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BENEFICIAL EFFECTS OF SELECTED ANTIOXDANTS IN EXPERIMENTAL DIABETES.

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ABSTRACT

The antioxidant effects of Vitamins C, E and N-acetyl-L-cysteine (N) on glucose disposal, reduced glutathione (GSH), malonyldialdehyde (MDA) and antioxidant enzymes glutathione peroxidase (GPX) and glutathione reductase (GRT) in the levels of streptozotocin-induced diabetic rats was investigated.
Glucose tolerance test (GTT) levels of untreated rats (group 1) were relatively elevated in STZ rats compared with rats fed antioxidant, supplemented diets, (Vit. C + E, group 3) and (N+C+E, group 4), although both antioxidant supplemented and untreated rats did not dispose off any appreciable quantity of glucose. A significant level ($P<0.05$) of GSH increase occurred in the red blood cells (RBCs) of diabetic rats on antioxidant supplement as compared with untreated diabetic rats. In contrast untreated diabetic rats (group 1) had significantly higher ($P<0.05$) MDA values of RBCs as compared with diabetic rats on supplementation of antioxidants (groups 2-4). The results of diabetic control rats showed that the concentrations of GSH-Px and GRT were increased as opposed to decreases in GSH levels suggesting that GSH regeneration by oxidized glutathione (GSSG) and the generation of $NADP^+$ from NADPH is theoretically insufficient under diabetic conditions.
This study therefore demonstrates that the levels of membrane lipid peroxidation in antioxidant supplemented diabetic rats RBC may have been blocked by vitamins C and E and by the replenishment of cellular GSH by N-acetyl-L-cysteine (N).

KEYWORDS: Lipid peroxidation, antioxidants, diabetes mellitus.

INTRODUCTION

Reactive oxygen species (ROS) are produced under diabetic conditions mainly through the glycation reaction (glucose derived reaction) (Hunt *et al*, 1991) that occurs in various tissues and may play a role in the development of complications in diabetes (Myint *et al*, 1995; Stahl and Sies, 1997). Erythrocyte (RBC) haemoglobin and erythrocyte oxygen accelerate lipid peroxide oxidation that may lead to RBC membrane damage and haemolysis. In erythrocytes and other tissues the enzymes glutathione peroxidase containing *selenium* (a prosthetic group) catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione (GSH) that in turn removes the H_2O_2 in a reaction catalysed by glutathione reductase (GRT) (Meister, 1985). In diabetic patients with vascular disease the level of lipid peroxidation is higher (Ihara *et al*, 1999). ROS are so destructive that they interact with and destroy, all molecules in the vicinity of their formation such as lipids, proteins and DNA (Emerit, 1994).

Most animals have their own antioxidant defense system that can be externally strengthened. Generally, most animal cells use vitamin C and glutathione together with a cycle of antioxidant enzymes to remove ROS and thereby protect themselves from metabolic processes of oxidative damage (Baynes, 1991). Vitamins C and E are well known dietary antioxidants while N-acetyl-L-cysteine (N) is a hydrogen peroxide scavenger (Satoh *et al*, 1979). The present study was undertaken to examine the effects of the above antioxidants on glucose disposal and the levels of reduced glutathione (GSH), malonyldialdehyde (MDA) and antioxidant enzymes glutathione peroxidase (GPX) and glutathione reductase (GRT) in streptozotocin-induced diabetes mellitus in rats.

MATERIALS AND METHODS

Wistar strain albino rats weighing (160-180g) were purchased from National Institute for Trypanosomiasis Research, Vom. The rats were divided into groups of four rats per cage. Diabetes was induced and confirmed in all the four groups of rats as previously described (Ohaeri, 2001).

