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Research Article

Effect of Irbesartan on Glucose Tolerance in a Type 2 Diabetes Mellitus Mouse Model

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Background: Angiotensin receptor blockers (ARBs) have been reported to affect glycaemic control in both animals and humans. A few studies have evaluated the effects of irbesartan on blood glucose levels and ARBs are recommended in patients with comorbid diabetes and hypertension. However, the effect of irbesartan on insulin resistance and glucose tolerance is inconclusive and contradicting.

Objectives: To evaluate the effect of irbesartan on blood glucose levels and glucose tolerance in diabetic and non-diabetic mice.

Materials and methods: Diabetes was induced in 18 obese BALB/c mice fed on high fat diet using alloxan monohydrate 150mg/kg via the intra-peritoneal route. Non-diabetic mice were assigned to three treatment groups (n=6/group) and each group received either of the following treatments: 20mg/kg irbesartan or 75mg/kg irbesartan or vehicle. Diabetic mice were also divided into three groups and each group received one of the three treatments mentioned above. Drug administration was done daily via oral gavage for 14 days. Blood glucose levels were measured on day 1 (baseline values), day 8, and day 13 of treatment. An oral glucose tolerance test was carried out on day 14 after administration of 50% dextrose at the dose of 1g/kg body weight. Blood glucose levels at 15, 30, 60 and 120 minutes were measured during the oral glucose tolerance test.

Results: Irbesartan at the dose of 20mg/kg (-39.44% ±8.96, p=0.0177) and 75mg/kg (-40.07% ±6.27, p=0.0111) significantly lowered blood glucose levels in diabetic mice. However, irbesartan 20mg/kg (-14.87% ±10.13, p>0.9999) and 75mg/kg (-9.07%±3.77, p>0.9999) did not significantly change blood glucose levels in non-diabetic mice. In non-diabetic mice there was only a modest difference in AUC in the irbesartan20mg/kg (AUC=28.73mmol/Lmin, p=0.6435), 75mg/kg (AUC=26.66mmol/Lmin, p>0.9999) compared to the non-diabetic control (AUC=26.63 mmol/Lmins). Although there was a slight improvement in glucose tolerance in diabetic mice, irbesartan 20mg/kg (AUC=55.35mmol/Lmin, p>0.9999) and 75mg/kg (AUC=45.54mmol/Lmin, p=0.1737) had no significant effect on glucose tolerance compared to the diabetic control group (AUC=63.53mmol/Lmin).

Conclusion: At standard treatment doses, irbesartan had a significant hypoglycaemic effect without significantly improving glucose tolerance in diabetic mice.

Key words: Irbesartan, glycemic effects, glucose tolerance, Type 2 diabetes, mouse model

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1. Introduction

Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are two major classes of drugs used in the management of hypertension that have similar efficacy(Strippoli et al., 2006).Their renoprotective effects have been observed to be better than other classes of antihypertensive agents (Strippoli et al, 2006, Sarafidis et al, 2008, Strippoli et al, 2005). Recent evidence suggests that the benefit of ARBs and ACEIs in patients with diabetes go beyond their renoprotective effects(Ram, 2011). They have been linked with positive glycaemic effects in

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diabetic mellitus (Sica, 2002, ALLHAT, 2002, Hansson et al, 1999).

Although ACEI and ARBs are now widely considered as hypoglycaemic in nature, there is conflicting evidence regarding their glycaemic effects. Some studies have reported no change in fasting blood glucose levels while other studies suggest that the hypoglycaemic effects are neither a class property nor dose-dependent (Negro et al, 2006, Benson et al, 2004, Munoz et al, 2009, Henriksen et al, 2001). In a study by Negro et al., telmisartan was observed to be superior to irbesartan in decreasing insulin resistance (Negro et al, 2006). Similarly, Benson et al., reported that telmisartan reduces fasting blood glucose levels (Benson et al, 2004). Significant decreases in HbAlc and fasting plasma glucose (FPG) were also observed after 6 months and 12 months treatment with telmisartan (Benson et al, 2004). However, Derosa et al., reported that 12 months treatment with irbesartan resulted in non-significant decreases in FPG and Hb1Ac (Derosa et al, 2006). Similarly, the study by Usuiet al., concluded that fasting plasma glucose and HbA1c were not affected by 6 months of treatment with telmisartan in diabetic patients (Usui et al, 2007).

