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Enhanced Hypoglycaemic Effects of Metformin by Valsartan in a Diabetic Rat Model

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The objective of this study was to establish the effect of valsartan on the hypoglycaemic effect of metformin. Forty-four 8-week-old Sprague-Dawley rats were fed on a high fat diet or standard diet for six weeks. Diabetes mellitus was induced by administering alloxan monohydrate 120 mg/kg intraperitoneally in rats on high fat diet. Valsartan had no significant effect on blood glucose levels in non-diabetic rats. Treatment of diabetic rats with a combination of metformin and valsartan at 5mg/kg (-10.8±8.5%), 15 mg/kg (-43.0±13.7%), and 30 mg/kg (-28.6±9.1%) for 14 days resulted in significant differences in the change in blood glucose levels compared to treatment with metformin alone (+9.5±9.1%) (F=4.351; d.f.=3;16; p=0.0201). Valsartan enhanced the hypoglycaemic effects of metformin in diabetic rats after two weeks of treatment. However, valsartan did not have significant effect on glucose tolerance in both non-diabetic and diabetic rats.

Key words: hypoglycaemia, valsartan, metformin, diabetes

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are the first-line therapy for patients with comorbid hypertension and diabetes mellitus because of their ability to slow down the decline in renal function [1]. Recently, the glycaemic effect of these drugs has been the subject of many studies [2-8]. Furthermore, ARBs and ACEIs have been shown to decrease the frequency of metabolic syndrome in patients with type 2 diabetes [9]. However, most studies have focused on the effect and mechanism of action of angiotensin receptor blockers (ARBs) on blood glucose levels.

Telmisartan has been shown to have agonistic activity on the peroxisome proliferator-activator receptor, PPAR-γ [10]. Similarly, irbesartan is a partial agonist at the PPAR-γ that has been observed to alleviate metabolic disorders in animal studies [10]. The glucose transport activity of ARBs may be independent of angiotensin 2 receptor blockade [11]. Irbesartan causes a significant increase in glucose transport and GLUT-4 translocation while valsartan has no effect on either glucose uptake or GLUT-4 translocation [12]. Current evidence from both animal models and clinical studies suggests that ARBs are insulin sensitizers [3,11,13]. However, only a few studies have investigated the influence of different ARBs on the efficacy of oral anti-diabetics.

In a study by Salama and colleagues, metformin showed higher blood glucose lowering effects when combined with telmisartan than when used as monotherapy [14]. In another study, treatment with valsartan for 26 weeks increased glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with impaired glucose metabolism [15]. In contrast, losartan showed no significant effect on the hypoglycaemic activity of glimepiride in a single-dose interaction study [16]. However, a significant change in blood glucose was observed during multi-dosing of the diabetic rats in the same study. This suggests that the interaction between an ARB and an oral antidiabetic agent may be more pronounced with time. However, no study has empirically demonstrated that the apparent synergistic effect of ARBs on oral antidiabetic agents is pronounced with continued co-administration of the two classes of drugs.

The hypoglycaemic effects of valsartan are well established [4,7,17-19]. Similarly, valsartan is postulated to improve the

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metabolism of glucose by enhancing the peripheral insulin sensitivity [4,9]. However, no studies have investigated the interaction between valsartan and oral anti-diabetics in humans or animal models. Given that ARBs have some structural differences which may influence their pharmacokinetic and pharmacodynamics properties, a class effect of ARBs on the hypoglycaemic efficacy of oral antidiabetic agents cannot be concluded based on results obtained from studies of telmisartan and losartan. Furthermore, it is important to establish whether the interaction between valsartan and oral antidiabetic agents is dose-dependent. The objective of this study was to determine the effect of valsartan on the hypoglycaemic effect of metformin using a rat model. Metformin was chosen because it is currently a first-line agent in obese patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Materials