The antioxidant diets were prepared by mixing commercial diet with antioxidants as shown (Table 1): Rats in Group 1 (control group) were kept on regular diet without any antioxidant. Group 2 rats were given 2.5 % N plus commercial diet. Group 3 rats were given 0.5 % vitamin C and 0.5 % vitamin E plus commercial diet while Group 4 rats were given 2.5 % N, 0.5 % vitamin C plus 0.5 % vitamin E. All rats were given food and water *ad libitum*. At the end of 28 days and after an overnight fast, rats were injected intraperitoneally with glucose (1.0g/kg body weight). Blood samples from tail veins were taken at various times (T0min - T120min) and blood glucose concentrations were measured by the glucose oxidase method (Trinder, 1969). Thereafter, the rats were sacrificed by decapitation and blood collected by cardiac puncture into a tube containing ethylene diamine tetraacetic acid (EDTA), mixed before centrifuging at 1500g for 5 minutes. The supernatant plasma collected into a clean sterile plastic container

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Table 1: Composition of Diets

Dietary Component	Normal diet %	N-acetyl-L-cysteine %	Vitamins C+E %	N-acetyl-L-cysteine+ Vits. C + E %
Crude-Protein	21	20.5	20.5	20.5
Nitrogen-Free extract	64.84	62.84	64.34	61.84
Fat	4.33	4.33	4.33	4.33
Ash	2.90	2.90	2.90	2.90
Crude fibre	3.33	3.33	3.33	3.33
Moisture	3.60	3.60	3.60	3.60
Vitamin C	—	—	0.50	0.50
Vitamin E	—	—	0.50	0.50
N-acetyl-L-cysteine	—	2.50	—	2.50
Total diet	100	100	100	100

Table 2: Effect of Antioxidants on Glucose Tolerance in Diabetic rats.

Time	Untreated	N	Vits. C + E	N+Vits.E+C	P-value
T0	12.1±1.23	11.5±1.17	11.8±1.19	11.5±1.58	(NS)
T30	30.40±0.58	21.3±0.45[a]	25.5±1.23[b]	22.4±1.22[a]	([a]P<0.05; [b]P<0.001)
T60	32.5±1.83	25.4±1.57[a]	27.5±1.23	26.4±1.55[a]	([a]P<0.05)
T120	29.5±0.82	24.3±1.24[a]	26.4±1.72	25.5±1.48[a]	([a]P<0.05)

Data are Means ± SEM (n=4).

Means on the same row with the same superscript are not significantly different (P>0.05).

and stored at –20° C was used within two days. The red blood cells were washed three times in 0.9 % sodium chloride and then haemolysed by exposure to nine parts of redistilled water followed by freezing (-20°C, 18 h) and thawing before analysis. Plasma and RBC GSH were determined according to the method of Beutler *et al* (1963) in which a stable yellow colour was developed when 5, 5'-dithiobis(2-nitrobenzoic acid) (DNTB) was added to sulphydryl coupounds. The colour of the reaction mixture was read specctrophotometrically at 412 nm.

Thiobarbituric acid-reacting compounds were assayed according to the method of Placer *et al*, (1966) and expressed as malonyldialdehde (MDA) equivalents in μmol litre^{-1} plasma and μmol g^{-1} haemoglobin in RBC haemolysate. GSH-PX and GRT were measured by the method of Beutler, (1984) using cumene hydroperoxide and reduced glutathione (GSH) as substrate with the loss of GSH following enzymatic reaction measured as enzyme activity and expressed in international units (μmol GSH oxidized min^{-1} at 25°C).

The significance of differences among groups with control values was determined using a one-way analysis of variance (ANOVA). The significance of change between control and each group was performed using Student's *t* test. P<0.05 was considered significant.

RESULTS

GTT levels of untreated rats (group 1) were relatively elevated in streptozotocin (STZ) rats (T60 min-32.5±1.83) compared with supplemented rats (N- group 2) (T60 min-25.4±1.57), (Vit. C + E, group 3) (T60 min-27.5±1.23) and (N+C+E, group 4) (T60 min-26.4±1.55), (Table 2.), although both antioxidant supplemented and untreated rats did not dispose off any appreciable quantity of glucose.

A significant level (P<0.05) of GSH increase occurred in the RBCs of diabetic rats on antioxidant supplement as compared with untreated diabetic rats. In contrast untreated diabetic rats (group 1) had significantly higher (P<0.05) MDA values of RBCs as compared with diabetic rats on supplementation of antioxidants (groups 2-4), Table 3. In general terms GSH-Px and GRT activities were decreased in diabetic rats on antioxidant supplement as against control rats (Table 4.).