Experimental studies using animal models have also produced equivocal findings. In a study by Munoz et al., amelioration of insulin resistance in adipose tissue was observed in male obese Zucker rats (fa/fa) treated with 50 mg/kg irbesartan for 6 months (Munoz et al, 2009). Similarly, angiotensin receptor blockade with olmesartan increased pancreatic insulin secretion and decreased glucose intolerance during glucose supplementation in a model of metabolic syndrome in rats(Rodriguez et al., 2012).In contrast, Aritomi et al., reported that use of valsartan alone did not cause a significant decrease in blood glucose levels in rats (Aritomi et al, 2012). However, in the same study significant hypoglycaemic effects were observed when valsartan was combined with cilnidipine (a calcium channel blocker) (Aritomi et al, 2012).

Both clinical and animal studies conducted to date have provided inconsistent evidence regarding the glycaemic effects of angiotensin receptor blockers in patients with co-morbid hypertension and diabetes mellitus. Therefore, there is need for more experimental evidence regarding the glycaemic effects of angiotensin receptor blockers. Irbesartan is a potent and selective angiotensin II subtype 1 receptor antagonist indicated for use in patients with hypertension, including those with type 2 diabetes mellitus and nephropathy (Bramlage et al, 2009, Ruilope and Segura, 2003). The objective of this study was to evaluate the effect of irbesartan on blood glucose control and glucose tolerance in diabetic and non-diabetic mice models.

2. Materials and Methods

2.1 Materials

Irbesartan tablets (Winthrop Pharmaceutics; Midrand, South Africa, Batch number: 3A699), normal saline, 5% dextrose, 50% dextrose, glucose monitoring machine kit where sourced from local suppliers. Standard normal mouse feed and high fat diet were obtained from a local stock feed manufacturer (National Foods Company of Zimbabwe). Alloxan monohydrate was purchased from Sigma Chemicals in Germany (Batch Number. BCBK4716V).

2.2 Study design and induction of Type I diabetes

Thirty six male BALB/c mice were purchased from a regulated breeder (Department of Veterinary Services, Ministry of Agriculture).

The mice were divided into two broad experimental groups. One group was fed on a standard mice diet (n=18) and the other group (n=18) was fed on a high-fat diet. Both groups were fed for six weeks. This was done to ensure induction of obesity (and insulin resistance) in the high fat diet group (Panchal and Brown, 2011). Diabetes mellitus was induced on 18 overnight-fasted obese mice using a single intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate dissolved in 0.9% w/v saline solution(Shankar and Mehendale, 2005). The mice were given 5% glucose solution for the three days post-diabetes induction to combat early phase hypoglycemia (Mishra A et al., 2010).

After three days of the injection, blood samples were drawn from tail vein of overnight fasted animals and fasting glucose levels were determined to confirm the development of diabetes (i.e. above 7mmol/l) (WHO, 2005).

2.3 Drug Administration

Non-diabetic mice were divided into three groups (n=6/group) with each group receiving one of the following treatments: 20mg/kg irbesartan or 75mg/kg irbesartan or vehicle (distilled water).

Diabetic mice were also divided into three groups and each group was treated with one of the following treatments: 20mg/kg irbesartan or 75mg/kg irbesartan or vehicle. The treatments were administered daily via oral gavage for 14 days. Irbesartan tablets were crushed and suspended in distilled water every morning just before administration. Blood glucose levels were measured on day 1(baseline), day 8, and day 13 of irbesartan administration.

