Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (Allium cepa Linn) as compared to standard drugs in alloxan diabetic rats

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Received 26 February 2002; revised 25 June 2002

Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide (SMCS) isolated from A. cepa and two standard drugs, glibenclamide and insulin were studied and compared in alloxan diabetic rats after using each of them for treatment for two months. These drugs ameliorated the diabetic condition significantly, viz. maintenance of body weight and control of blood sugar in rats. Further they lowered the levels of malondialdehyde, hydroperoxide and conjugated dienes in tissues exhibiting antioxidant effect on lipid peroxidation in experimental diabetes. This is achieved by their stimulating effects on glucose utilization and the antioxidant enzymes, viz. superoxide dismutase and catalase. The probable mechanism of action of SMCS and glibenclamide may be partly dependent on the stimulation of insulin secretions and partly due to their individual actions. In the amelioration of diabetes the standard drugs showed a better action, but as an antioxidant SMCS proved to be a better one.

Oxidation and production of free radicals are an integral part of metabolism. At high concentrations, free radicals can initiate lipid peroxidation and also damage other important molecules including proteins, carbohydrates and DNA. Free radicals and lipid peroxides are generated in vivo under various pathological conditions including hyperglycemia. With respect to the role of lipid peroxidation in diabetes, it has been postulated that the etiology of the complications of diabetes involves oxidative stress, as a result of hyperglycemia. It has been demonstrated that glucose can undergo oxidation in blood when catalyzed by trace metals, generating free radicals, hydrogen peroxide and reactive ketoaldehydes. Although the rate of glucose autoxidation is slow, it is relevant to the tissue damage in diabetes.

Living organisms have developed complex antioxidant systems to control production and reduce damage from free radicals. Endogenous components include GSH and Se-glutathione peroxidase, Fcatalase, NADPH, ubiquinol-10, Mn, Cu, Zn-SOD and uric acid. Decreased levels of antioxidants are reported in diabetes.

Some natural products like cysteine sulfoxide derivatives, thiosulfimates, flavanoids and beta-carotene are also reputed for their free radical scavenging actions. As S-methyl cysteine sulphoxide (SMCS) isolated from onion showed significant antidiabetic and hypolipidemic activities, a study of its effects as compared to that of standard drugs on lipid peroxidation in diabetic rats was envisaged. A brief description of the experimental procedures and the results are presented here.

Materials and Methods
SMCS was prepared from fresh onions using ion-exchange resins. Fresh onion was sliced into boiling water to inactivate the enzyme alliinase, which converts the sulfoxide amino acids (alliins) to their aliin-type thiosulfinate compounds. It was then ground with 80% methanol, filtered and centrifuged. SMCS was isolated from the clear supernatant by ion-exchange chromatography using three successive columns of ion-exchange resins, viz. Amberlite IR 120 supplied by Romali, Bombay, India; Amberlite IRC 50 and Amberlite IRA 93 supplied by Sigma Chemical Company, USA. All the onion amino acids were first adsorbed on IR 120 column of a strong cation exchanger, and eluted with 0.1N ammonium hydroxide. The fractions were analysed by paper chromatography using butanol/acetic acid/water (12:3:5v/v) system. Fractions containing the amino acids were pooled and concentrated on a rotary evaporator at 40°-43°C whereby ammonia was also removed.

The concentrate was passed through IRC 50 column of weakly acidic cation exchanger. The neutral effluent obtained from the column was collected, con-
Their fasting blood glucose was determined in moderately diabetic rats by 13%. A smaller dose of 200mg/kg was given to the animals. After a fortnight rats with moderate diabetes exhibited glycosuria as detected by Benedict’s test and hyperglycemia with a fasting blood glucose (FBG) of 190-280 mg/l00 ml were selected and divided into four groups of six. Their initial body weights, glycosuria and FBG were determined before the start of the experiment. A control group of six normal rats were also taken. The rats were maintained on laboratory rat feed (Gold Mohur) supplied by Lipton Indian Ltd., Bangalore, India and treated as given below:

- **Group I** Normal rats fed with normal saline 10 ml/kg/day
- **Group II** Diabetic rats fed with normal saline as above
- **Group III** Diabetic rats treated with SMCS 200mg/kg/day as a solution in normal saline
- **Group IV** Diabetic rats treated with glibenclamide 500 μg/kg/day as a suspension in normal saline
- **Group V** Diabetic rats injected (sc) with plain insulin 5 IU/kg/day in normal saline

Glibenclamide was supplied by Boehringer Knoll Ltd., Bombay, India and insulin by Boots Company Ltd., India. SMCS and glibenclamide were administered orally with a gastric tube. Water was given to the rats *ad libitum*. The experiment was carried out for 60 days. Their food intake was measured daily. Their fasting blood glucose and urine sugar were assessed after one month, and also after two months. Then their final weights were taken and they were decapitated. Their liver and heart were collected for the estimation of malondialdehyde (MDA), hydroperoxides (HP), conjugated dienes (CD), super oxide dismutase (SOD) and catalase by standard methods. Data were analysed statistically by Student’s *t*-test and Analysis of Variance (ANOVA) along with Duncan’s multiple range test.

