JOURNAL OF APPLIED TOXICOLOGY, VOL. **17**(6), 409–413 (1997)

Influence of Atenolol and/or Metformin on Glutathione and Magnesium levels in Diabetic Rats

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Key words: metformin; atenolol; glutathione; magnesium; diabetes.

Recently there has been growing interest in studying the differences between different classes of antihypertensive drugs in preventing cardiovascular events in diabetic patients. Hypomagnesemia is common in diabetes mellitus, and correlates to its chronic complications and the associated alteration of the antioxidant enzyme activity. Depletion of reduced glutathione (GSH) in the blood has been demonstrated with myocardial injuries associating hypomagnesemia. A previous study has demonstrated a beneficial effect of metformin hydrochloride (Met), an antihyperglycemic drug, on both magnesium (Mg) and GSH levels in diabetic animals. The purpose of this study was to investigate the effect of oral atenolol, metformin (50 and 60 mg kg^{-1} day^{-1}, respectively) and their combination for 14 days on Mg and GSH levels in blood, liver and heart of diabetic male Wistar rats, as these two parameters have been shown to be altered in diabetics and linked to myocardial ischemic injuries. The results of this investigation showed a state of low levels of Mg and GSH in both blood and liver of the diabetic animals. Treatment with atenolol alone did not change these levels significantly, however administration of metformin or atenolol/metformin increased significantly the GSH levels in both liver and blood, and returned the liver Mg content back to normal values. © 1997 by John Wiley & Sons, Ltd. *J. Appl. Toxicol.*, Vol. 17(6) 409–413 (1997)

(No of Figures: 0. No of Tables: 4. No of References: 32)

INTRODUCTION

Diabetes mellitus and hypertension frequently coexist, and in many practices may be the most commonly encountered combination of chronic diseases. Hypertension is clearly a central factor in diabetic heart disease, and the risk of cardiovascular deaths approximately doubles when diabetes is complicated by hypertension.[1] Assessment of cardiovascular risk in the diabetics has to take into account the fact that both elevated blood pressure and diabetic mellitus are known independently to increase the likelihood of cardiovascular diseases.[2] Recently there has been growing interest in insulin resistance and its relationship to hypertension.[3]

The American Diabetes Association sponsored a Consensus Development Conference in May 1993, to emphasize the importance of early detection and management of hypertension in diabetics. They demonstrated that diabetic patients, as a group, are more prone to the side-effects of antihypertensive medication. Furthermore, research is recommended to establish the differences between different classes of antihypertensive drugs in preventing cardiovascular events in diabetic patients.[4]

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Reduced glutathione (GSH), an antioxidant agent, is synthesized mainly in the liver.[5] Its importance in the control of the development of free radicals has been demonstrated.[6] Depletion of GSH increases the susceptibility of animals against toxins and oxidative stress, and the role of active oxygen-derived radicals in oxidative damage of myocardial ischemic injury is most likely established.[7] Marked depletion in GSH concentrations has been reported in diabetics.[8]

Magnesium (Mg), the second most abundant intracellular element, plays a key role in cellular metabolism. It is essential for many steps in glycolysis and is an obligatory co-factor in the enzyme reactions of GSH synthesis.[9,10] Hypomagnesemia has been reported in diabetic patients.[11] The correlation between Mg deficiency and various cardiovascular diseases, such as coronary artery diseases, hypertension and cardiac arrhythmia, has become well established.[12,13] Recent findings have implicated a role for increased oxidative stress during magnesium deficiency-induced injury.[14] Such changes improved with various drugs, such as vitamin E, propranolol and probucol, with a suggestion of the possible role of free radical participation in this model.[15–17]

Metformin (Met), 'a biguanide antihyperglycemic drug', is effective in the treatment of hyperglycemic obese and non-obese non-insulin-dependent diabetics (NIDD). It has no blood sugar lowering effect in non-diabetic subjects, and has been shown to reduce blood pressure in hypertensive patients.[18] The main mechanism of action of Met to achieve decreased glycemia

CCC 0260–437X/97/060409–05

Received 8 April 1996
Accepted (revised) 22 July 1997

in NIDD subjects is attributed to an enhancement of insulin action, possibly due in part to increased insulin receptor binding, but more importantly through an effect at the post-receptor level.[19,20] A previous investigation showed that Met has a beneficial effect on magnesium and GSH levels in diabetic rats.[21]

The cardioprotective effect of beta-blockers may be attributable to their antihypertension, antiarrhythmic and antiplatelet actions. Atenolol (Atn) is a beta-blocker, classified as cardioselective (β_1-selective), and is reported to lack intrinsic sympathomimetic activity (ISA). Beta-blockers without ISA have been shown to reduce cardiovascular morbidity and mortality. Atenolol is currently employed by diabetic patients in the management of hypertension, ischemic heart disease and tachycardia.[22,23] Its effect on Mg and GSH levels is not well studied.

