

Protective Effect of Aspirin in Relation to IGF-1 in Streptozotocin Induced Type-II Diabetic Rats

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The present study aimed at investigating the protective effects of aspirin in relation to insulin like growth factor-1 (IGF-1) in streptozotocin (STZ) induced type-2 diabetic rats.

Materials and Methods: Rat pups were divided into four groups; on the 5th day of their age, group-I pups received citrate buffer solution and served as the normal group; group-II, treated only with streptozotocin (80 mg/kg, i.p), served as the diabetic group; groups-III & IV, treated with aspirin (10 mg/kg/day, p.o) for one month (5-35 days) and two months (5-65 days) after streptozotocin, served as the treated groups. On the 35th and 65th days, blood samples were collected from all animals and fasting blood sugar, fasting insulin, IGF-1, insulin resistance and insulin sensitivity levels were estimated.

Results:

Conclusions: The study indicates that aspirin pretreatment seems to protect the pancreas from damage caused by STZ and maintains glucose levels in diabetic rats, while increasing insulin sensitivity and reducing insulin resistance, which may indicate an involvement of an insulin like pathway, particularly IGF-1.

Key Words: Insulin like growth factor-I, Streptozotocin, Aspirin, Type-2 diabetes

Received: 07.02.2009 **Accepted:** 16.03.2009.

Introduction

Type-2 diabetes mellitus is a metabolic disorder, characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin.¹ One of the major characteristics of type 2 diabetes mellitus, is insulin resistance; if the insulin resistance results from oxidative damage, then it could be predicted that chronic oxidative stress would lead to hyperinsulinaemia if plasma glucose is clamped at normal level by infusing the required insulin. Increased oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and anti-

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weeks, animals with blood glucose levels above 150 mg/dL were considered diabetic.¹⁰

Study design

The 5 day-old rat pups (neonates) were divided into four groups as follows; Group I-14 pups, group II-8 pups, group III-7 pups, and group IV-9 pups. Group I pups received citrate buffer solution and served as the normal control group; group II were treated only with streptozotocin (80 mg/kg, *i.p.*), and served as the diabetic controls, group-III treated with aspirin (aspirin dissolved in small volume of ethanol and mixed with milk) 10 mg/kg/day, *p.o.*, for one month (5-35 days) after streptozotocin, served as the treated group for one month, and group IV, treated with aspirin (aspirin dissolved in small volume of ethanol and finally mixed with milk) (10 mg/kg/day, *p.o.*) for two months (5-65 days) after streptozotocin, served as the treated group for two months. On days 35 and 65, blood samples were collected from all animals and fasting blood sugar levels, fasting insulin levels, fasting IGF-1 levels, insulin resistance and insulin sensitivity levels were estimated for one month and two months. Insulin resistance was assessed using the previously validated homeostasis model assessment for insulin resistance, calculated from the fasting insulin and fasting glucose concentrations according to the formula:¹¹

$$\text{HOMA-IR} = \frac{\text{FI in mU/L or } \mu\text{U/mL} \times \text{FPG in mg/dL}}{405}$$

Similarly, insulin sensitivity was assessed using the previously validated homeostasis model assessment for insulin sensitivity, calculated from the fasting insulin and glucose concentrations according to the formula:¹¹

$$\text{HOMA-S} = 1/\text{HOMA-IR}.$$

Statistical analysis

All variables are expressed as means±SD. Group differences of continuous variables were compared using ANOVA followed by

Newman-Keuls test; for all analyses performed using Graph Pad Prism 4 (Version. 4) $p < 0.05$ was considered to be statistically significant.

Results

35th day blood analysis

Pups treated with streptozotocin per se and in combination with aspirin for one month and for two months showed significantly raised body weight, fasting blood glucose and insulin resistance levels when compared to the normal control group of pups ($p=0.0005$, $p<0.0001$, $p<0.0001$) respectively. Pups treated with streptozotocin alone and in combination with aspirin for one month and two months showed significantly lowered fasting insulin and insulin sensitivity levels when compared to the normal control group of pups ($p<0.0001$, $p<0.0001$ respectively). Pups treated with aspirin for one month had significantly raised IGF-1 levels but the two month-treatment group showed significantly lowered IGF-1 levels when compared to normal pups ($p<0.0001$) (Table 1).

65th day blood analysis

Pups treated with streptozotocin per se and in combination with aspirin for one and two months had significantly raised body weight, fasting blood glucose and insulin resistance levels when compared to the normal control group of pups ($p=0.0006$, $p<0.0001$, $p=0.0030$) respectively; those treated with streptozotocin alone and in combination with aspirin for one month and for two months showed significantly lowered fasting insulin and insulin sensitivity levels when compared to the normal control group of pups ($p<0.0001$, $p=0.0068$) respectively. Pups treated with aspirin for one month had significantly raised IGF-1 levels, while the two month treatment pups had significantly lowered IGF-1 levels when compared to normal pups ($p<0.0001$) (Table 2).

