Role of IGF-I in aspirin pretreatment in streptozotocin induced type-II diabetic rats

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Citation

Abstract

In the present study, we made an attempt to investigate role of insulin like growth factor-I (IGF-I) in aspirin pretreatment in streptozotocin induced type-2 diabetes mellitus in rats. Rat pups were divided in to four groups, on 5th day of their birth, group-I pups were received citrate buffer solution served as normal, group-II were treated only with streptozotocin (80mg/kg, i.p) served as diabetic, group-III & group-IV were treated with aspirin (10mg/kg/day, p.o) for one month (5-35 days) and two month (5-65) after streptozotocin served as treated groups. On 36 and 66 day, blood samples were collected from all animals and fasting blood sugar, fasting insulin, IGF-I, insulin resistance and insulin sensitivity levels were estimated. Results of 36 & 66 days blood samples of pups treated with streptozotocin alone and in combination with aspirin for one month and two months were shown significantly raised body weight, fasting blood glucose and insulin resistance levels (P=0.0005, p<.0001, p<.0001, P=0.0006, p<.0001, P=0.0030) and significantly lowered fasting insulin and insulin sensitivity levels when compared to the normal control pups (p<.0001, p<.0001, p<.0001, p<.0001, p<.0001, P=0.0068) respectively. Pups treated with aspirin for one month were shown significantly raised IGF-I levels but two months treatment were shown significantly lowered IGF-I levels when compared to the normal pups (p<.0001). The present study indicates that aspirin pretreatment seems to protect pancreas from damage caused by STZ and maintains glucose levels in diabetic rats and increases insulin sensitivity and reduces insulin resistance, this may a involvement of insulin like pathway particularly IGF-I.

INTRODUCTION

Type-2 diabetes mellitus is a metabolic disorder with characteristics of hyperglycemia and insufficiency of secretion or action of endogenous insulin [1]. Insulin resistance is one of the major characteristics of type 2 diabetes mellitus. If the insulin resistance can result from oxidative damage, then a prediction would be 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 antioxidant defenses, is a widely accepted participant in the development and progression of diabetes and its complications [2]. Hyperglycemia was also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway to generate free radicals [3]. Free radicals can be generated in glucose oxidation, which is believed to be the main source of free radicals, which are not degraded by catalase or glutathione peroxidase, and in the presence of transitional metals, can lead to production of extremely reactive hydroxyl radicals [4]. Aspirin is a

derivative of salicylic acid, used as a NSAID, anti thrombotic, antioxidant and anti diabetic drug has new approach in type2 diabetes. Salicylates inhibit serine/threonine caused insulin resistance and IKK-β activity and restore insulin sensitivity, both in-vitro and in vivo. Salicylate alters the phosphorylation patterns of IRS proteins, resulting in the decrease serine phosphorylation, increased tyrosine phosphorylation, and improved insulin action [5]. The principal mechanism of action of aspirin for its pharmacological actions is inhibition of arachidonate cyclooxygenase [6]. Cyclooxygenase (COX) is of two types namely COX-I & COX-II. COX-I is a constitutive enzyme expressed in most tissues including blood platelets and is involved in cell-cell signaling and tissue homeostasis. COX-II is induced in inflammatory cells when they are activated and is believed to be the enzyme that produces the prostanoid mediators of inflammation. Aspirin and also most of the non-steroidal anti inflammatory drugs (NSAIDS) in current use are inhibitors of both isoenzymes (COX-I &COX-II), though they vary in the degree of inhibition of each [7]. Many of the antioxidants have the capability of decreasing the blood sugar levels. Free radicals play a major

role in most of the diabetes and cardiovascular diseases. Aspirin with its antioxidant properties is considered to be beneficial in such disorders [8]. The insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3), insulin receptor substrate 1 (IRS1), insulin receptor substrate 2 (IRS2), and the vitamin D receptor (VDR) genes have been proposed as being directly or indirectly involved in insulin- related pathways. Polymorphisms of these genes have been identified, some of which have been shown to have effects on insulin resistance and/or colon cancer risk [9]. In this study we make an attempt to investigate whether aspirin plays any important role in preventing type-II diabetes mellitus in relation to IGF-I in rats. In view of the above facts, aspirin having antioxidant, antidyslipidimic and anti diabetic properties. We made an attempt to investigate whether aspirin plays a role in preventing non insulin diabetes mellitus.

MATERIALS AND METHODS
MATERIALS

Aspirin pure substance was a kind gift from Natco Pharma Limited, Hyderabad, India. Diphenyl picryl hydrazyl and streptozotocin were purchased from Sigma, St. Louis, USA. Glucose kit was purchased from Excel diagnostics limited, Hyderabad. Ethanol (analytical grade) purchased from E. Merck Limited, Mumbai, India.