The present study was designed to investigate whether Atn treatment can modify the concentrations of GSH and Mg in experimental diabetic animals, as these two parameters have been shown to be altered in diabetics and linked to myocardial ischemic injuries.[7,8,12] The influence of concurrent administration of Met with Atn has been studied in this investigation as Met could improve GSH, Mg and glucose levels in diabetic animals, as reported.[21] This may explain in part the Atn cardioprotective action and may help to achieve a more wise prescribing of these two agents in diabetics.

MATERIAL AND METHODS

Animals and treatment

Male Wistar rats (270 ± 40 g) were purchased from Charles River Breeding Laboratory and were kept in an environmentally controlled room. The temperature was maintained at 23–25°C and 50–55% relative humidity and the light was set on a 12-h light/dark cycle. Water and food were available *ad libitum*. The animals were randomly divided into four groups of five rats each and were under observation for 1 week. Then diabetes was induced by the administration of a single dose (60 mg kg^{-1} i.p.) of freshly prepared streptozotocin (STZ) (Sigma Chemical Company, St Louis, MO) 48 h prior to treatment. During the experimental period, the animals were monitored by periodic testing for fasting serum glucose, and those with levels < 8.5 mmol l^{-1} were considered non-diabetics. In all groups, the drugs were administered orally by gavage daily for 14 days and fasted overnight before the blood was withdrawn. The control groups received deionized water and other groups were treated with Atn, Met (50 and 60 mg kg^{-1} day^{-1}, respectively), and their combination. In this study Wistar rats were preferred because they are characterized by a narrow spectrum of normal blood sugar,[24] and STZ was used because it seems to be ineffective on GSH levels.[8]

Body and organ weight ratio

At the end of the experiment the body weight was recorded. The animals were then sacrificed and their hearts and livers immediately removed and weighed, and the organ/body weight ratios were calculated.

Chemical analysis

Reagents of analytical grade were obtained from Fisher Scientific and Sigma Chemical Company. The concentrations of Mg were measured in serum and tissues by a Varian atomic absorption spectrophotometer, using an air–acetylene flame.[25] The GSH concentrations in blood and tissues were measured using DTNB (5,5′-dithiobis-2-nitrobenzoic acid), as described.[26] Fasting serum glucose was monitored colorimetrically using a Sigma diagnostic kit.

Statistical analysis

Results were expressed as means ± SEM. Significant differences between the groups were determined by one-way analysis of variance (ANOVA) with Scheffe's test at a significance of $P < 0.05$.

RESULTS

Fasting serum glucose and body and organ weights

The effect of Atn, Met or their combination on fasting serum glucose is shown in Table 1. During the experimental period, the animals were monitored by periodic testing for fasting serum glucose, and those with levels < 8.5 mmol l^{-1} were considered non-diabetics. A significant increased serum glucose level was observed after 48 h of induction of diabetes. Treatment with Atn alone did not affect this level during the time studied. Monitoring the glucose level in the Met-treated group revealed that the serum glucose was without any significant change 7 days after the treatment compared to the positive control group. After 14 days of treatment, although the serum glucose was still significantly higher than the normal control, the values were significantly less than the positive control group. When both drugs were used in combination, Atn maintained the blood sugar-lowering effect of Met.

Body and organ weights

Diabetic animals, in all diabetic groups, had a lower mean body weight compared with the normal group. Heart wet weight did not show any changes in any of the groups, while liver wet weight in all diabetic groups was lower than that of the normal rats. The heart/body and liver/body weight ratios were without any significant changes compared to the control (Table 2).

Magnesium levels in serum, liver and heart

Table 3 shows the effect of Atn, Met or Atn/Met on serum, liver and heart magnesium levels. Serum magnesium was decreased significantly in diabetic rats. There were no significant differences in serum Mg between the three treated groups. Liver magnesium

Table 1. Effect of atenolol and/or metformin on serum glucose[a]

	Normal control	Diabetic			
		+ve Control	Atn	Met	Atn/Met
Day 0	7.47 ± 0.13	21.82 ± 0.82[b]	21.7 ± 0.84[b]	23.9 ± 0.6[b]	22.12 ± 0.8[b]
Day 7	7.38 ± 0.09	21.76 ± 0.90[b]	20.9 ± 0.6[b]	19.22 ± 0.8[b]	19.2 ± 0.5[b]
Day 14	7.43 ± 0.05	21.7 ± 0.51[b]	20.8 ± 0.8[b]	16.8 ± 0.6[b,c]	15.3 ± 0.23[b,c]

[a] Values are mean ± SEM (mmol l^{-1}) from five fasting male Wistar rats per group after oral treatment (mg/kg^{-1} day^{-1}) with Atn (50) and or/Met (60) for 14 days, and sacrificed 24 h after the last treatment. Diabetes was induced by a single dose of STZ (60 mg kg^{-1} i.p.) 48 h prior to treatment.
[b] Significantly different compared to the normal control group, ANOVA ($P < 0.05$).
[c] Significantly different compared to the +ve control group, ANOVA ($P < 0.05$).

