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The possible mechanism of action of metformin in renal ischemia reperfusion in rats

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Abstract:

Background/Aim: Renal ischemia plays an important role in renal impairment and transplantation. Metformin appears to have anti-inflammatory and antioxidant effects, The aim of this work is to examine these effects on renal ischemia reperfusion (I/R) in rats.

Methods:Four equal groups of rats:

Control, Sham, (I/R) and Metformin treated I /R groups.

A renal I/R injury was done by a left renal pedicle occlusion to induce ischemia for 45 min followed by 60 min of reperfusion with contralateral nephrectomy. Metformin pretreated I/R rats in a dose of 200 mg/kg/day for three weeks before ischemia induction.

Nitric oxide (NO), tumor necrosis factor alpha (TNF α) , catalase (CAT) and reduced glutathione (GSH) activities were determined in renal tissue, while creatinine clearance (CrCl) , blood urea nitrogen (BUN) were measured and 5 hour urinary volume and electrolytes were estimated . *Results:* these results showed significant increase in NO,TNF α, BUN , CrCl and significant decrease in urinary volume , electrolytes, CAT and GSH activities in the I/R group than those in the control group. Metformin decreased significantly NO, TNF α, BUN and Cr Cl while increased urinary volume, electrolytes , CAT and GSH activities.

Conclusion: metformin produced anti-inflammatory renoprotective effect on Cr Cl and diuresis in renal I/R injury.

Keywords: *renal I/R-metformin-rats*

Introduction:

Renal ischemia followed by reperfusion leads to acute renal failure and sepsis in both native kidneys and renal allografts(1,2). Renal I/R results in formation of reactive nitrogen species, oxidative stress, ATP depletion, increase calcium intracellular (3) and usually is followed by harmful inflammatory response and tissue damage (4).

The inflammatory reaction started by renal I/R is the source of pro inflammatory cytokines and monocytes infilteration in the kidney that have been known to be the major source of TNF α which stimulates production of reactive oxygen species and is able to up regulate its own expression (5) and the expression of other genes involved in the inflammatory response (6) leading to a reduction of the glomerular filteration rate (GFR) (7).

The presence of proinflammatory cytokines in I/R leads to the up regulation of inducible NO (iNO) that reacts with superoxide to generate peroxynitrite which harms tissues (8).

Thus based on the pathophysiology in renal I/R, it was hypothesized a possible effect of metformin in renal I/R.

Metformin is a biguanide used in type 2 diabetes, it inhibits hepatic glucose production and increases peripheral insulin sensitivity(9).

The mode of action of metformin is incompletely understood however, it appears to have anti-inflammatory and antioxidant effects involved in its beneficial effects on insulin resistance and polycystic ovary syndrome (10,11).

This work aimed to investigate the possible mechanism and pharmacological effects of metformin on renal I/R.

Material and Method

Drugs and Chemicals: metformin powder (sigma, St louis, Mo). Chemicals for assay CAT and GSH activities were purchased from sigma (St louis, Mo). TNF-α Kit was purchased from G enzyme, (Cambridge, MA, USA). NO detection chemicals were from(R&D system Inc. USA).

Animals and experimental protocol:

Sprague – Dawley rats weighing 200-250gm were used.

Animals were handled with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health and the approval from Animal Ethic Committee of the institution(Egypt).

The rats were fed with a standard rat chow and allowed to freely drink water. The rats were anaesthetized with thiopental (50mg/kg intraperitoneally) and the body temperature was kept at 36-38°C by placing the rats under light source. The abdominal region was shaved with a safety razor and sterilized with povidine iodine solution.

A midline incision was made and a right nephrectomy is done after ligating the pedicle. A non traumatic vascular clamp was applied to the left renal pedicle.

The control group (n=10): the rats in this group served as control

The sham group (n=10): the rats in this group were sham operated with

the exposure of the left renal pedicle,but were not subjected to any I/R and were pretreated orally with metformin.

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I/R group (n=10) : ischemia was applied for 45 min followed by 60 min of reperfusion(12).