**Results**

The daily food consumption by different groups of rats was more or less the same (8-10g/rat/day). The changes in their body weights, however differed significantly. The diabetic rats showed a significant reduction in their body weights and a significant increase in blood sugar level (*P < 0.001*) after two months. After the first month of treatment, the body weights of the treated groups were more or less maintained. However after two months, the treated groups showed a significant (*P < 0.001*) decrease in blood sugar and urine sugar and a just significant increase (*P < 0.05*) in body weight against the untreated control (data not presented).

Urine sugar showed a gradational decrease (++++ to +) during two months treatment. In the diabetic control group, the body weight steadily decreased and the FBG increased during the period. Loss of weight was prevented and the FBG was significantly reduced by treatment. The urine sugar for all the treated groups was controlled to negligible levels (traces +) at the end of two months treatment. The effects of treatment was in the order insulin > glibenclamide > SMCS.

Figure 1 shows the levels of MDA, HP and CD in the liver and heart of normal rats, diabetic control and treated rats. The liver of the diabetic control rats showed elevated levels of MDA, HP and CD (*P < 0.001*) as compared to normal levels. These parameters were significantly lowered in the treated groups (*P < 0.05 - 0.001*). Both insulin and SMCS showed to be better than glibenclamide in this respect. The decreases of MDA in the treated groups were 11.6%, 8% and 21% for SMCS, glibenclamide and insulin respectively. The corresponding reductions of HP in these groups were 34%, 23% and 31% respectively. The order of reductions of CD was the same as that for HP, i.e with SMCS giving the highest reduction of 12%, followed by insulin with 10% and glibenclamide with 8% reductions.

In the case of heart, only CD showed a significant (*P < 0.001*) increase of 62% in the diabetic control...
rats. Treatment with the drugs significantly lowered this parameter by 15-18% (P < 0.05-0.01).

On the contrary, MDA and HP levels were just significantly (P < 0.05) lower in the diabetic rats by 7% and 13% respectively. Treatment with the three drugs showed only a non-significant change in the former. However, on treatment with SMCS, glibenclamide and insulin the HP levels showed a just significant increase (P < 0.05).

FIGURE 2 shows the activities of SOD and catalase in the liver and heart of normal rats, diabetic control and treated diabetic rats. The enzymes showed significant decreases in their activities both in liver and heart of the diabetic control rats (P < 0.001). On treatment with each of the drugs the activities of these enzymes increased to significant levels (P < 0.05-0.001) except that for SOD in the heart of glibenclamide treated group.

Discussion

The results show that although all the three drugs have antidiabetic action, only SMCS has a definite antioxidant action. SMCS seemed to be only a mild antidiabetic drug but a strong antioxidant agent, as it most effectively reduced the peroxidation products, viz. MDA, HP and CD in both liver and heart of the treated diabetic rats.

The rise of the lipid peroxidation level observed in the diabetic rats agrees with the results of several other studies conducted on diabetic rats and human subjects. Under normal conditions some amount of peroxidation also occurs. Scavenging enzymes like SOD, catalase and glutathione peroxidase metabolize the superoxide ions thus formed to harmless intermediates. A change in this condition in diabetes leads to overproduction of superoxide ion and hydrogen peroxide which in turn form the harmful hydroxyl radical. As found in this study a decrease has been observed in the activities of SOD and catalase in some tissues of diabetics by Sekar et al. and also in a previous study from this department which may explain the reasons for the over production of lipid peroxide in diabetes. As observed in the beneficial effects of S-allylcysteine sulfoxide (SACS) isolated from Allium sativum LINN in diabetic condition, here also we find that SMCS isolated from Allium cepa LINN is superior to glibenclamide and insulin for the antioxidant effects. The lower effects of SMCS as compared to SACS may be because of the unsaturated bonds present in the latter.

![Graph showing the effect of long-term treatment with SMCS, glibenclamide, and insulin on lipid peroxidation. Values presented are mean of 6 rats. ANOVA calculated. Duncan's procedure: Range for the 0.05 level. Significance of the results are given in the text.](image-url)
A lowered level of MDA and HP with the heart tissue of diabetic rats despite a decrease in the activities of SOD and catalase may be explained thus: under conditions of stress, the heart tissue utilizes glucose in preference to fatty acids for its energy production. The decreased utilization of fatty acids may be one of the factors for the decreased formation of lipid peroxides as well as the observed decreases in the activities of SOD and catalase in heart. Secondly an enhanced action of glutathione S-transferase as reported by some workers in heart may also contribute to a decrease in the level of lipid peroxides in that tissue. This enzyme detoxifies fatty acid hydroperoxides to nontoxic alcohols.

The antioxidant activity of the sulfoxide R-S(O)-cysteine is critically dependent on the nature of the R side chain. SMCS and the sulf oxide isolated from garlic, viz. SACS are good scavengers of free radicals and this may be due to their stimulatory action on different antioxidant enzymes. Thus treatment of diabetic rats with SMCS might have ameliorated the deteriorated condition of diabetes by increasing the activities of SOD and catalase independently or through a stimulated secretion of insulin that eventually lead to a control of lipid peroxidation. Glibenclamide treatment might have gained the control of diabetes via its insulin secretory action and that too to a considerable extent. According to the papers presented at an international symposium on Alliums 2000, the senior author and many others proved that both garlic and onion are full of active principles that protect us from diabetes, cancer and coronary heart disease.

Acknowledgement

The authors acknowledge the authorities of the University of Kerala for providing all the facilities for this research and particularly for the grant of a research fellowship to the first author (K K).
References