Table 2. Effect of atenolol and/or metformin on organ/body weight ratio[a]

	Normal control	Diabetic			
		+ve Control	Atn	Met	Atn/Met
Body wt. (g)	305 ± 2.03	234 ± 2.8[b]	235.8 ± 3.6[b]	239 ± 2.1[b]	136.6 ± 3.2[b]
Liver wt. (g)	13.6 ± 0.1	9.5 ± 0.2[b]	10.92 ± 0.08[b]	10.9 ± 0.08[b]	10.92 ± 0.09[b]
Heart wt. (g)	0.86 ± 0.06	0.85 ± 0.01	0.78 ± 0.01	0.81 ± 0.007	0.8 ± 0.01
Organ/body wt.					
Liver	4.44 ± 0.05	4.12 ± 0.13	4.64 ± 0.05	4.6 ± 0.07	4.62 ± 0.07
Heart	0.33 ± 0.002	0.36 ± 0.003	0.33 ± 0.004	0.34 ± 0.002	0.34 ± 0.003

[a] Values are mean ± SEM from fasting five male Wistar rats per group after oral treatment (mg kg^{-1} day^{-1}) with Atn (50) and or/Met (60) for 14 days, and sacrificed 24 h after the last treatment. Diabetes was induced by a single dose of STZ (60 mg kg^{-1} i.p.) 48 h prior to treatment.
[b] Significantly different compared to the normal control group, ANOVA ($P < 0.05$).

Table 3. Effect of atenolol and/or metformin on magnesium in diabetic rats[a]

	Normal control	Diabetic			
		+ve Control	Atn	Met	Atn/Met
Serum (mmol/l^{-1})	0.84 ± 0.06	0.66 ± 0.06[b]	0.66 ± 0.06[b]	0.69 ± 0.06[b]	0.68 ± 0.04[b]
Liver (mmol/kg^{-1} wet wt.)	0.93 ± 0.04	0.66 ± 0.02[b]	0.77 ± 0.02	0.92 ± 0.03[c]	0.95 ± 0.03[c]
Heart (mmol kg^{-1} wet wt.)	1.18 ± 0.03	1.17 ± 0.02	1.14 ± 0.02	1.13 ± 0.02	1.14 ± 0.02

[a] Values are mean ± SEM from five fasting male Wistar rats per group after oral treatment (mg kg^{-1} day^{-1}) with Atn (50) and or/Met (60) for 14 days, and sacrificed 24 h after the last treatment. Diabetes was induced by a single dose of STZ (60 mg kg^{-1} i.p.) 48 h prior to treatment.
[b] Significantly different compared to the normal control group, ANOVA ($P < 0.05$).
[c] Significantly different compared to the +ve control group, ANOVA ($P < 0.05$).

concentration was significantly decreased in diabetic rats. Atenolol treatment slightly, but not significantly, increased this level compared to the positive control group, whereas treatment with Met and Atn/Met increased liver Mg content significantly and returned the values back to normal levels. No significant changes in heart Mg content could be detected in all diabetic groups compared to the normal control group.

Levels of GSH in blood, liver and heart

The changes in the GSH levels in rat blood, liver and heart are presented in Table 4. After 14 days of induction of diabetes, a significant decrease in blood and liver GSH levels was observed. Treatment with Atn alone did not significantly change these values compared to the positive control group. On the other

Table 4. Effect of atenolol and/or metformin on glutathione in diabetic rats[a]

	Normal control	Diabetic			
		+ve Control	Atn	Met	Atn/Met
Serum (mg %)	41.3 ± 0.7	25.76 ± 1.1[b]	27.3 ± 1.2[b]	35.2 ± 1.17[c]	36.95 ± 0.35[c]
Liver (μmol/g^{-1})	6.72 ± 0.25	4.27 ± 0.2[b]	4.3 ± 0.14[b]	5.68 ± 0.06[c,d]	5.68 ± 0.07[c,d]
Heart (μmol g^{-1})	1.16 ± 0.03	1.18 ± 0.02	1.18 ± 0.02	1.17 ± 0.01	1.17 ± 0.01

[a] Values are mean ± SEM from five fasting male Wistar rats per group after oral treatment (mg kg^{-1} day^{-1}) with Atn (50) and or/Met (60) for 14 days, and sacrificed 24 h after the last treatment. Diabetes was induced by a single dose of STZ (60 mg kg^{-1} i.p.) 48 h prior to treatment.
[b] Significantly different compared to the normal control group, ANOVA ($P < 0.05$).
[c] Significantly different compared to the +ve control group, ANOVA ($P < 0.05$).
[d] Significantly different compared to the Atn-treated group, ANOVA ($P < 0.05$).

hand, administration of Met alone increased significantly the blood GSH level compared to the positive control group and significantly increased the liver GSH contents compared to both the positive control and Atn-treated groups. Administration of Atn and Met in combination achieved the same effect as Met alone. The GSH content of the heart was without any significant changes in any of the groups.

