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Berberine protects against metformin-associated lactic acidosis in induced diabetes mellitus

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ABSTRACT

Objective(s): Causality of occurrence of metformin-associated lactic acidosis (MALA) is a clinical problem. Currently, there is no drug available to prevent MALA. The present study was conducted to evaluate the protective effect of Berberine (BBR) against MALA in induced diabetic rat model.
Materials and Methods: A sample of 75 healthy male Wistar rats was randomly selected according to inclusion and exclusion criteria. 75 male Wistar rats were randomly divided into a control and 4 experimental groups. Streptozotocin (STZ) in citrate buffer (pH 4.5) at a dose of 45 mg/kg was injected for induction of diabetes mellitus and rats achieving fasting blood glucose >250 mg/dl were included. Blood samples were collected 18 hr after the last dose of metformin and berberine. Ethical approval was taken before the study was conducted. Staistix 10.0 (USA) software was used for data analysis.
Results: Berberine decreased MALA. Metformin, metformin + BBR 50 mg/kg bwt, and metformin + BBR 100 mg/kg bwt showed serum lactate as 1.87±0.4 mmol/lL, 1.62 ± 0.44 mmol/l and 1.47± 0.45 mmol/l, respectively (P=0.0001). Insulin resistance and liver enzymes were improved in BBR treated rats.
Conclusion: The present study reports berberine protects against MALA in streptozocin-induced diabetes mellitus.

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Introduction

Diabetes mellitus (DM) is estimated to reach almost 600 million by the year 2035 as reported by World Health Organization (1, 2). Metformin is a biguanides drug, used as first-line for type 2 diabetics (T2DM). It is preferred as a first line drug because of its mortality and morbidity decreasing potential due to cardiovascular events (3, 4). Except for gastrointestinal side effects, the safety profile of metformin is well established. However, clinicians are scared of one rare side effect of metformin therapy; the lactic acidosis (LA) caused most probably by mitochondrial dysfunction. Causality of occurrence of LA with metformin therapy is debatable. Risk of metformin-associated LA (MALA) ranges from 2 to 9/100 000 patients (5, 6).

However, spontaneous reporting of LA is growing (5-7). MALA carries 50% mortality which is very high (7). While other studies have reported 26 to 30% mortality (5, 7, 8). The present study was conducted to analyze the effects of Berberine (BBR) against MALA.

BBR is a natural herbal agent, which is the active incipient of the Chinese herb *Coptis chinensis* Franch which has been used since time immemorial. Chemically, BBR is an isoquinoline alkaloid. Its chemical structure is different from existing anti-diabetic drugs such as sulfonylureas, metformin, sitagliptin, etc. BBR has been used as a traditional remedy for gut disorders. As part of folk medicine, the BBR has been used as a remedy for DM since ancient times (9, 10).

In this context, the present experimental study was designed to evaluate and analyze the efficacy of BBR in MALA in a male Wistar albino rat model.

The present study is the first type of its design which was conducted to assess whether BBR is effective against MALA, which is a medical emergency if such effect is proved it may benefit the diabetic community.

Materials and Methods

The present experimental study was conducted at the Animal House of Faculty of Medicine and Allied Medical Sciences (FMAMS), Isra University, Hyderabad,

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Sindh, Pakistan from January 2016 to July 2016. A sample of 75 healthy male Wistar rats was randomly selected according to inclusion and exclusion criteria. Animals were bought from Jinnah Postgraduate Medical Center (JPMC), belonging to Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA. They were cross bred at the Animal house of the Basic Medical Sciences Institute (BMSI), JPMC, Karachi, Pakistan.

Animal housing

Animals were handled according to the animal ethics guidelines of the National Institute of Health (NIH) and animal ethics committee of the institute. Animals were acclimatized for one week. Stainless steel cages with automatic nozzles were used for housing of rats. Pellet diet and water was available freely. Access to food and water was *ad libitum*. 12 hr light-dark cycle was maintained with 25±2 °C temperature.

