables were examined

Urea level (blood urea level of rats was measured by modified Berthelot's reaction using Urea Enzymatic colorimetric Kit (Diamond Diagnostics, Egypt)

Creatinine level (Serum levels creatinine was measured by Jaffe's method using Creatinine Colorimetric Kit (Diamond Diagnostics, Egypt).

MDA (Determination of renal tissue malondialdehyde levels was performed using colorimetric kit supplied from (Bio diagnostic, Egypt).

Data analysis using statistics

Results are expressed as means ± Standard error of mean (SEM). GraphPad Prism software v. 8.0.2 (GraphPad) and the Statistical Package for Social Sciences, SPSS version 22 were used for statistical analysis and graph plotting.
(**SPSS, Chicago, IL, USA**).

One-Way analysis of variance **(ANOVA**) test is used to compare of parametric quantitative data between more than two groups followed by **Tukey's post hoc test** that used to compare the two groups**.** Statistical significance was defined as **(P value < 0.05).**

Results

Impact of ischemia/reperfusion on serum urea, creatinine.

The induction of I/R resulted in increased blood urea level and creatinine level (table 1).

Regarding MDA levels, it was increased in the sera of the I/R rats.

Impact of Metformin on Urea and creatinine levels

Administration of metformin to I/R group significantly lowered the serum urea and Creatinine (table 1).

Table (1): The mean value ± SEM of serum urea, creatinine in different groups:

Group	Control group (mean±SEM) (N=10)	Ischemia/ reperfusion group (mean±SEM) (N=20)	Metformin–treated group (mean±SEM) (N=20)	P 1	P 2	P 3
Urea (mg/dl)	35.61 ± 0.95	82.67± 1.47	71.57 ± 3.39	0.0001**	0.0001**	0.0001**
Creatinine (mg/dl)	0.82 ± 0.038	1.02 ± 0.052	0.76 ± 0.033	0.003**	0.001**	.5

P 1- p value of I/R & Control
P 2- p value of Met & I/R
P 3 - value of Met & control
** Highly significant

Impact of Metformin on MDA level

Metformin administration also caused a significant lower level of serum MDA when compared to the I/R group (table 2).

Table (2): The mean value ± SEM and significance of MDA level of different studied groups in kidney tissue:

Group	Control group (mean ± SEM) (N=10)	Ischemia/ reperfusion group (mean ± SEM) (N=20)	metformin–treated group (mean ± SEM) (N=20)	P 1	P 2	P 3
MDA (nmol/mg tissue)	0.68 ± .057	0.74± .024	0.42 ± .011	0.425	.0001**	.001**

P_{1}-p value of I/R & Control
P_{2}- p value of Met & I/R
P_{3}- value of Met & control
** Highly significant

Discussion

Acute kidney injury (AKI) is still major issue in healthcare systems which cause a heavy burden across the world [14]. One of the main causes of AKI is ischemia-reperfusion damage (IRI), which results from problems with several kinds of diseases and surgical interventions that impair renal blood flow[15]. Significant alterations in kidney architecture and function are brought about by IR [16].

Under stressful conditions, autophagy is triggered in order to promote the recycling of (damaged) macromolecules, organelles, or protein aggregates into components of cells that play a role in energy production and stress

response systems[17]. As a result, it has been noted that under stressful conditions, autophagy protects renal cells [18].

Serum levels of urea and creatinine were found to rise considerably in reactions to renal ischemia/reperfusion when compared to the control group, and to fall in the metformin-treated group when compared to the ischemia/reperfusion condition. These findings indicate a somewhat improved renal function, indicating that metformin significantly lowered the degree of renal damage [19].

The prior findings are consistent with [13]. This individual discovered that elevated blood urea nitrogen (BUN) and serum creatinine levels were observed after I/R damage and could be used to assess renal function and the extent of injury. In addition, these findings were consistent with prior research that found an increase in creatinine clearance, blood urea nitrogen (BUN), serum uric acid, and damage to tissue following I/R, related to alterations in serum biochemical parameters caused by kidney damage [20].

Metformin is now the first-line anti-hyperglycemic drug for patients just diagnosed with type 2 diabetes, particularly obese diabetic patients[21]. Hence, metformin has been suggested as an organ-protective medication during situations where IR injury (IRI) occurs, such as transplantation, and could help prevent IRI exceeding its glucose-lowering effects [22].
Our results showed significant increase in renal MDA concentration in I/R rats and metformin could significantly decrease the level of MDA.

This finding aligns with a prior research study that suggested metformin may reduce the rise in reactive oxygen species (ROS) and malondialdehyde (MAD) formation in acute renal damage [23]. An increasing body of research indicates that autophagy defends the kidney against oxidative stress in the event of acute ischemic kidney injury, chronic kidney disease, and even ageing [24], and improves the antioxidant defense mechanisms (SOD) [25].

Conclusion

Finally, using an in vivo I/R damage model, we have established at the very initial time the fact metformin exerts a protective role in I/R injury and stress conditions in the kidneys.

Acknowledgements

Resources for funding

This study did not obtain specific funding from governmental or private funding individuals.

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