Forty-four male Sprague-Dawley rats were obtained from the University of Zimbabwe Animal House. Valsartan tablets (Norvartis Pharmaceuticals, Johannesburg, South Africa), metformin tablets (Remedica Ltd, Limassol, Cyprus), normal saline, dextrose, glucose powder, glucometer, and glucometer strips were sourced from a licensed local pharmaceutical wholesaler. Alloxan monohydrate was obtained from Sigma Chemicals (Darmstadt, Germany). High fat diet (19.4% maize germ meal high starch, 12.0% wheat feed, 65.7% soya full fat, 1.2% limestone flour bimco, 1.4% monocalcium phosphate, 0.3% salt-fine, 0.05% premix) and standard diet (28.5% proteins, 13.5% fats (ether extract), 58.0% carbohydrates) were obtained from National Foods Company Pvt. Ltd. (Harare, Zimbabwe).

Animal husbandry

The rats were kept in cages (six rats per cage) and fed on pelleted food. The animals were allowed free access to fresh water *ad libitum.* The rats were subjected to a constant daily cycle of 12 h of light and 12 h darkness and controlled temperature (22-24°C) and humidity (50-60 % RH). The study was approved by the Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC/361/14). The animals were handled and treated following the principles outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86-23 Rev. 1985).

Induction of type 2 diabetes

The rats were divided into two groups. The first group with a total of 20 rats was fed a standard diet while the second group with a total of 24 rats was fed a high-fat diet for six weeks. The high fat diet was to ensure induction of obesity and insulin resistance. Diabetes mellitus was induced on 24 overnight-fasted rats using a single intraperitoneal injection of 120 mg/kg body weight of alloxan monohydrate dissolved in 0.9% w/v saline solution [20]. The rats were kept for the next three days on 5% glucose solution, in their cages, to combat early phase hypoglycaemia [21,22]. Three days after alloxan monohydrate injection, blood samples were drawn from the tail veins of the overnight fasted animals and fasting glucose levels were determined to confirm the development of diabetes (i.e., above 7.8 mmol/l [>140 mg/dl]) [22].

Drug administration

Table 1 shows a summary of the experimental groups. The drugs were administered by oral gavage. Suspensions of each drug were prepared everyday using distilled water and the doses of each rat were calculated using a United States Food and Drug Administration (US FDA) approved formula: Animal dose = Clinical human dose × conversion factor for rats (6.2) [23].

Drug administration was continued for 15 days once daily and fasting blood glucose measured on days 1, 8 and 15 after diabetic induction. Blood glucose levels were determined using a glucometer by collecting blood samples through tail tipping technique. Blood samples were collected on the specified days after an overnight fast before the next doses of the drugs were administered.

Table 1: Experimental groups used in the study

Group	Diet	Drug treatment	Key
1	Standard diet	Valsartan 5mg/kg/day	Val5+SD
2	Standard diet	Valsartan 15mg/kg/day	Val15+SD
3	Standard diet	Valsartan 30mg/kg/day	Val30+SD
4	High fat diet	Distilled water + Metformin 50mg/kg	Met Control
5	High fat diet	Valsartan 5mg/kg/day + Metformin 50mg/kg	Val5+Met+HFD
6	High fat diet	Valsartan 15mg/kg/day + Metformin 50mg/kg	Val15+Met+HFD
7	High fat diet	Valsartan 30mg/kg/day + Metformin 50mg/kg	Val30+Met+HFD

Glucose tolerance test

An Oral Glucose Tolerance Test (OGTT) was carried out on the last day of the experiment. Following a fast, a glucose load was administered at a dose of 1 g/kg and blood glucose was measured over a span of 2 h at 15, 30 and 120 min. The dose chosen was considered appropriate because at higher doses (>2g/kg) the amount of glucose to which the lean tissue is exposed to in an obese rat is disproportionately high compared with that in a non-obese rat with similar lean mass. Consequently, obese rats could be misdiagnosed as being glucose intolerant because they receive more glucose for the same lean body mass [24].