ANIMALS

Four pregnant female Wistar rats, weighing between 300-350g were obtained from Mahaveer Enterprises, Hyderabad. The rats were housed individually in acrylic cages in standard environmental conditions (20-250C), fed with standard rodent diet and water ad libitum. The rats were delivered within 1-2 days. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use. The experiment was planned after getting the approval from the Institutional animal ethical committee.

INDUCTION OF DIABETES

On 8.00 AM, the rat Pups were received a single 80 mg/kg intraperitoneal injection of streptozotocin (Sigma, St. Louis, MO) in 0.1 M sodium citrate buffer, pH 4.5. Control nondiabetic animals were fasted and received citrate buffer alone. After 8th week, animals with blood glucose levels greater than 150 mg/dl were considered diabetic [10].

STUDY DESIGN

Rat pups (neonates) were divided in to four groups

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

$$HOMA\text{-}IR = FI \text{ in mU/l or } \mu U/ml \times FPG \text{ in mg/dl} / 405$$

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

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

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, a P value < 0.05 was considered to be statistically significant. All analyses were performed using Graph Pad Prism 4 (Version. 4).

RESULTS
8TH WEEK ANALYSIS

Pups treated with streptozotocin alone and in combination with aspirin for one month and two months were shown 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[Table-1] Pups treated with streptozotocin alone and in combination with aspirin for one month and two months were shown significantly lowered fasting insulin and insulin sensitivity levels when compared to the normal control group of pups (P<0.0001, P<0.0001)

respectively[Table-1] Pups treated with aspirin for one month were shown significantly raised IGF-I levels but two months treatment were shown significantly lowered IGF-I levels when compared to the normal pups (P<0.0001) [Table-1&Figure1-5]

10TH WEEK ANALYSIS

Pups treated with streptozotocin alone and in combination with aspirin for one month and two months were shown 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[Table-1] Pups treated with streptozotocin alone and in combination with aspirin for one month and two months were shown 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 were shown significantly raised IGF-I levels but two months treatment were shown significantly lowered IGF-I levels when compared to the normal pups (P<0.0001) [Table-1&Figure 1-4,6]
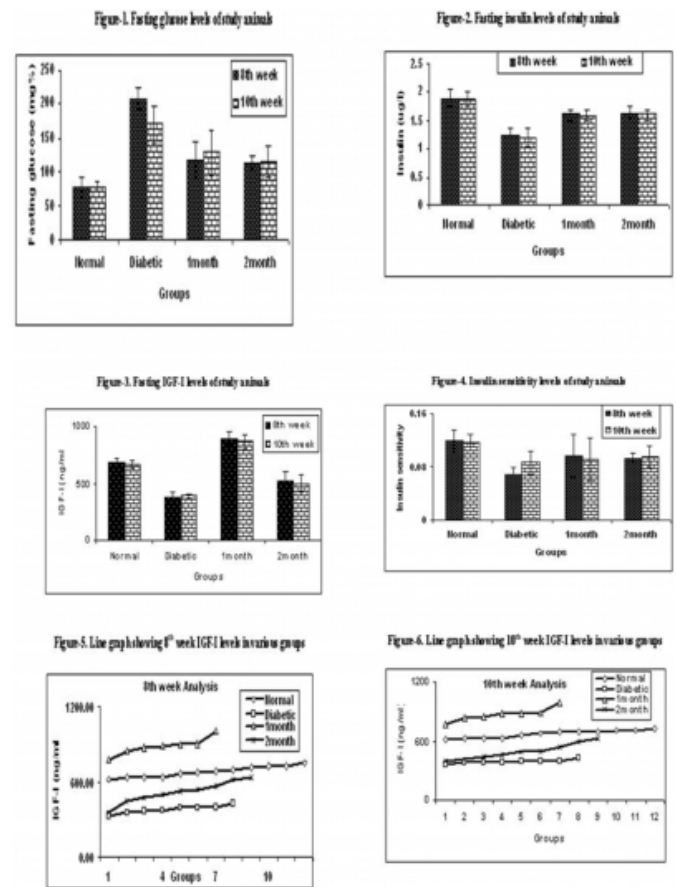
Figure 1