Inclusion criteria

Male Wistar rats of 10 weeks age, healthy, and weighing 150–200 g were included.

Exclusion criteria

Female Wistar rats, diseased, sick, and lazy male Wistar rats were strictly excluded.

Animal groups

75 male Wistar rats were randomly divided into the control and 4 experimental groups;

Group A (n=15): Normal healthy rats taken as controls. 0.9% Normal saline
Group B (n=15): Streptozocin induced Diabetic rats
Group C (n=15): Diabetic rats + Metformin (100 mg/kg bwt) (11) daily for 28 days
Group D (n=15): Diabetic rats + Metformin (100 mg/kg bwt) + BBR (50 mg/kg bwt)[12] daily for 28 days
Group E (n=15): Diabetic rats + Metformin (100 mg/kg bwt)[11]+ BBR (100 mg/kg bwt)[12] daily for 28 days.

Induction of diabetes mellitus

Streptozotocin (STZ) in citrate buffer (pH 4.5) at a dose of 45 mg/kg body weight (mg/kg bwt) was injected subcutaneously to 12 hr fasting rats. 48 hr were lapsed for stabilization of blood glucose. Rats which showed elevated fasting blood glucose (>250 mg/dl) were included (11).

Chemicals

Metformin was purchased from the Merck Pharmaceutical Company. Berberine hydrochloride was purchased through the pharmacy department of the institute. Tweens 80 was used along with sterile water for injection of the prepared formulation.

Blood sampling

Blood samples were collected 18 hr after the last dose of metformin and BBR. Blood was collected by cardiac puncture. Each sample was collected in a gel tube (without anti-coagulant) and incubated for 15 min at room temperature. Disposable syringe (BD, USA) was used for cardiac puncture. Blood was centrifuged at 3000 *rpm* for 15 min. Clear sera were stored at -20°C for biochemical analysis.

Biochemical testing

Blood glucose- fasting and random, HbA1c, fasting insulin, serum bilirubin, liver enzymes, serum lactate, and serum creatinine were detected on a Roche Biochemical analyzer (Cobas e 411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Homeostasis model assessment (HOMA) was calculated as HOMA insulin resistance (HOMA-IR) = fasting insulin × fasting glucose/22.5 (13, 14).

Ethical approval

Ethical approval was taken before the study was conducted. Performa was designed for data collection.

Statistical analysis

Statistix 10.0 (USA) software was used for data analysis. One way analysis of variance with descriptive statistics and *post hoc* Bonferroni test were used for data analysis. Results were presented as mean± SD. Statistical analysis was performed at 95% confidence interval ($P \leq 0.05$).

Results

The present experimental study was conducted with a sample of 75 Wistar male albino rats. Group A comprised of control rats. Experimental groups (streptozocin-treated) included B, C, D, and E. Blood glucose, glycated HbA1c, serum lactate, fasting blood glucose, fasting insulin, and HOMA-IR (%) showed positive results in the BBR treated animals.

Serum lactic acid was reduced by co-administration of BBR in metformin-treated animals. Metformin (group C), metformin + BBR 50 mg/kg bwt (group D), and metformin + BBR 100 mg/kg bwt (group E), revealed serum lactate as 1.87±0.4 mmol/l, 1.62±0.44 mmol/l and 1.47±0.45 mmol/l, respectively (Table 1) (P= 0.0001). Fasting glucose, fasting insulin and HOMA-IR model showed amelioration in BBR treated rats. Insulin resistance was decreased in high dose BBR treated rats. Liver aminotransferase (ALT, AST), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), ϒ-glutamyl transferase (ϒ-GT), serum bilirubin, and serum creatinine also revealed statistically significant amelioration in BBR treated animals (Table 2).