Table 1. Effects of aspirin on various biochemical parameters at 5th week

Parameter	Normal	Diabetic	Aspirin treatment		P value
			One Month	Two Months	
Sex (M/F)	4/8	3/5	2/5	4/5	
Body weight(g)	104.5±14.3	118.7±15.5	138.5±18.6	106.6±16.5	0.0005
Glucose (mg/dL)	77.0±14.1	207.2±14.3	117.1±28.0	112.8±10.5	<0.0001
Insulin (µg/L)	1.8±0.1	1.2±0.1	1.5±0.0	1.6±0.1	<0.0001
IGF-1 (ng/ml)	681.7±40.7	381.0±32.1	884.7±67.9	522.8±87.0	<0.0001
HOMA-IR	8.6±1.2	14.8±1.9	11.2±3.1	10.9±1.1	<0.0001
HOMA-S	0.12±0.02	0.07±0.01	0.10±0.03	0.09±0.01	<0.0001

All variables are expressed as means ± SD. Group differences of continuous variables were compared using ANOVA followed by Newman Keuls test. For all analyses, a P value < 0.05 was considered to be statistically significant.

Table 2. Effects of aspirin on various biochemical parameters at 10th week

Parameter	Normal	Diabetic	Aspirin treatment		P value
			One month	Two months	
Sex (M/F)	4/8	3/5	2/5	4/5	
Body weight (g)	99.5±20.9	130.6±29.9	144.2±19.2	111.1±15.1	0.0006
Glucose (mg/dL)	77.7±7.3	170.4±26.6	127.7±34.1	115.7±23.4	<0.0001
Insulin (µg/L)	1.8±0.1	1.1±0.1	1.5±0.0	1.5±0.1	<0.0001
IGF-1 (ng/ml)	670.5±37.3	393.6±18.8	868.7±65.3	497.2±76.4	<0.0001
HOMA-IR	8.6±0.8	12.0±2.4	12.0±3.4	10.9±1.8	0.0030
HOMA-S	0.12±0.01	0.09±0.02	0.09±0.03	0.09±0.02	0.0068

All variables are expressed as means ± SD. Group differences of continuous variables were compared using ANOVA followed by Newman Keuls test. For all analyses, a P value < 0.05 was considered to be statistically significant.

Discussion

Results of our study are consistent with those of the Yaun et al. study that reported salicylates inhibit IKK-B activity and restore insulin sensitivity, both in vitro and in vivo.⁵ Hundal et al. reported that treatment of nine type 2 diabetic patients for 2 weeks with high dosages of aspirin (7 g/day), resulted in reduced hepatic glucose production and fasting hyperglycemia and increased insulin sensitivity.¹² Micossi et al. reported aspirin stimulates insulin and glucagon secretion and increases glucose tolerance in normal and diabetic subjects.¹³ Seino et al. reported that acetyl salicylic acid (ASA) alleviates glucose intolerance in maturity onset diabetics by a direct enhancement of insulin secretion.¹⁴ Our study also indicates that following one

month treatment with aspirin, blood glucose levels and insulin resistance levels were significantly reduced and levels of insulin and insulin sensitivity improved significantly.

There is a great deal of evidence that aspirin / NSAIDs affect insulin resistance; it has been long known that salicylates have a hypoglycemic effect and reduce fasting blood glucose in diabetic persons.¹⁵⁻¹⁹ High doses of salicylates have been shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing insulin signaling.²⁰ In patients with type 2 diabetes, aspirin treatment has been shown to reduce fasting plasma glucose, total cholesterol, C-reactive protein, triglycerides, and insulin clearance; aspirin reduced hepatic glucose production and improved insulin-stimulated peripheral glucose uptake by 20%.²¹ The influence of aspirin/NSAID on

insulin resistance appears to be independent of COX-2 inhibition, involving instead inhibition of nuclear factor- κ B and InB and/or activation of peroxisome proliferator-activated receptors.²⁰

An interaction between aspirin and IRS1 in antagonizing effects of tumor necrosis factor- α (TNF- α) has also been reported. TNF- α , a major cause of insulin resistance in obesity and inflammation, has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin signaling cascade, one of which is insulin receptor substrate.²²⁻²⁶ IRS1 is the major cytoplasmic substrate of the insulin receptor in most insulin sensitive tissues and is necessary for maintenance of metabolic homeostasis. Aspirin has been shown to inhibit the TNF- α -induced serine phosphorylation of IRS1 through inhibition of multiple serine kinases, including IB kinase.²¹

Our findings are however consistent with other animal studies, demonstrating low insulin sensitivity in mice with liver specific deletion of the IGF-1 gene that is reversed by treatment with recombinant human IGF-1^{27,28} IGF-1 has hypoglycemic effects and en-

hances insulin sensitivity in both experimental and human subjects, due to its type-1 receptors and/or hybrid insulin / IGF-1 receptors.²⁹

High levels of IGF-1 in the 1-month treated aspirin group when compared to the two month treated group, demonstrate that short term therapy seems to be beneficial. A short course of preconditioning of hepatocytes with aspirin is better than long term treatment, in the production of IGF-1. Mechanisms of increased levels of IGF-1 in the one month aspirin treated group however are not very clear

In conclusion, the present study indicates that aspirin pretreatment seems to protect the pancreas from damage caused by STZ, maintains glucose levels in diabetic rats, increases insulin sensitivity and reduces insulin resistance. The insulin like pathway, particularly IGF-1, may be involved in the protection provided by aspirin treatment in type-2 diabetes. The mechanism of increased levels of IGF-1 in the one-month treated group when compared to the two-month treated group is not clear and further studies are required to prove this hypothesis.

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