2.4 Glucose tolerance test

An Oral Glucose Tolerance Test (OGTT) was carried out on day 14 after commencing irbesartan dosing. Following an overnight fast, a glucose load was administered at a dose of 1g/kg and blood glucose levels were measured over a span of 2 hours (15 minutes, 30 minutes, one hour and 2 hours). A dose of 1g/kg of glucose was chosen because in higher doses (>2g/kg) the amount of glucose to which the lean tissue is exposed in an obese mouse will be disproportionately high compared with that in a non-obese mouse with similar lean mass thus obese mice could be misdiagnosed as being glucose intolerant simply because they receive more glucose for the same lean body mass (Andrikopoulos et al., 2008). Measurement of blood glucose was done using an SD CodeFree™ Blood Glucose Meter (SD Biosensor, Inc Korea). The meter uses the glucose oxidase method to determine glucose levels.

2.5 Statistical Analysis

GraphPad® Prism Version 6.0 (California, USA) software was used for data analysis. Blood glucose levels and area under the curves (AUCs) were compared across the treatment groups using one away ANOVA. AUC is a measure of glucose exposure or rate of glucose utilisation. A high value of AUC represents poor glucose control. AUC was determined by the trapezoidal rule (a numerical integration method used to approximate the integral or the AUC) using GraphPad® Prism Version 6.0 (Shi-Tao. et al, 1991). Where there was statistical significant difference, Bonferonni post-hoc test was used during paired comparisons. The significance level was set at α=0.05.

2.6 Ethical considerations

The study was approved by the Joint Parirenyatwa Hospital and College of Health Sciences Research Committee (JREC), University of Zimbabwe (approval number: **JREC/125/14**). Animals were handled following the principles outlined in the Zimbabwe Animal Experimentation Act of 1963.

3. Results

Table 1 shows the actual blood glucose levels recorded at baseline, on day 8, and day 13 of treatment in both diabetic and non-diabetic mice.

Table 1: Actual blood glucose levels before the dosing on specific days

Experimental group	Baseline (day 1) (mmol/L) Mean±SEM	Day 8 (mmol/L) Mean±SEM	Day 13 (mmol/L) Mean±SEM
Non-diabetic mice			
Control	6.43±0.32	6.18±0.25	6.10±0.10
Irbesartan 20 mg/kg	6.42±0.22	6.77±0.13	5.9±0.21
Irbesartan 75 mg/kg	6.47±0.20	6.77±0.48	6.32±0.22
Diabetic mice			
Negative Control	13.85±3.01	11.25±2.15	12.23±2.58
Irbesartan 20 mg/kg	14.98±2.07	12.80±2.69	9.34±2.48
Irbesartan 75 mg/kg	14.30±2.66	11.25±2.29	9.42±2.58

Effect of irbesartan on blood glucose levels in non-diabetic mice

Figures 1 and **2** show the percentage change from baseline in blood glucose levels in non-diabetic mice on day 8 and day 13, respectively. On day 8, only the control group had a decrease in blood glucose (Mean= -3.62%; SEM=1.18). The other treatment groups showed an increase in blood glucose levels: 20mg/kg (Mean= +6.20%;SEM=4.76), and 75mg/kg (Mean=+5.39%; SEM=8.78). There was no significant difference in the change in blood glucose levels on day 8 in non-diabetic mice that were administered 20mg/kg irbesartan, 75mg/kg irbesartan, and control group ($F=0.8819$; d.f.=2;15; $p=0.4344$). On day 13, all the three groups had reduced blood glucose values. The group administered 20mg/kg irbesartan had the greatest change in blood glucose levels (Mean= -14.87%; SEM=10.13), followed by the group administered 75mg/kg irbesartan (Mean= -9.06%; SEM=3.77) with the control group showing the least change (Mean= -7.33%; SE=1.77). However, there was no significant difference in the change in blood glucose levels between mice that were administered 20mg/kg irbesartan, 75mg/kg irbesartan, and the control group ($F=0.6900$; d.f.=2;15; $p=0.6837$).