Table No – 1: Effects of aspirin on various biochemical parameters

Parameter	Normal		Diabetic		Aspirin treated for				P-Value	
					One Month		Two Months			
	8th Week	10th Week	8th Week	10th Week	8th Week	10th Week	8th Week	10th Week	8th Week	10th Week
Sex (M/F)	4M/8F	4M/8F	3M/5F	3M/5F	2M/5F	2M/5F	4M/5F	4M/5F		
Body Weight(g)	104.58 ± 14.37	99.58 ± 20.94	118.75 ± 15.53	130.63 ± 29.93	138.57 ± 18.64	144.29 ± 19.24	106.67 ± 16.58	111.11 ± 15.16	0.0005	0.0006
Glucose (mg%)	77.09 ± 14.15	77.75 ± 7.33	207.26 ± 14.33	170.46 ± 26.61	117.19 ± 28.04	127.75 ± 34.19	112.87 ± 10.54	115.71 ± 23.43	P<0.0001	P<0.0001
Insulin (μg/ml)	1.89 ± 0.14	1.86 ± 0.14	1.21 ± 0.17	1.19 ± 0.16	1.59 ± 0.09	1.57 ± 0.09	1.62 ± 0.12	1.59 ± 0.11	P<0.0001	P<0.0001
IGF-I (ng/ml)	681.76 ± 40.75	670.59 ± 37.38	381.07 ± 32.10	393.62 ± 18.84	884.76 ± 67.98	868.72 ± 65.37	522.82 ± 87.03	497.20 ± 76.40	P<0.0001	P<0.0001
HOMA-IR	8.6 ± 1.29	8.63 ± 0.87	14.89 ± 1.99	12.06 ± 2.44	11.25 ± 3.10	12.06 ± 3.45	10.92 ± 1.14	10.91 ± 1.84	P<0.0001	0.0030
HOMA-S	0.12 ± 0.02	0.12 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.10 ± 0.03	0.09 ± 0.03	0.09 ± 0.01	0.09 ± 0.02	P<0.0001	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.]

Figure 2

Figure-1. Fasting glucose levels of study animals

Figure-2. Fasting insulin levels of study animals

Figure-3. Fasting IGF-I levels of study animals

Figure-4. Insulin sensitivity levels of study animals

Figure-5. Line graph showing 8th week IGF-I levels in various groups

Figure-6. Line graph showing 10th week IGF-I levels in various groups

DISCUSSION

Yaun et al., 2001 reported Salicylates inhibits IKK-B activity and restore insulin sensitivity, both invitro and invivo [5]. Hundal et al., 2001 reported treatment of nine type 2 diabetic patients for 2 Weeks with high dosages of aspirin (7g/day) resulted in reduced hepatic glucose production and fasting hyperglycemia and increased insulin sensitivity [12]. Micossi et al., 1947 reported aspirin stimulates insulin and glucagon secretion and increases glucose tolerance in normal and diabetic subjects [13]. Seino et al., 1982 reported that acetyl salicylic acid (ASA) alleviates glucose intolerance in maturity onset diabetics by a direct enhancement of insulin secretion [14]. Our present study also indicates that the aspirin treated for one month shown that significantly reduced blood glucose levels, significantly improved levels of insulin, significantly improved levels of insulin sensitivity and significantly lowered insulin resistance levels.

There is a great deal of evidence that aspirin / NSAIDs have effects on insulin resistance. It has been long known that salicylates have a hypoglycemic effect and that they reduce fasting blood glucose in diabetic persons [15161718 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]. Aspirin/NSAID influence on insulin resistance appears to be independent of COX-2 inhibition, instead involving inhibition of nuclear factor-nB and InB and/or activation of peroxisome proliferator-activated receptors [20].

An interaction between aspirin and IRS1 in antagonizing effects of tumor necrosis factor- ▢ (TNF- ▢) has also been reported. TNF- ▢, 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 23 24 25 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- ▢-induced serine phosphorylation of IRS1 through inhibition of multiple serine kinases, including IB kinase [21].

However, our findings are consistent with the animal studies demonstrating low insulin sensitivity in mice with liver specific deletion of the IGF-I gene that is reversed by treatment with recombinant human IGF-I [27 28] which was raised by the aspirin treatment for one and two months. IGF-I has hypoglycemic effects and enhances insulin sensitivity in both experimental and human subjects it is due to its type-1 receptors and / or hybrid insulin / IGF-I receptors [29].

High levels of IGF-I in one month treated aspirin group when compared to two month treated group, is important to note that short term therapy seems to be beneficial. Preconditioning of hepatocytes with aspirin on short course is better than long term treatment in production of IGF-I. Mechanism of increased levels of IGF-I in one month aspirin treated group is not well understood.

In conclusion, the present study indicates that aspirin pretreatment seems to protect pancreas from damage caused by STZ and maintains glucose levels in diabetic rats and increases insulin sensitivity and reduces insulin resistance. The protection offered by the aspirin treatment in type -2 diabetes, there may a involvement of insulin like pathway particularly IGF-I. The mechanism of increased levels of

IGF-I in one month treated group when compared to two month treated group is not well understood and further studies are required to prove this hypothesis.

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