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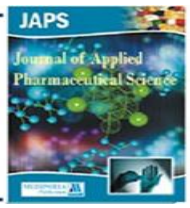

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Subtherapeutic Dose of Prazosincan Ameliorate Metformin Induced Lactic Acidosis in Renal Compromised Rats

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ABSTRACT

Incidence of lactic acidosis caused by metformin is rare but this can occur in renal compromised individuals because of metformin accumulation. The drug enters the liver through organic cationic transporter 1(OCT1) and reduces oxygen consumption in mitochondria resulting in reduced lactate clearance and lactic acidosis. In the present study, we investigated that inhibition of the transport of metformin in the liver could reduce the blood lactate levels. Eighteen healthy male albino rats were selected for the study. Group 1- control group included 6 rats, they were given normal saline for 10 days by i.p injection. Group 2- Twelve rats were induced nephrotoxicity by gentamicin at the doses of 40mg/kg given by i.p. route . Group 3- six rats from group 2 were given metformin according to human doses of 1000mg/day and group 4- included six rats from group 2 received metformin and prazosin at subtherapeutic dose i.e. according to 1mg/day human dose. Blood urea, serum creatinine and total urinary albumin were found to be significantly ($p<0.0001$) increased in group 2 as compared to control group. Resting blood lactate levels were significantly($p<0.0001$) high in group 3 animals as compared to controls and addition of low dose of prazosin in group 4 further reduced the resting blood lactate levels as compared to group 3($p<0.0001$). So, the present study concludes that metformin can ameliorate the chances of developing lactic acidosis in renal compromised individuals if low dose of prazosin is added in the treatment.

Keywords: OCT 1, metformin, lactic acidosis, prazosin, nephrotoxicity

INTRODUCTION

Biguanides are a class of drugs widely used as oral antihyperglycemic agents for the treatment of type 2 diabetes mellitus but they are associated with lactic acidosis, a lethal side effect. Nowadays, metformin is the only member of this class available for use today(Bailey and Turner, 1996). Metformin increases the activity of AMP dependent protein kinases (AMPK) by phosphorylation (Zhou *et al.*, 2001). Activated AMPK stimulates fatty acid oxidation, glucose uptake &nonoxidative metabolism & it reduces lipogenesis& gluconeogenesis. The net result is increased glycogen storage in skeletal muscle, increased peripheral sensitivity to insulin, reducing gastrointestinal absorption & hepatic glucose production (Hundal *et al.*, 2000; Borst and Snellen, 2001).

Molecular mechanism by which metformin activates AMPK is not known, it is thought to be indirect possibly by reducing intracellular energy stores. Consistent with this merformin has been shown to inhibit cellular respiration by specific action on mitochondrial complex I resulting in reduction of O2 consumption & glucose production in isolated hepatocytes in a concentration dependent manner. Excessive inhibition of mitochondrial respiration may cause lactic acidosis. Lactic acidosis is a severe adverse effect of biguanides&phenformin was withdrawn from the market in 1970s for this reason (Kwong and Brubacher, 1998). Like phenformin, metformin has been associated with lactic acidosis. The most concrete evidence of this is from cases of metformin overdose where high circulatory levels of the drug were associated with high plasma lactate & academia. However, estimated incidence of lactic acidosis attributable to metformin use is 3-6 per lac patient years of treatment(Lalau and Race, 2001; Scarpello and Howlett, 2008).Lactic acidosis is observed in patients with renal dysfunction because renal secretion is the major elimination route of metformin(Davidson and Peters, 1997). It is known now that metformin is good substrate of rat OCT 1(Wang *et al*., 2002). OCT1, is abundantly expressed in liver & to some extent in kidney at basolateral membrane. Although, no significant reduction was observed in urinary excretion of metformin, the hepatic uptake of metformin was reduced markedly in OCT-/- mice(Wang *et al*., 2002). Therefore, Wang *et al*., 2003, concluded that hepatic uptake of biguanides plays an important role in lactic acidosis. Keeping in mind these results, we designed the present study, wherein, OCT 1 in liver was inhibited by a drug, prazosin. Prazosin has very high affinity towards OCT 1(Koepsell H, 2004; Koepsell H and Endou H, 2004; Hayer ZM *et al.*, 2002). It is a non specific α blocker used in hypertension, benign prostatic hyperplasia, raynaud's phenomenon, raynaud's disease & in refractory cases of congestive heart failure. Contraindicated in patients with known sensitivity to quinazoline, prazosin. The present study, therefore, has been designed to explore the protective effect of prazosin at subtherapeutic doses, in ameliorating the blood lactate levels in renal compromised rats.

MATERIAL & METHODS

The experimental protocol was approved by Institutional Animal Ethical Committee.

Drugs

Metformin (glyciphage by Franco Indian), Prazosin (prazopress by Sun Pharma) and Gentamicin (Genticyn by AHPL) made available from hospital pharmacy.

Study design

Eighteen healthy male albino rats were selected & housed in two groups. They were acclimatized to the laboratory for a week under 12:12 light dark cycle. The animals were fed on standard pellet diet & water ad lib.

Group 1: Six rats were administered equivalent volume of 0.1 ml i.p. of NS for 10 days.