Data analysis

Data were analysed using GraphPad® Prism Version 6.0 (California, USA). Data are reported as mean ± standard error of mean (SEM). The Student's t-test was used to conduct paired comparisons of weight changes and glucose levels during the induction of obesity and diabetes mellitus induction, respectively. One way ANOVA was used to test the differences in the mean percent change between different treatment groups. Mann-Whitney's test and Kruskal-Wallis' tests were used for non-normally distributed data in place of the Student's t-test and one way ANOVA, respectively. Where applicable, Bonferroni's *post-hoc* test was conducted for paired comparisons. The significance level was set at α=0.05.

RESULTS

Induction of diabetes mellitus

Diabetes was successfully induced in 24 rats using a high fat diet and intraperitoneal administration of alloxan monohydrate. Table 2 shows mean weight changes in diabetic and non-diabetic rats. The rats given high fat diet had a higher weight gain (mean=83.8±3.86) compared to those given standard diet (mean=62.6±3.15) [$p<0.001$]. Figure 1 shows a comparison of the blood glucose levels of diabetic rats and non-diabetic rats before the rats were assigned to different treatment groups. The diabetic rats had higher blood glucose level (mean±SE =23.05±1.40) than the non-diabetic rats (mean±SE =5.81±0.17) [$p<0.0001$].

Table 2: Mean weight and percentage changes in rats on different diets

Variable	High fat diet (n=24)	Standard diet (n=20)
Mean(±SD) baseline weight (grams)	148.3±18.8	124.6±16.5
Mean(±SD) weight after 6 weeks (grams)	268.7±21.4	200.62±15.8
Mean(±SD) % weight change	83.8±27.6	62.63±14.1

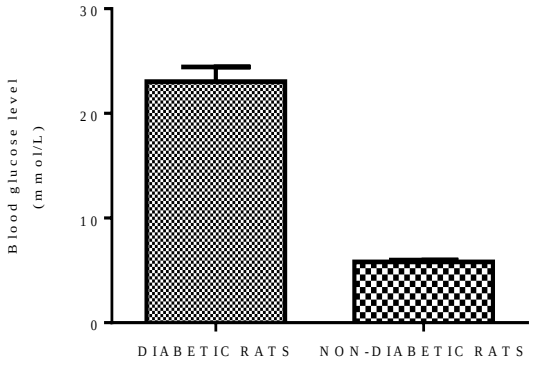


Figure 1. Comparison of blood glucose levels in diabetic and non-diabetic rats before treatment.

Effect of valsartan in non-diabetic rats

Figure 2 and Table 3 show the percentage change in blood glucose levels in non-diabetic and diabetic rats from baseline to day 8 and day 15, respectively. Elevated blood glucose levels were observed on the 8th day of treatment with low, medium, and high dose valsartan. However, there were no significant dose-relationship was observed on the effect of valsartan on blood glucose levels in non-diabetic rats (F=3.021; d.f.=2;17; p=0.0754). In contrast, continued treatment with valsartan for fifteen days resulted in reduction in blood glucose levels. However, the decrease in blood glucose levels was not significantly different in the three doses (F=1.450; d.f.= 2.17; p=0.2623).

Effects of valsartan on the hypoglycaemic effect of metformin

Figure 2 and Table 3 show the percentage change in blood glucose levels on day 8 and day 15, in diabetic rats treated with metformin alone and in combination with varying doses of valsartan, respectively. On day 8, all the four treatment groups showed a decrease in blood glucose levels. The group treated with medium dose valsartan (15 mg/kg) plus metformin (50 mg/kg) recorded the highest decrease in blood glucose (mean=-39.7%; SE=15.5; median=-54.4%), followed by the group treated with high dose valsartan (30 mg/kg) and metformin (mean=-23.2; SE=10.0; median=-10.9%), and the group treated with low dose valsartan (5 mg/kg) and metformin (mean=-22.3%; SE=7.9; median=-25.1%), and lastly by the group that was treated with metformin monotherapy (mean=-2.2%; SE=8.9; median=-8.3). However, there was no significant interaction between valsartan and metformin on day 8 of treatment (χ^2=2.890; p=0.4088).

