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Impact of Selenium on Alcohol Induced Hypercholesterolemi: A Probe into its Mechanism

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Abstract: ***HMGCoAreductase is the rate limiting enzyme in cholesterol synthesis. Hypercholesterolemia is associated with selenium deficiency. The present study showed that high dose of selenium reduced cholesterol level and also reduced the activity of HMGCoAreductase in alcohol induced hypercholesterolemia condition. In vitro studies showed that, activation occurs at a particular concentration.(4-13mM) below and above this concentration, inhibition is observed. Feeding sodium selenite increased the hepatic selenium content. High dose of selenium induce the expression of selenoproteins. These selenoproteins may be involved in the modulation process.***

Keywords: selenium, HMGCoAreductase and alcohol

1. Introduction

Hypercholesterolemia and cardiovascular disorders have shown to be associated with selenium deficiency [18, 20] . Low serum selenium is associated with a decrease of liver microsomal activity and serum HDL-C concentration [12].Selenium has a crucial role in controlling the effects of thyroid hormone on fat metabolism [2].

The major rate limiting step in the biosynthetic pathway of sterols is considered to be NADPH dependent reduction of 3-hydroxy-3-methyl-glutarylCoenzymeA to mevalonate. HMGCoAReductase catalysis this reaction and is stringently feedback regulated *in vivo* by cholesterol and other mevalonate derived non steroids. The activity of reductase is regulated by three distinct mechanisms. First one is the level of gene expression, hence depletion of cholesterol enhances the synthesis of reductase mRNA. The second regulatory mechanism involves the rate of degradation of HMGCoAreductase which is also modulated by the supply of cholesterol. There is the phosphorylation-dephosphorylation of the reductase which causes inactivation and activation of the reductase. Variations in cholesterol have no effect on the phosphorylation of the enzyme by AMP activated proteinkinase [5].

Many hypolipedemic agentslike vitamin [C [8], lovastatin [11] are known to be inhibitors of HMGCoAReductase. Cholestryamine, a bile acid sequesterent, dietary fiber from black gram have been reported to increase the activity of HMGCoAReductase [13]

Nassie et al [14] reported that hypercholesterolemia associated with se deficiency was due to the increased activity of HMG CoA reductase activity in the liver microsome. Existing reports on selenium are inadequate to explain its hypolipedemic action. Hence we partially purified HMGCoAreductase from the rat liver and studied the modulatory effects of Se *in vitro*. We also studied the effect of Se in the alcohol induced hyperlipidemia in rats.

2. Materials and Methods

Animals: Normal male albino rats of Sprague dawley strain bred in our animal house weighing between 180-210g were divided into four groups of six each as follows,

Group I Control Rats
Group II Ethanol group(950mg/ethanol/100g body weight)
Group III Selenium group (0.05mg sodium selenite/100g body weight)
Group IV Ethanol + Selenium group (500mg ethanol/100g body weight+0.05mg sodium selenite/100g body weight) .

Rats were fed with rat feed (Lipton India Ltd) . Food and water were given ad libitum. Sodium selenite and ethanol were administered as detailed above. Sodium selenite freshly dissolved in water and ethanol diluted in the ratio (1:1) were given orally by gastric tube for 45 days. Animals were fasted overnight and sacrificed. Liver was dissected for the preparation of microsomal fraction and kept in ice cold containers. HMGCoAReductase was purified and assayed from the microsomes as described by the method of Don A Klenise*et al* [3] for *in vitro* studies. Cholesterol was estimated by Abel'ls [1] method and HMGCoA activity in the vivo studies was assayed by estimating the ratio of HMGCoA/Mevalonate by the method of Rao and Ramakrishnan [15] .

Spectrophotometric method of Assay:
The HMGCoAreductase activity in solubilized fractions is assayed spectrophotometrically by measuring the rate of decrease in absorbance at 340nm due to the oxidation of NADPH. The reaction mixture in a volume of 0.5ml contains potassium phosphate buffer, pH7, 50µmol, dithiothreitol 2µmol, NADPH, 0.3µmol, DL-HMGCoA 0.15µmol and enzyme 0.2-400µg of protein. The reaction mixture was preincubated in a 2mm light path glass cuvette without HMGCoA present for 5min at 37 ^{0}C. The assay for enzyme activity was then carried out by the addition of HMGCoA in the reaction mixture at 37 ^{0}C. in a recording spectrophotometer. The initial velocity of the reaction is

measured and the net rate of NADPH oxidation was determined by subtracting the rate of its oxidation in the absence of HMGCoA from the rate observed with both substrates present.

Effect of Se:
The optimum conditions selected for the assay was temperature at 37 ^{0}C and pH at 7. The HMGCoA concentration was 0.15µm. Concentration of sodium selenite ranged from 1-31mM.

The enzyme was pre incubated with sodium selenite for 10min at 37 ^{0}C and the reaction was then carried out by the method of Don A Kleinsek *et al* [11] . Sodium selenite solution was made fresh in distilled water.

Se content in total liver and microsome:
The liver and hepatic microsomal fractions were ashed in asilica crucible and dissolved in a mixture of acids (10:4:1 mixture of nitric acid: Perchloric acid:sulfuric acid). The se content was estimated using the Trace Analyser (Perkin, Model2380)

Statistical Analysis
Data were analysed by One way analysis of variance (ANOVA). All the values are expressed as means ±SD. P values of 0.05 or less were considered significant.All analysis was performed on computer using the statistical package SPSS.

3. Results

Se content in total liver and microsomes
Se content was decreased in liver of ethanol treated group in comparison with control. But it was not detected in the microsome fraction of ethanol treated rats. In Se supplemented group the content was increased both in liver and microsomes in comparison with control and ethanol groups.