DISCUSSION

Increased cellular oxidative stress due to hyperglycaemia in diabetes mellitus can cause glycation of membrane proteins (Ihara *et al*, 1999; Baynes, 1991). The development of vascular disease and other diabetic complications may also be promoted by oxidative stress

lipid peroxidation could be suggested as beneficial to streptozotocin-induced diabetes in rats.

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Table 3. Effect of Antioxidants on Lipid Peroxidation in experimental diabetic rats.

	RBC		WHOLE-BLOOD	PLASMA
	GSH (μmol/gHb)	MDA (nmol/gHb)	GSH (μmol/g protein)	MDA (μmol/g protein)
Control	0.579±0.023	0.747±0.052	0.653±0.014	0.443±0.012
N	0.838±0.021^{a}	0.423±0.038^{a}	0.994±0.021	0.237±0.001
Vit.C+E	0.603±0.012	0.589±0.052	0.698±0.010	0.325±0.002
N+C+E	0.721±0.012^{a}	0.539±0.014^{a}	0.845±0.025	0.303±0.001

Data are mean ±SEM (n=4).

Means on the same column with the same superscript are not significantly different (P>0.05).

Table 4: Effects of Antioxidant Supplement on some blood Enzymes in Diabetic rats.

	RBC		PLASMA	
	GSH-PX (μmol/g)	GRT (μmol/g)	GSH-PX (μmol/g)	GRT (μmol/g)
Control	13.750±0.052	10.325±0.044	0.497±0.033	0.547±0.020
N	8.675±0.032^{a}	7.318±0.027	0.336±0.012	0.523±0.015
Vit.C+E	10.568±0.027	9.686±0.036^{a}	0.257±0.021	0.366±0.022
N+C+E	9.523±0.031^{a}	9.753±0.020^{a}	0.358±0.017	0.486±0.014

Data are mean ±SEM (n=4).

Means on the same column with the same superscript are not significantly different (P>0.05).

(Giuliano *et al*, 1996; Aoki *et al*, 1992). In insufficiency of the antioxidant system, haemolytic states that are often provoked by certain food factors or drugs may develop.

MDA is thought to disturb aminophospholipid organization in the membrane bilayer of RBCs while GSH is known to scavenge free radicals and reactive oxygen intermediates (Aoki *et al*, 1992; Meister, 1985). The data shown in Table 3 suggest that an increase in lipid peroxidation is mediated, at least in part by GSH depletion and increased MDA in STZ diabetic rats. This can be construed to indicate that RBC haemoglobin and RBC oxygen accelerated lipid peroxidation may lead to damage of the RBC membranes and their haemolysis. Thus GSH depletion can further disrupt cellular defenses against oxidative stress resulting in lipid peroxidation (Jain and McVie, 1994).

MDA is an end product of fatty acid peroxidation. Oxygen radicals exert cytotoxic effects that cause peroxidation of membrane phospholipids that result in accumulation of products such as malonialdehyde (MDA) causing altered membrane permeability and lipid peroxidation (Myint *et al*, 1995; Ihara *et al*,1999; Emerit, 1994). This study therefore demonstrates that the levels of membrane lipid peroxidation in antioxidant supplemented diabetic rat RBC may have been decreased by vitamins C and E and N through the replenishment of cellular GSH (Table 3).

Intracellular GSH level is maintained through the reduction of GSSG by NADPH and de novo GSH synthesis (Meister, 1985).

The results of diabetic control rats showed that the concentrations of GSH-Px and GRT were increased in (Table 4.) as opposed to decreases in GSH levels (Table 3) suggesting that GSH regeneration by GSSG and the reaction of NADPH to $NADP^{+}$ is theoretically insufficient under diabetic conditions. In contrast, supplementing the diets of diabetic rats with selected antioxidants of Vitamins C, E and N-acetyl-L-cysteine increased GSH levels and reduced the concentrations of GSH-Px and GRT.

In conclusion, antioxidant supplementation in