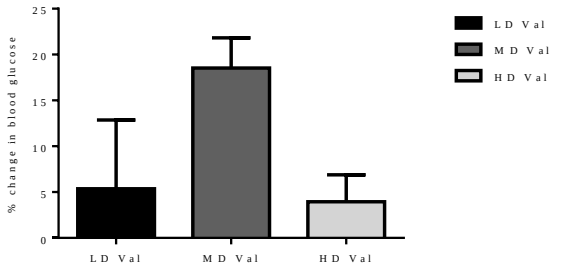
On day 15, the combination of medium dose valsartan and metformin recorded the highest decrease in blood glucose (mean=-43%; SE=13.7; median=-40.2%), followed by the combination of high dose valsartan and metformin (mean=-28.6%; SE=9.1; median=-29.1) and the combination of low dose valsartan and metformin (mean=-10.8%; SE=8.5; median=-10.9%). The metformin group, unlike other groups showed a slight increase in blood glucose in the diabetic rats (mean=+9.5%; SE=9.1; median=4.9%). There was a significant interaction between valsartan and metformin on day 15 (F=4.351; d.f.=3;16; p=0.0201). The decrease in blood glucose was higher in the combination of 15 mg/kg valsartan with metformin (-43%) compared to metformin only (9.5%) (p=0.0114). However, there was no significant dose-response relationship across the three doses of valsartan on both day 8 (F=3.021; d.f.=2;17; p=0.0754) and day 15 (F=1.450; d.f.= 2;17; p=0.2713) of treatment.

Oral glucose tolerance test

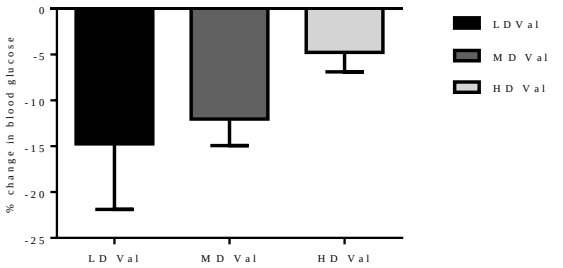
Figure 3 shows the AUCs after a glucose tolerance test in non-diabetic and diabetic rats, respectively. There was no significant difference in the glucose tolerance among non-diabetic rats administered low dose valsartan (5 mg/kg), medium dose valsartan (15 mg/kg) and high dose valsartan (30 mg/kg) (F=2.857; d.f.=2;17; p=0.0852). The AUCs were similar in the three doses of valsartan 5 mg/kg (mean=25.6 mmol/Lmin; SE=0.63), 15 mg/kg (mean=23.11 mmol/Lmin; SE=0.66) and 30 mg/kg (mean=23.12 mmol/Lmin; SE=1.02). There was also no significant difference in the glucose tolerance of diabetic rats administered combination of low dose valsartan (5 mg/kg) with metformin, combination of medium dose valsartan (15 mg/kg) with metformin and combination of high dose valsartan (30 mg/kg) with metformin and metformin with distilled water (F=2.714; d.f.=3;16; p=0.0794). However, the metformin control group had the highest AUC (mean=124.5 mmol/Lmin;

SE=5.0). The combination of medium dose valsartan (15 mg/kg) and metformin had the lowest AUC (mean=71.12 mmol/Lmin; SE=17.40), followed by the combination of high dose valsartan and metformin (mean=81.94 mmol/Lmin; SE=14.63) and lastly the combination of low dose valsartan and metformin (mean=99.5 mmol/Lmin; SE=10.94).