Effect of irbesartan on blood glucose levels in diabetic mice

Figure 3 shows the percentage change in blood glucose levels in diabetic mice after 8 days of drug administration. All the groups showed a decrease in blood glucose levels with the group administered 75mg/kg irbesartan (Mean= -22.19%; SEM=4.65) showing the highest decrease followed by the positive control (P/control) group (Mean= -16.99%; SEM=3.10). There was no significant difference in the blood glucose changes between the 20mg/kg irbesartan group, 75mg/kg irbesartan group, and the P/control group ($F=1.240$; d.f.=2;14; $p=0.3245$).

Figure 4 shows the percentage change in blood glucose levels in diabetic mice after 13 days of drug administration. All the groups showed a decrease in blood glucose levels with the group administered 75mg/kg irbesartan showing the highest decrease (Mean= -40.07%; SEM=6.27). The P/control group showed the least decrease (Mean= -11.36; SEM=1.59). There was a significant difference in the change in blood glucose levels between diabetic mice that were administered 20mg/kg irbesartan, 75mg/kg irbesartan, and the P/control group ($F=7.674$; d.f. =2;14; $p=0.0056$).

A Bonferroni post-hoc test showed that the decrease in blood glucose levels was higher in the 20mg/kg irbesartan group (-39.44%) than in the P/control group (-11.36%) ($p=0.0177$). There was also a significant difference in the change in blood glucose levels in diabetic mice that were administered 75mg/kg irbesartan (-40.07%) compared to P/control group (-11.36%) ($p=0.0111$).

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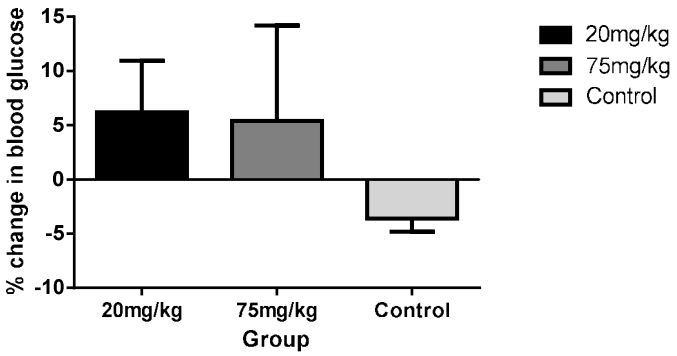

Figure 1: Percentage change in blood glucose from baseline to day 8 in non-diabetic mice; Data are expressed as the mean ± SEM

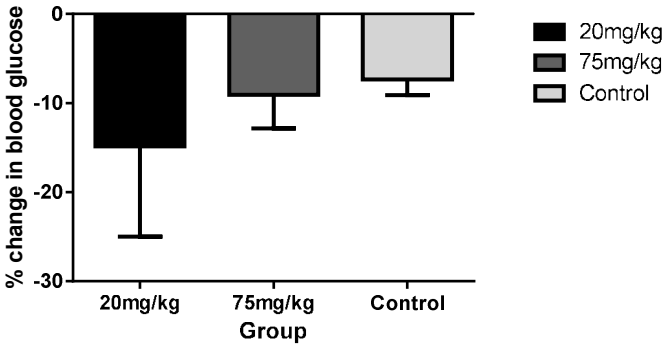

Figure 2: Percentage change in blood glucose from baseline to day 13 in non-diabetic mice; Data are expressed as the mean ± SEM