DISCUSSION

The exact relationship between the degree of glycemic control and development of cardiovascular complications in NIDD patients has continued to be controversial, and any treatment intended to improve glycemic control might be beneficial for other risk factors for cardiovascular diseases.[27,28] Metformin has been shown to be a possible candidate for such therapeutic potential. It decreases blood glucose and improves insulin resistance, lipid profile and hypertension in diabetics.[18–20] Optimal management of hypertension for the prevention of coronary artery diseases requires more than normalization of the blood pressure if the hazard of coronary events is to be reduced. Beta-blockers have undesirable effects on glucose and lipid metabolism. It is important to balance against these disadvantages the positive benefits that beta-blockers have, therefore Atn is currently employed by diabetic patients in the management of hypertension and ischemic heart diseases.[22,23] The potential to combine Atn and Met to obtain a more cardioprotective effect may be a potentially important therapy.

In this report, induction of diabetes by STZ was confirmed by periodic testing of fasting serum glucose. Treatment with Atn alone did not affect the high serum glucose levels in the diabetic animals. On the other hand, treatment with Met for 14 days reduced serum glucose significantly, whereas it did not reverse the values completely to the normal levels. Concurrent administration of Atn with Met kept the benefit of Met alone without any changes. This is in accordance with other investigators who reported that Atn did not augment the blood glucose in the diabetics, unlike some other beta-blockers such as propranolol, which impairs glucose tolerance.[29]

In recent years, there has been growing interest in Mg deficiency and its correlation with cardiovascular diseases, hypertension, insulin resistance and free-radical tissue defense mechanism. Diabetes mellitus, hypertension and hypokalemia are now considered to be risk factors of hypomagnesemia.[9,11] The results of this study are in agreement with others who demonstrated a state of hypomagnesemia in diabetics.[30] Atenolol unlike non-cardioselective beta-blockers, neither impairs glucose tolerance nor reduces potassium concentrations in diabetics.[29] This may be linked to our findings that treatment with Atn alone did not alter blood Mg levels in the diabetic animals.

It has been demonstrated that diabetes mellitus is associated with a reduced Mg liver concentration,[10] which is in accordance with the results of this report. This may be linked with the fact that the intracellular hepatocyte Mg level is quite sensitive to the alteration in total Mg content, as reported by other investigators.[30] Treatment with Atn alone slightly, but not significantly, increased this level, while the administration of Met or Atn/Met induced a significant increase in the hepatic concentration of Mg, but not in blood. This may be attributed to a Met–insulin-like action, as suggested by Nordin and Baily.[10,31] They demonstrated that after control of diabetes with insulin therapy, the plasma Mg may be consistently decreased because it is taken up by soft tissues.

Magnesium concentration in the myocardium is resistant to the alteration in total body Mg content. It has been reported that of all the soft tissues, heart uptake of Mg is the most rapid,[30] while it is not readily easy to lose Mg from the heart even in the case of hypomagnesemia. Moreover, it has been demonstrated that the reduction in myocardial protein synthesis in Mg-deficient animals is not due to any local cellular deficiency, but rather should be seen as a response to reduced serum level.[32] These observations may explain our findings of normal concentrations in the heart of both treated and not treated animals.

The significant low levels of GSH in blood and liver of our diabetic animals are in accordance with Saleh *et al.*[8] They demonstrated that these low levels could be corrected by insulin therapy. In this investigation, oral administration of Atn alone did not affect these alterations. On the other hand, treatment with Met or Atn/Met improved the GSH and Mg levels in both

blood and liver. These observations, when taken with the fact that Mg is essential for GSH synthesis, would suggest a possible role for Met–insulin-like action in this process, as reported by Ewis and Abdel-Rahman.[21]

In conclusion, the administration of Atn, although a known protective to the cardiovascular system, did not show any measurable beneficial effect on the altered levels of GSH and Mg in diabetic animals. The observed responses associating the concurrent administration of Met with Atn could be attributed to the treatment with Met by itself. Further investigations are recommended to establish the mechanism of this therapeutic potential.

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