Group 2: Twelve animals received 40 mg/kg/day (Kosek JC *et al.*, 1974) i.p. of gentamicin for 10 days to induce nephrotoxicity

Group 3: Out of group 2 animals, 6 rats were given metformin at the doses of 1gm/day human dose by i.p. route

Group 4: Out of group 2 animals, 6 rats were given metformin along with prazosin at the human doses of 1mg/day

Biochemical study

Blood samples from rat tail vein were collected from group 1 & 2 after 10days & examined for blood urea, serum creatinine, total urinary protein& resting blood lactate levels by using lactate analyser (YSI model 1500 SPORT)

Blood samples again collected from rat tail vein after 10 days from group 3 & 4 & examined for resting blood lactate levels.

Statistical analysis

Statistical analyses were carried out by applying student 't' test using Graph Pad Prism 5 software. Values of $p<0.05$ is considered statistically significant.

RESULTS

After gentamicin administration, nephrotoxicity was induced in group 2 rats, there was significant change in the body weights & resting blood lactate levels (table I). Nephrotoxicity was reflected by their significant changes in blood urea, serum creatinine& total proteins in urine ($p<0.0001$) (table II). Blood lactate levels in renal compromised rats were significantly ($p<0.0001$) high as compared to nephrotoxic rats, after administration of metformin for 10 days in group 3. Blood lactate levels in the group (group 4) who have received metformin &prazosin, returned to near normal levels(table III)

Table. 1: Body weights & resting blood lactate levels in control & nephrotoxic rats.

Group	n	body weight(gms)	Resting blood lactate (mmol/l)
Group 1	6	189.17± 11.58	1.11 ± 1.50
Group 2	12	156.33 ± 8.33*	1.47 ± 0.29**

Group 1- control rats
Group 2- nephrotoxic rats
Values are mean ± SD , *p<0.0001, **p<0.01 as compared to control.

Table. 2: Comparison of renal function tests in control & nephrotoxic rats.

Groups	n	Blood urea(mg/dl)	Serum creatinine (mg/dl)	Total urinary Protein(mg/dl)
Group 1	6	24.70± 1.88	0.97± 0.04	3.74± 0.70
Group 2	12	67.24± 3.71*	5.02 ± 0.68*	7.20± 0.73*

Group 1- control rats
Group 2 -nephrotoxic rats
Values are mean ± SD, *p<0.0001 as compared to their respective values of control

Table. 3: Comparison of resting blood lactate levels in metformin received renal compromised rats (group 3) & metformin + low dose prazosin received renal compromised rats(group 4).

Groups	n	Resting blood lactate levels(mmol/l)
Group 2	12	1.47 ± 0.29
Group 3	6	4.70 ± 0.44*
Group 4	6	1.59 ± 0.12**

Group 3- renal compromised rats who have received metformin. Group 4- renal compromised rats who have received metformin +low dose of prazosin. Values are mean ± SD, *p<0.0001 as compared to group 2, **p<0.0001 as compared to group 3.

DISCUSSION

Lactic acidosis (LA), is a common cause of metabolic acidosis (Luft FC, 2001; Stacpoole PW, 1993). It is a life threatening condition characterized by low arterial pH (<7.35) and elevated blood lactate levels (>5mmol/L) and more than 50% patients died when LA took place under phenformin administration (Brown et al, 1998; Kwong and Brubacher, 1998).The mechanisms that are thought to be responsible for LA by biguanides are twofold: 1. Increased lactate production in peripheral tissues(Borst and Snellon, 2001) and 2. Inhibition of lactate metabolism/transport in the liver and other tissues such as heart & muscle.Though, LA incidence caused by metformin is negligible(Kamber et al, 2008) still it can occur as a result of renal dysfunction. El-Mir *et al.,* 2000 demonstrated that metformin reduced the oxygen consumption in a concentration dependent manner in isolated rat hepatocytes. The relationship between inhibition of oxygen consumption by the metformin & the inductibilty of blood lactate was investigated to determine the correlation between them by Wang *et al.,* 2003. There was a clear linear correlation indicating that the reduction of oxygen consumption can be used as an index of LA.

In present study, animals were made renal compromised by administration of gentamicin. There are several studies, where gentamicin is used to induce nephrotoxicity(Kannappan N *et al.,* 2010;Vijayakumar and Naidu 2000). Aminoglycosides have long been one of the commonest causes of drug induced nephrotoxicity(Moore RD *et al.,* 1987). The drug induce conspicuous & characteristic changes in lysosomes of proximal tubular cells consistent with accumulation of polar lipids (myeloid bodies)(Begg and Barclay, 1995; De Broe ME *et al.,* 1984). These changes are accompanies by signs of tubular dysfunction or alterations. Accumulation of metformin occurred in nephrotoxic rats but this could not be uptaken by the liver as transporter OCT 1 was inhibited. Prazosin was used for inhibiting this transporter as it has highest affinity for it. Prazosin was used at low doses that were subtherapeutic *i.e.* human dose of 1mg/day. Low doses were chosen to avoid the commonest side effect of the drug i.e. postural hypotension. Pagilwar S *et al.,* 2009 also demonstrated the use of prazosin as anti-inflammatory at subtherapeutic doses in rats. OCT 1 inhibition was reflected by decreased blood lactate levels as compared to the group who did not receive prazosin. This finding was in accordance with Wang *et al.,* 2002 who demonstrated that blood lactate levels were high in wild type mice as compared to OCT (-/-) mice.

CONCLUSION

The present study concludes that addition of sub-therapeutic dose of prazosin with metformin in type 2 diabetes mellitus could be of value in ameliorating the incidence of lactic acidosis in renal compromised individuals. Intrahepatic concentration of metformin is probably responsible for LA by reducing oxygen consumption in hepatocytes and prazosin reduces metformin concentration by inhibiting OCT 1, a hepatic uptake transport.

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