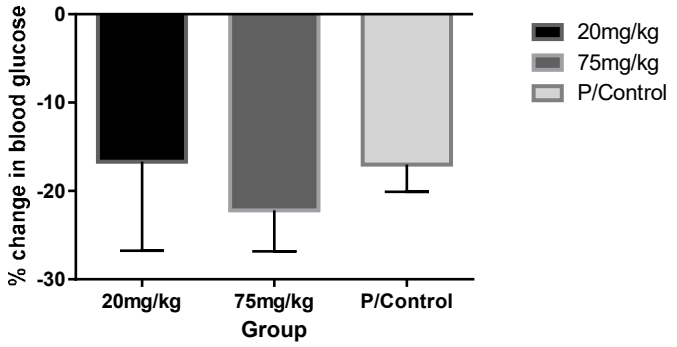

Figure 3: Percentage change in blood glucose from baseline to day 8 in diabetic mice; Data are expressed as the mean ± SEM

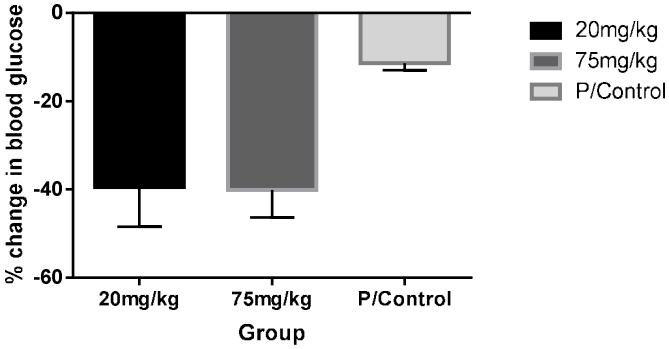

Figure 4: Percentage change in blood glucose from baseline to day 13 in diabetic mice; Data are expressed as the mean ± SEM

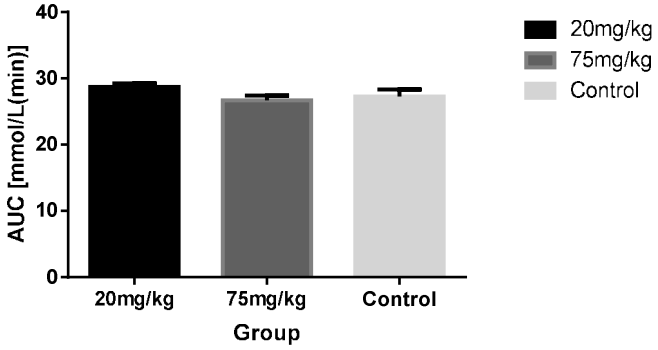

Figure 5: Effect of irbesartan on oral glucose tolerance in non-diabetic mice; Data are expressed as mean ± SEM

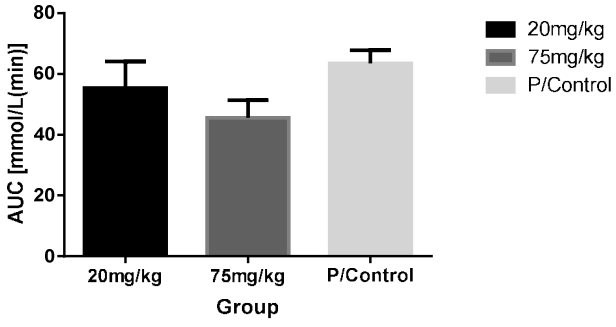

Figure 6: Effect of irbesartan on oral glucose tolerance in diabetic mice; Data are expressed as mean ± SEM

Effect of irbesartan on glucose tolerance in both diabetic and non-diabetic mice

Figure 5 and **6** show the area under the curve (AUC) obtained during the OGTT in non-diabetic and diabetic mice, respectively. In non-diabetic mice, the AUCs of the different treatment groups were almost similar, 20mg/kg irbesartan group (Mean=28.73mmol/L; SEM=0.52), 75mg/kg irbesartan group (Mean=26.66mmol/L; SEM=0.74) and Control (Mean=27.24mmol/L; SEM=1.07).