Concentration of Cholesterol in liver and Serum
The concentration of cholesterol was significantly increased in Se supplemented group in comparison with control and ethanol group. Co administration of se along with alcohol reduced the alcohol induced hypercholesterolemia.

Activity of HMGCoAredcuctase (*In vivo*)
For this study we have measured HMGCoA/Mevalonate ratio. Hence lower the ratio indicates higher activity. In vivo studies showed that Se supplementation brought down the activity of HMGCoAreductase in comparison with control and alcohol group.

Effect of Se on HMGCoAreductase (*In vitro*)
The HMGCoAreductase activity was inhibited between sodium selenite concentration 1-4mM. There was an increase in the activity, in the concentration range 4-13mM. Further increase in the concentration of sodium selenite inhibited the activity of reductase.

4. Discussion

We have observed in the present study, that exogenous high dose of Se reduced cholesterol level and also reduced the activity of HMGCoA in alcohol induced hypercholesterolemic condition. But in the in vitro studies, there is an activation in the activity of the enzyme at a particular concentration, below and above this concentration inhibition is observed. Inhibition at higher concentration may be due to high metal ion content. The discrepancy in the in vivo and and in vitro experiments suggests that Se may not be modulating directly HMG CoA activity

It may be involved in the phosphorylation-dephosphorylation system of HMGCoAreductase or in the functional aggregation of its subunits. Selenite and other redox-active seleno compounds can modify proteinkinaseC (PKC) in the test tube [7] . Gopalakrishna [7] reported that PKC is inactivated by low concentration of selenite, which is involved in the phosphorylation-dephosphorylation reactions of HMGCoAreductase.

Hepatic microsomal HMGCoAreductases from rats under various dietary conditions display different kinetic properties [10]. Feeding sodium selenite increased the hepatic selenium content. But Se concentration was undetectable in the alcohol group.High dose of selenium induce the expression of many selenoproteins [19] . These seleno proteins may be involved in the modulation process. So the activation observed under the *in vitro* condition may not be the actual picture under*in vivo* conditions. Several laboratories [4, 6, 16, 17] have demonstrated that rat liver HMGCoAreductase is allosterically inhibited by the disulfide formation between vicinal sulfhydryl groups on the enzyme. Se substitutes for sulfur in may biomolecules when it is in excess [9]. These incorporations may bring about structural changes.hence it can be concluded that elemental selenium has no role in modulating HMGCoAreductase activity *in vitro*. But exogenous supply of Se can reduce alcohol induced hypercholesterolemia.

5. Acknowledgement

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Table 1: Selenium content in total liver and microsome

	Se content in total liver (ppm/g tissue)	Se content in microsomes (ng/g tissue
I Control	91.76± 3.68	1.246± 0.07
II Ethanol	32.60± 1.24[a]	Not detected
III Sodium selenite	223.00± 8.24 [b]	4.017± 0.15[bd]
IV Ethanol+Sodium selenite	122.76± 4.43[ce]	2.086± 0.08[ce]

Values expressed as mean±SD

a p< 0 05 between control and ethanol groups
b p<0 05 between control and selenium groups
c p<0 05 between control and selenium+ ethanol grous
d p<0 05 between ethanol and selenium group
e p<0 05 between ethanol and selenium + ethanol goups

Table 2: Concentration of cholesterol in liver and serum (mg/100g tissue and mg/100ml serum)

	Se content in total liver (ppm/g tissue)	Se content in microsomes (ng/g tissue
I Control	339.76± 12.47	77.50± 2.99
II Ethanol	377.18± 14.01[a]	92.50± 3.55 [a]
III Sodium selenite	304.75± 10.81 [b]	67. 80± 2. 49[bd]
IV Ethanol+Sodium selenite	331.77± 12.23 [e]	80.2± 2.83[ce]

Foot notes same as in table 1

Table 3: Activity of HMGCoArecuctase (*in vivo*)

	Liver(HMGCoA/Mevalonate)
I Control	4.38± 0.16
II Ethanol	3.87± 0.15[a]
III Sodium selenite	4.99± 0.17[bd]
IV Ethanol+Sodium selenite	4.30± 0.19[ce]

Foot notes same as in table 1

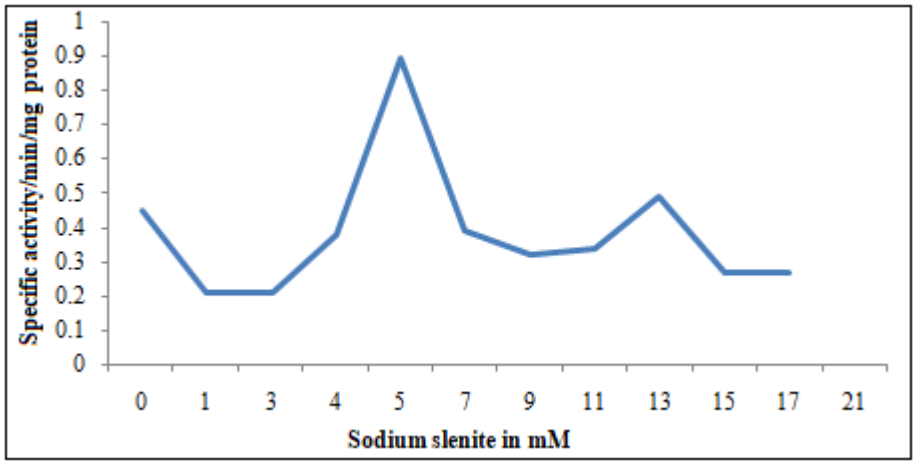

Fig: Effect of selenium on HMGCoAReductase (*In vitro*)