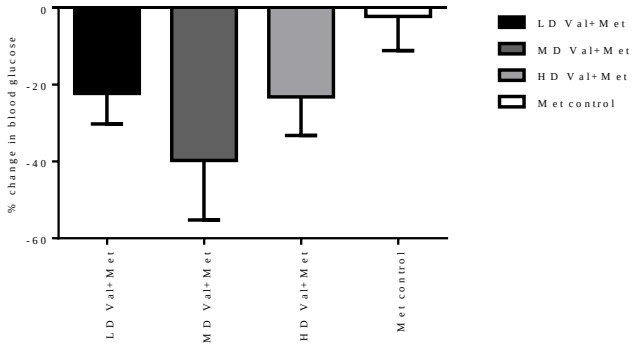
Percentage change in blood glucose in non-diabetic rats: Day 8



Percentage change in blood glucose in non-diabetic rats: Day 15



Percentage change in blood glucose in diabetic rats: Day 8



Percentage change in blood glucose in diabetic rats: Day 15

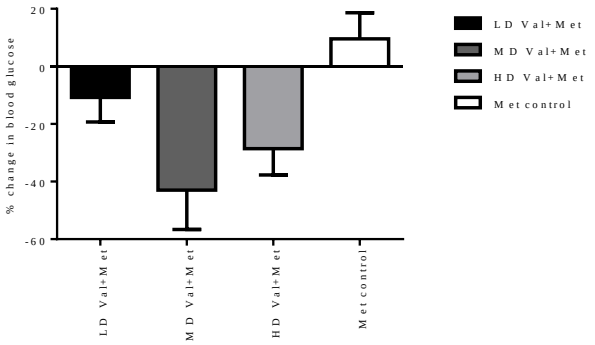


Figure 2. Percentage changes in blood glucose levels from baseline to day 8 and day 15 in in non-diabetic and diabetic rats.

Table 3: Blood glucose levels at baseline, day 8, and day 15

Treatment group	Baseline (mmol/L) Mean±SE	Day 8 (mmol/L) Mean±SE	Day 15 (mmol/L) Mean±SE
Non-diabetic rats			
Valsartan 5mg/kg	6.1±0.5	6.3±0.1	5.0±0.1
Valsartan 15mg/kg	5.5±0.2	6.5±0.1	4.8±0.1
Valsartan 30mg/kg	5.8±0.2	6.0±0.1	5.6±0.2
Diabetic rats			
Valsartan 5mg/kg +metformin 50mg/kg	21.7±2.2	17.3±2.7	19.7±2.8
Valsartan 15mg/kg +metformin 50mg/kg	19.8±2.5	11.6±3.7	11.3±3.6
Valsartan 30mg/kg +metformin 50mg/kg	23.6±3.5	17.9±3.7	17.2±4.2
Metformin 50mg/kg	28.6±1.8	27.6±1.6	30.9±1.5

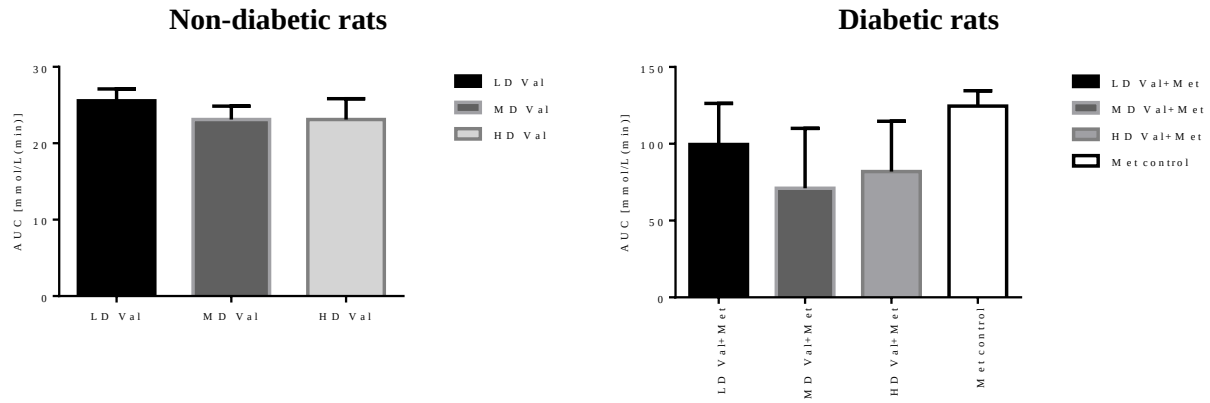


Figure 3. Area under the curve in OGTT of non-diabetic and diabetic rats.