Table 1. Blood glucose, serum lactate, and insulin resistance in controls and experimental rats (n=75)

Parameters	Group A Controls	Group B Diabetics	Group C Diabetics+ Met	Group D Diabetics+ Met+ BBR (low dose)	Group E Diabetics+ Met+ BBR (high dose)	*P*-value
Blood glucose (R) (mg/dl)	132.3±14.0	348.1±55.4	290.4±78.0	244.1±67.8	209.3±71.6 †	0.0001
Glycated HbA1 (%)	4.9±0.8	11.9±1.2 †	11.0±1.2	10.3±1.6 †	10.2±1.7	0.001
Serum lactate (mmol/l)	0.63±0.14	1.20±0.44	1.87±0.40	1.62±0.44	1.47±0.45	0.049
Fasting glucose (mg/dl)	106.0±12.4	190.9±35.5	171.7±41.9 †	160.7±38.2 †	146.5±35.4	0.019
Fasting Insulin (µU/l)	19.9±4.4	32.0±1.9 †	30.1±4.2 †	26.7±7.8	22.3±9.0	0.0001
HOMA-IR (%)	2.1±0.3	4.7±0.4	4.4±0.8	3.8±1.2	3.2±1.4	0.035

† *P* >0.05; Met: metformin; BBR: Berberine

Table 2. Liver enzymes, serum bilirubin, and serum creatinine in controls and experimental rats (n=75)

Parameters	Group A Controls	Group B Diabetics	Group C Diabetics+ Met	Group D Diabetics+ Met+ BBR (low dose)	Group E Diabetics+ Met+ BBR (high dose)	*P*-value
ALT (U/l)	34.8±7.8	68.8±15.4	65.6±12.5	58.9±9.1	52.1±6.7	0.0001
AST (U/l)	27.5±4.1	50.3±11.5	38.1±12.6 †	33.2±5.0 †	31.8±4.6	0.009
ALP (U/l)	75.9±17.0	144.9±35.0	114.7±47.0	105.6±34.6	93.3±33.8	0.0293
LDH (U/l)	111.3±18.2	173.1±26.9	154.7±35.1	133.7±28.4 †	121.5±7.8	0.001
GGT (U/l)	35.5±6.3	78.9±5.3	57.7±22.9	55.3±22.1	46.2±20.0	0.0291
S. bilirubin (mg/dl)	0.6±0.1	1.5±0.2	1.4±0.3	1.3±0.4	1.2±0.3	0.001
S. creatinine (mg/dl)	0.8±0.1	1.4±0.3	1.1±0.2	1.0±0.3	0.9±0.2	0.001

† *P* >0.05; Met: metformin; BBR: Berberine

Discussion

The present study demonstrates for the first time that BBR protects against MALA. The exact role of metformin in the lactic acidosis is not understood. The Cochrane group (15) comparative outcomes study of metformin intervention versus the conventional approach (COSMIC) study, (16) and the United Kingdom prospective diabetes study (17) have disputed the existence of lactic acidosis in the presence of metformin and hence the term metformin-induced lactic acidosis has subsequently been changed to MALA. Metformin reduces pyruvate dehydrogenase activity and mitochondrial transport of reducing agents, and thus enhances anaerobic metabolism. This shift to anaerobic metabolism, in the presence of reduced insulin, increases production of precursors of the Kreb`s cycle (15-17).

The fasting glucose, fasting insulin, and HOMA-IR model showed improvement with a decrease in Insulin resistance by BBR. A significant decrease in liver aminotransferase, ALP, LDH, YGT, and serum bilirubin proves the hepatoprotective effect of BBR.

Anti-diabetic activity of BBR has been suggested by previous studies, (18-20) A clinical study reported BBR exerts glycemic regulatory potential in T2DM. Three months therapy with BBR decreased fasting blood glucose from 11.6 to 6.6 mmol/L, as reported by the previous study (18). A previous study used 0.3–0.5 g of BBR thrice a day and reported a decrease in fasting and random blood glucose by 21% and 27%, respectively (19). Yet another previous study[20] reported total cholesterol reduction by 23%, triglyceride by 40% and fasting blood glucose by 31% with 500 mg of BBR three times a day in T2DM.