There was no significant difference in the glucose tolerance across the three treatment groups (F=1.734; d.f.=2;15; p=0.2102).In diabetic mice, the 75mg/kg irbesartan group had the lowest AUC (Mean=45.54mmol/L; SEM=5.81) reflecting an improvement in glucose tolerance compared to the P/control group (Mean=63.43mmol/L; SEM=4.44). The AUC of the 20mg/kg irbesartan group was also lower than the P/control group reflection of a possible improvement in glucose tolerance (Mean=55.35mmol/L; SEM=8.78).

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However, there was no significant difference in the glucose tolerance across the three treatment groups (F=2.139; d.f.=2;14; p=0.1547).

4. Discussion

Analysis of blood glucose levels in non-diabetic mice after eight days and 13 days of treatment in all the three groups (20mg/kg and 75mg/kg irbesartan and control) did not show any significant changes or decreases. These findings are consistent with a study by Onishi et al., who observed that irbesartan at doses of 50mg and 100mg in haemodialysis hypertensive patients does not cause any significant changes in blood glucose levels (Onishi et al, 2013). Similarly, Maeda et al., concluded that irbesartan at 50mg/kg does not cause any significant changes in blood glucose levels in non-diabetic mice (Maeda et al, 2014). The findings in the present study give further evidence on the safety of irbesartan in non-diabetic hypertensive patients. In the diabetic mice, the pattern of blood glucose level changes was different from that observed in the non-diabetic mice. The 20mg/kg and 75mg/kg irbesartan doses decreased blood glucose significantly compared to the control group on day 13. The changes in blood glucose levels were consistent with findings from a study by Henriksen et al., who observed that 25mg/kg and 50mg/kg irbesartan significantly lowers blood glucose levels in obese Zucker rats (Henriksen et al, 2001). Irbesartan was also shown to reduce blood glucose in diabetic rats partly by increased insulin secretion as a result of increased blood flow to the pancreas (Huang et al, 2006). On day eight however, in the non-diabetic mice, there was a slight increase in blood glucose levels in the 20mg/kg and 75mg/kg irbesartan groups, possibly as a result of stress related stimulation of the sympathetic nervous system (Surwit. et al, 1992).

The effect of irbesartan on glucose tolerance in non-diabetic mice was also evaluated in this study using the oral glucose tolerance test. Similar to the blood glucose level analysis on day 8 and day 13 in the non-diabetic mice, the OGTT in all the three groups did not show any significant differences. This is suggestive of irbesartan not having any significant effects on blood glucose levels, glucose tolerance and insulin sensitivity in non-diabetic animals. In diabetic mice, both doses of irbesartan in diabetic mice did not significantly improve the glucose tolerance compared to the control group. Although there was no statistical difference between the two doses of irbesartan, there was generally an improvement in glucose tolerance compared to the control group. The findings of the present study were not consistent with the findings by Henriksen et al. (2001) who reported that the 50mg/kg irbesartan administered for 21 days to diabetic obese Zucker rats improves glucose tolerance as evidenced by significantly lower AUCs compared to control obese diabetic Zucker rats (Henriksen et al, 2001).The lack of significant improvement in glucose tolerance in the current study might be explained by a shorter irbesartan treatment period.

From the findings in this study the use of irbesartan in co-morbid diabetes and hypertension should be encouraged because of its potential positive effects on glycaemic control. However, blood glucose levels should be measured regularly for those diabetic patients using antidiabetic agents and irbesartan since irbesartan theoretically would augment their hypoglycaemic effects. The present study forms a basis for further research on animals to confirm the mechanism by which irbesartan reduces blood glucose levels significantly in diabetic mice and not in non-diabetic mice. The study also forms the basis for further research into the pharmacodynamic interactions of irbesartan and commonly prescribed antidiabetic agents first in animals then in humans.

5. Conclusion

In the present study, irbesartan significantly reduced blood glucose levels in diabetic mice and not in non-diabetic mice. However, a two week treatment with irbesartan did not significantly improve glucose tolerance in both diabetic and non-diabetic mice.

Conflict of Interest Declaration

The authors declare no conflict of interest.

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