I/R + Metformin group (n=10) : the rats were pretreated orally with metformin at a dose (200 mg/kg) daily (13) for three weeks before the induction of ischemia. The control and the I/R groups, received a comparable volume of vehicle physiological saline. Each rat (after induction of renal I/R in I/R group) was caged in one metabolic cage (Nalgene metabolic cage. Nalge company,

New York 14602-0365), that designed for collection of urine throughout 5 hour test.

The collected urine was considered as one reading for each of control, sham, I/R *and* Metformin groups. At the end of 5 hour, the animals were sacrificed, the left kidneys were removed and blood collected from rat hearts and stored at-20°C until detection of BUN and

Cr CL (U ×V/ P).

U= Concentration of creatinine /1ml urine, V =Volume of urine /min P =Concentraiton of creatinine in 1ml blood.

Assay of urine volume, Na^+ , CL^- excretion was also done (14-*17*)

The renal tissue was stored at −70°C until biochemical analyses. Kidneys were thawed and homogenized (10%W/V) with 0.15 KCl at 4°C then centrifuged at 10,000g for 1.5h. The supernatant was used as the source of experimental product. NO level was determined according to the method of (18).

Glutathione (GSH) assay was determined by the method of (19). The catalase activity was performed according to (20) . Protein oxidation was determined according to Levine et al., (21) Protein levels were measured according to(22).

All the measurements were done by UV spectrophotometer

Statistical analysis:

All data are expressed as means ± standard deviation of mean ANOVA Test was used followed by Tukey post hoc test.

A P value of ≤ 0.05 was statistically significant.

Results:

BUN and CrCl levels in the I/R group were significantly higher than in control rats ($p<0.05$) table (1).

Table 1: Creatinine clearance **(**Cr Cl) and blood urea nitrogen(BUN) levels in control and test groups. Mean ± SD.

Groups	CrCl (ml/min)	BUN mg/dl
Control group	1.30 ±0.11	14.30±0.25
Sham group+metformin	1.27±0.09	15.70±0.19
I/R group **P1**	1.85±0.25 <0.001***	28.00±0.62 <0.001***
I/R+metformin group **P2** **P3**	1.55±0.22 0.001** 0.028*	18.10±1.00 <0.001*** <0.001***

P1: Statistical significance between control group and saline treated I/R group.
P2 Statistical significance between control group and Metformin treated I/R group.
P3 Statistical significance between saline treated I/R group and Metformin treated I/R group.

When metformin was administered before I/R, BUN and CrCl levels were still significantly higher than control group but their elevation were significantly lower in comparison to I/R group alone ($P<0.05$).

TNF α and NO levels were significantly higher in the I/R group than those of the control group (Table 2). Pre treatment with metformin significantly lowered their levels in comparison to I/R group ($P<0.05$).

On the other hand,CAT and GSH activities significantly decreased in I/R group on comparing to the control group (Table 3). Metformin pre treatment increased CAT and GSH activities in comparison to the I/R group($P<0.05$).

As shown in table (3) Metformin administration produced significant increase of the 5 hours urinary volume and electrolytes excretion compared to I/R group ($P<0.05$).

Table 2: Tumour necrosis factior α (TNF α)and inducible nitric oxide (iNO)levels in control and test groups. (Mean ± SD).

Groups	TNF α (pmol/mg tissue)	iNO nmol/ mg tissue
Control group	17.60 ±5.98	2.54 ± 0.82
Sham group+ metformin	16.70 ±5.50	2.35 ±0.80
I/R group P1	54. 00±6.02 <0.001***	4.50±0.89 <0.001**
I/R+ metformin group P2 P3	39 ± 14.01 <0.001*** 0.006**	3.53±0.95 0.02* 0.03*

P1: Statistical significance between control group and saline treated I/R group.
P2 Statistical significance between control group and Metformin treated I/R group.
P3 Statistical significance between saline treated I/R group and Metformin treated I/R group

Table 3: Catalase(CAT) and reduced glutathione (GSH) activities and 5h urine volume& electrolytes* sodium(Na^+), chloride(CL^-)* in control and test groups.(Mean ± SD).