DISCUSSION

The purpose of this study was to assess whether there was any pharmacodynamic interaction between valsartan and metformin. The study also evaluated the effect of the low, medium, and high doses of valsartan on the hypoglycaemic effects of metformin. All the doses of valsartan reduced blood glucose levels after two weeks of treatment in the non-diabetic rats. However, the reduction in blood glucose levels was not statistically significant possibly due to the short period of therapy. In contrast, in a study by Chan *et al*., a significant decrease in blood glucose level was observed after a single intravenous injection of valsartan 0.2 mg/kg was administered to rats [25]. Their findings may suggest that the route of valsartan administration has an effect on blood glucose level. The inhibition of the renin angiotensin system improves glucose tolerance and insulin sensitivity [26]. A retrospective study by Sverre *et al*. on non-diabetic hypertensive patients also showed a significant decrease in blood glucose levels and a decrease in risk of developing diabetes mellitus after valsartan treatment [7]. Similarly, in a study by Top *et al*., non-diabetic hypertensive patients had increased insulin sensitivity after taking 80 mg of valsartan for three months [18].

The hypoglycaemic effects of metformin were enhanced by valsartan in diabetic rats. The enhanced hypoglycaemic effect of metformin may be as a result of a pharmacodynamic interaction as suggested by Antitha *et al*. who observed enhanced hypoglycaemic effects of metformin in combination with statins [27]. A more developed hypothesis regarding the synergistic effect of valsartan on metformin is that it may increase insulin sensitivity [15,28]. The blockade of the renin-angiotensin system by ARBs leads to remodelling of islet beta cells which will improve insulin secretion. This may explain why medium doses of valsartan lowered blood glucose more than high doses in combination with metformin [29]. High doses of valsartan might fatigue the beta cells and this may in turn negatively affect the remodelling of islet beta cells [29]. In the study by Salama *et al*., the combination of metformin and telmisartan in diabetic rats restored blood glucose levels back to normal levels. Telmisartan is also a full PPARγ agonist [10] and has a chemical structure similar to rosiglitazone [14]. These properties may explain the superiority of the combination of telmisartan and metformin to that of valsartan and metformin in reducing blood glucose levels.

In this study, an oral glucose tolerance test was carried out to determine the effect of valsartan 5 mg/kg, 15 mg/kg and 30 mg/kg on insulin sensitivity. Although not statistically significant, valsartan appears to have slightly improved glucose tolerance in diabetic rats during the two weeks of treatment. The high dose valsartan improved glucose tolerance better than low dose valsartan. These findings are consistent with a randomised controlled trial by Zijl *et al*. which observed that 26 weeks of therapy with valsartan 320 mg (equal to 30 mg/kg in rats) increases glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with impaired glucose metabolism [15]. Similarly, these findings are consistent with other studies that reported that low doses of valsartan (1

mg/kg) improved glucose tolerance after 3 weeks of therapy in KK-Ay mice [4,30]. In a study on irbesartan by Henriksen *et al*., a dose-response increase in glucose tolerance and mediated-glucose transport was observed [31]. The difference in the effect of ARBs on insulin sensitivity may be due to a difference in species of animals used and the duration of therapy in the two studies.

CONCLUSION

Valsartan enhanced the hypoglycaemic effects of metformin in diabetic rats after two weeks of treatment. However, valsartan did not have significant effect on glucose tolerance in both non-diabetic and diabetic rats.

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