Study on the Effects of Alloxan Induced Diabetes and Insulin on Blood Level of Vitamin C

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Introduction

The specific capillary lesion of diebetes mellitus microangiopathy, affects primarily the basement membrane of retinal and glomerular capillaries and also some neurological tissues. But small vessels are always involved in other organs. It may be due to over production of basement membrane tissue due to altered carbohydrate metabolism or leakage and accumulation of plasma constituents on the basement membrane. Despite the suggestion, that all diabetic neuropathy is metabolically caused, vascular lesions are also a causative factor in the production of peripheral menifestations of diabetic neuropathy [1]

Again basement membrane is made up of fibrillar net-work of protein similar to that of collagen, the biosynthesis of which needs hydroxylation ractions.[2] It has also been suggested that, as vitamin C is implicated in collagen synthesis, the low cellular levels of vitamin may be connected with microvascular connective tisse changes which occur in the diabetics and believed to be responsible for the organ damage seen in the condition[3]. Ginter and his colleagues have noted that vitamin C is lower in plasma and leucocytes of diabetics than control [4].

Diabetes mellitus has got disturbances of all kinds of vitamin metabolism including vitamin C. Biochemical assessment of vitamin C nutritional status is derived mainly from measuring ascorbic acid levels in serum and leucocytes. The latter are generally considered more colsely related to tissue stores of vitamin C. Serum levels of vitamin C are therefore, most commonly used in evaluating individuals population groups [5].

Considering all these evidences, it is suggested that there may

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have some relationship between the blood level of vitamin C and vascular impairment and complications of diabetes mellitus which depends also on duration of the disease. Therefore, the present work has been undertaken to study the effects of alloxan induced diabetes mellitus of short and long duration on blood level of vitamin C.

Marterials and Methods

Long Evans strain of Norweigian rats, aged 2-3 months, each weighing 150-200 gms and of both sexes were used. They were divided into 3 groups.

Group I : Consisted of 19 rats. Each rat received 2 ml of intraperitoneal injections of normal saline (vehicle) for 3 consecutive days. Of these 19 rats, 10 were sacrificed after 2 weeks and the remaining 9 after 6 weeks.

Group II : Consisted of 17 rats. Each rat received intraperitoneal injection of alloxan in a dose of 200 mg/kg body weight for 3 consecutive days. Of these 17 rats, 8 were sacrificed after 2 weeks and 9 after 6 weeks.

Group III : Consisted of 14 rats. They received alloxan injection as in group II. On the third day (after induction of diebetes as indicated by hyper-glycemia) each rat received injection NPH (N = Neutral solution, P = Protamin Zinc solution, H = Hagedorn) insulin subcutaneously in a dose of 2 units/day each for 2 and 6 weeks. Of these 14 rats, 7 were sacrificed after 2 weeks and the remaining 7 after 6 weeks. Administration of insulin was stopped for 24 hours before sacrificing the animals.

In all experimental cases, blood was collected after overnight fasting for estimation of sugar and vitamin C. Blood sugar level was measured by Folin and Wu method described by Hawk [6] and vitamin C was determined by 2-4 dinitrophenylhydrazine method described by Lowery et al [7].

Results

Bloods Sugar Level

Administration of alloxan caused a significant ($P < 0.001$) increase in fasting sugar level sacrificed both after 2 weeks and 6 weeks (Table-1). Administration of NPH (N = Neutral Solution, P = Protamin Zinc Solution, H = Hagedorn), insulin in alloxan treated diabetic rats caused a significant ($P < 0.001$) reduction in fasting sugar level sacrificed both after 2 weeks and 6 weeks (Table-1).

Table 1. Fasting blood sugar level in different groups of experimental rats.

	(mg/100 ml) (Mean ± SE)	
	After 2 weeks	sAfter 6 weeks
Group-I	91±2.02	93.1±3.12
Group-II	304±16.37	482 ± 9.15
Group-III	102 ± 2.57	112 ± 3.04

*** P < 0.001 in a test significance of difference from control.

SE = Standard Error.

Table 2. Plasma vitamin C level in different groups of experimental rats.

	Level (mg/100 ml) Mean ± SE	
	After 2 weeks	After 6 weeks
Group-I	2.8 ± 0.22	2.6 ± 0.31
Group- II	2.2 ± 0.21	1.1 ± 0.18
Group-III	2.3 ± 0.19	1.4 ± 0.15

*** P < 0.001 in a test of significance of difference from control.

SE = Standard Error.

Plasma Vitamin C Level

In alloxan induced diabetic rats, there was no singificant change in plasma level of vitamin C sacrificed after 2 weeks as shown in Table-II. But the plsma level of vitamin C was significantly (P < 0.001) decreased in diabetic rats sacrificed after 6 weeks both untreated and treated with insulin.

Discussion

In the present study, a decreased ($p<0.001$) plasma level of vitamin C by alloxan induced diabetic rats through long duration (6 weeks) was observed. But a small decrease (statistically not significant) in plasma vitamin C level occur in diabetes mellitus of short duration (2 weeks). Administration of insulin failed to restore the level of vitamin C to

normal in diabetic rats sacrificed after 6 weeks.

Based in [8] observed vitamin C deficiency in diabetes mellitus and he concluded that the degree of C-hypo-vitaminization was directly proportional to the serverity and duration of the disease. There was evidence [9] from animal experiment that the ability of insulin to decrease blood glucose level can be enhanced by high dose of vitamin C.

The actual cause of decreased level of plasma vitamin.C in alloxan induced diabetic rats after 6 weeks obtained in the present work is not clear. However, it may be suggested that the decreased level of vitamin C in the study would be due to (I) increased consumption f this vitamin in diabetes mellitus, (II) depletion of tissue stores of vitamin C and (III) increased excretion of this water soluble vitamin in urine. However, none of the above factors have been investigated in this study. The slight decrease in short term diabetic rats might be due to tissue store of vitamin.C in rats which try to maintain the observed blood level of vitamin C. But in diabetes of long duration, depletion of tissue store might cause significant decrease in plasma level of vitamin C.

Summary

A significant ($P < 0.001$) decrease in plasma vitamin C level was observed in long term (6 weeks) alloxan induced diabetic rats. But no such significant change was observed in short term (2 weeks) alloxan induced diabetic rats. After administration of insulin, no significant change in plasma vitamin C level was observed both after 2 weeks and 6 weeks incomparison to the diabetic group of same duration.

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