MALA is a medical emergency which carries 50% mortality rate, (2, 21) hence there is a need to search for new remedies to overcome the problem. Some of the previous studies concluded that MALA is not life threatening in the long term, (21) while other studies suggested MALA patients must be treated by dialysis to prevent morbidity and mortality (22, 23). However, it is an established fact that MALA is rare but life threatening (24). Within 2 years of clinical use of metformin in the USA, 2-9 cases of MALA per 100,000 were reported with mortality rate as high as 50% (25). Pathophysiology is not understood and remains to be elucidated (26). One suggested mechanism is interference with liver lactate clearance by metformin through inhibition of complex I of the respiratory chain (25, 27). Later on it was reported that lactic acidosis was associated with mitochondrial dysfunctions through inhibition of mitochondrial protein synthesis (28). Thus above scientific investigations and interpretations point towards in-depth studies on the effects of the metformin.

Surely, the present is the first report on the investigation of combined effects of BBR and metformin in Streptozocin-induced diabetes in which the serum lactate was found low in BBR treated rats. Together with the lactic acidosis, it was also thought essential to investigate the other important biochemical parameters to correlate the functioning of the vital organs like liver (liver transaminase) and kidney (serum creatinine) in diabetic rats.

Hence the present study was conducted to analyze the effect of Metformin and BBR combination on different biochemical parameters with special reference to serum lactate. Diabetic rats were used in this study to resemble the likely pathophysiological condition in humans; use of Metformin in diabetes will benefit the pharmacodynamic effects of Metformin (29). A 28-day diabetic rat experimental model was designed on the basis of the reports where metformin induces lactic acidosis from the first week of therapy (30).

The magnitude of increase in the levels of hepatocyte markers like ALT and AST is well correlated with abnormal liver function associated with diabetes (31, 32). These findings of the present study may be important in a metformin-associated toxicological scenario. In the present study, diabetes was induced first and metformin treatment was started afterward to mimic the human pathophysiology of DM so that changes associated with Metformin or its combinations with BBR would be closer to the changes which can be correlated in humans. The significant decrease in lactic acid in diabetic animals which were co-treated with Metformin+BBR demonstrates the potential additive effects. This is a marker for BBR potential to mitigate the lactate levels in Metformin exposed animals probably by altering the mitochondrial pathways as reported previously (27). Hence, the present study postulates that BBR combined with metformin most probably will be a good combination in preventing mitochondrial dysfunction.

Metformin toxicity study in healthy rats (33, 34) revealed no changes in renal function, which supports our present study as serum creatinine levels were within normal limits. Serum creatinine is a reliable marker of renal function. In the present study, BBR and metformin showed nephroprotective and hepatoprotective effects. The finding on metformin is in agreement with previous studies (33, 34). Metformin alone treated rats also revealed a significant decrease in serum ALT and AST levels compared to the diabetic control. The decreasing trend in liver aminotransferase by co-administration of BBR and metformin may be interpreted as having hepatoprotection offered by both drugs. The present study postulates that a) BBR might have a direct antagonistic effect against metformin, b) BBR might antagonize the poisoning effects of metformin on the pyruvate dehydrogenase enzyme and complex I of respiratory chain, c) BBR may accelerate the lactate oxidation by mitochondria, and d) The most important fact noted in present study was an improvement in fasting insulin induced by BBR. Insulin accelerates the oxidation of serum lactate by mitochondria.

Conclusion

The present study reports BBR protects against MALA in streptozocin-induced DM. It is concluded that the augmented fasting insulin induced by BBR, might have accelerated the oxidation of serum lactate by mitochondria thus lowering its serum levels. Improved serum insulin brings a balance between lactate oxidation by mitochondria and glycolysis pathway.

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