Groups	CAT U\mg tissue	GSH nmol\mg pr	5h urine		
			Volume ml\kg\5h	Na^+ mmol\kg\5h	CL^- mmol\kg\5h
Control group	2267±68.90	7.35±0.65	13.50±1.01	3.12±0.22	4.23±0.200
Sham group+metformin	2270±70.01	6.93±0.60	12.90±1.00	3.00±0.19	4.02±0.21
I/R group P1	1800±151.02 <0.001***	3.60±0.64 <0.001***	6.00±0.53 <0.001***	1.42±0.01 <0.001***	1.99±0.02 <0.001***
I/R+metformin group P2 P3	2193±98.70 0.067 (ns) <0.001***	5.89± 0.76 <0.001*** <0.001***	10.00±1.55 <0.001*** <0.001***	2.60± 0.03 <0.001*** <0.001***	2.95±0.03 <0.001*** <0.001***

P1: Statistical significance between control group and saline treated I/R group.
P2 Statistical significance between control group and Metformin treated I/R group.
P3 Statistical significance between saline treated I/R group and Metformin treated I/R group.

Discussion

Lipid peroxidation is related to I/R induced tissue injury. Production of inducible NO synthase (NOS) under lipid peroxidation and inflammatory conditions results in the induction of NO which react with O2 liberaling peroxy nitrite. NO itself inactivates the antioxidant enzyme system CAT and GSH(23).

Alteration in NO synthesis have been observed in other kidney injuries as nephrotoxicity and acute renal failure induced by endotoxins (24). These studies showed that treatment with iNOS inhibitors improved renal function and decreased peroxy nitrite radicle which is believed to be responsible for the shedding of proximal convoluted tubules in I/R (25).

Other studies have shown that TNF α produced from the

pro inflammatory cytokines in renal I/R may lead to renal injury either by direct action via apoptosis or through inflammatory cells mediated renal tissue injury (26). Anti TNF α therapy leads to reduction of renal damage(27).

Super oxide radicles presence due to I/R are changed into H202 by dismutation with antioxidant enzyme system CATand GSH resulting in reduction of their activities (28).

This study showed a decrement in CAT and GSH activities in the I/R group compared with the control, this decrease is the result of their consumption by free radicles which are involved in I/R. Metformin administration increased CAT and GSH activities in comparison with the I/R due to the anti oxidant effect of metformin (29).

In addition, the present results demonstrated that oral administration of metformin to I/R rats group produced significant increase of urine volume and urine excretion of Na^+ and Cl^-. this could be explained on the basis that metformin is considered to have a stimulatory effect and could activate AMPK in the kidney. This AMPK or the energy sensor AMP activated protein kinase is a protein kinase which orchestrates the regulation of energy generation and energy consuming pathways (30). It is highly expressed in the kidney and is involved in a variety of physiological and pathological processes including ion transport, fluid and electrolytes homeostasis, diabetic nephropathy , Diabetes mellitus disease, ischemia, renal hypertrophy and inflammation (31,32).

On activation of AMPK, it acts to restore energy homeostasis by phosphorylating multiple substrates that act both to stimulate energy production and minimize energy and adenosine triphosphate ATP consumption(33).

Woolhead et al., found that AMPK activation with phenformin inhibited Quabain sensitive transepithelial sodium transport and metformin treatment attenuated the down regulation of basolateral membrane Na^+ -K^+ ATPase expression following ischemia in canine kidney cells (34). These results suggest that AMPK activation may enhance ischemic preconditioning in renal tissue ,more over there may be differential effects of AMPK depending on the setting and time course of activation (35). AMPK activation had been described by Sag et al (36) to have anti inflammatory action through inhibition of TNF α, IL1B induced NF-KB gene expression , iNO synthase and cyclooxygenase 2 in stimulated macrophages.

These results showed the anti inflammatory, the anti oxidant and renal ionic transport homeostasis effects of metformin on the renal injury followed I/R . The assay of AMPK in renal I/R is needed in further studies.

CONFLICT OF INTEREST NOTIFICATION

There are NO actual or potential conflicts of interest.

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