Selenomethionine effects on polyamine catabolism in renal tissue of hepatectomized rats

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Citation

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Abstract

Background/Aims. Selenium metabolism and polyamine biosynthesis are linked in their common requirement for S-adenosylmethionine. During the period of liver regeneration, many growth factors that affect kidney metabolism appear in circulation. The idea of the present study was to elucidate the influence of Se-Met on polyamine metabolism in renal tissue of hepatectomized rats.Methods: Male albino Wister rats, were used for this study. Partial hepatectomy was performed by the resection of two-thirds of liver tissue mass (67%). One group of hepatectomized animals received Se-Metionine intraperitoneally, in a single daily dose of 2.5 μg per 100g body weight, and the other, as well as control group, received 0.9% NaCl instead of the drug for seven days. The last dose of selenomethionine was applicated on the 7th day after hepatectomy, one hour before sacrificing. Results: The supplementation of Se-methionine to hepatectomized animals causes the decrease of PAO activity in renal tissue. The amount of MDA follows PAO activity; it increases after operation in kidney tissue and decreases under SeMet treatment. Diamine oxidase activity increases under the influence of Se-methionine in renal tissue of hepatectomized rats. Conclusion: During regeneration, polyamine metabolism in renal tissue modifies. Se-methionine suplementation has a protective effect.

INTRODUCTION

Selenomethionine (Se-Met) is a component of selenoproteins which are present in great amounts in plants like a broccoli, garlic, onions and, also, in fish meet [1]. A great number of experimental and apllied investigations point out the fact that the ingestion of Se-Met with food inhibits the apearance of malignant diseases, especially lung, colon and prostate carcinomas [2]. The biological mechanisms underlying the cancer chemopreventive effects of Se-Met supplementation have yet to be elucidated [3].

Polyamines, spermine, spermidine and putrescine are naturally-occurring cellular components essential for physiological functions of all living cells [4]. In mammalian cells polyamines derive from amino acids L-arginine (via L-ornithine) and L-methionine in the form of S-adenosyl-methionine (SAM), by a series of enzymatic reactions. SAM serves as a propylamine donor for the biosynthesis of polyamines, spermidine and spermine Selenium metabolism and polyamine biosynthesis are linked in that they both require S-adenosylmethionine as a co- factor [5]. .

Concerning the numerous literature suggestions about the protective effects of Se-methionine and the significance of polyamines in cell metabolism, the examinations of Se-methionine supplementation effects on polyamine metabolism in regenerating rat liver tissue 7 days after two-third partial hepatectomy were done in our laboratory [6]..

The idea of the present study was to elucidate the influence of Se-Met on polyamine metabolism in renal tissue of hepatectomized rats treated with Se-Met for seven days, through investigation of polyamine oxidase (PAO) and diamine oxidase (DAO) activities, and by the estimation of malondialdehyde (MDA) levels, the crucial marker of lipid peroxidation. Our results confirmed that during the regenerative period of liver tissue PA metabolism in renal tissue was modified.

MATERIAL AND METHODS

Male albino Wister rats, weighing 150-180 g, were used for

this study. The following experiments were conducted in accordance with the principles and procedures of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996).

Partial hepatectomy was performed by the resection of two-thirds of liver tissue mass (67%), according to the classical technique of Higgins and Anderson [7], under Kethalar anesthesia, through a midline abdominal incision and consisted in the removal of the median and left lateral lobes of the liver. Operations were done under clean, but not sterile conditions. After surgery, animals were housed in individual cages under controlled and uniform conditions of light and temperature. They had free access to their respective diet until the time of killing.

Immediately after operation, the hepatectomized animals were divided into two groups: the animals that received Se-Met ("Sigma") intraperitoneally, in a single daily dose of 2.5 g per 100g body weight, and those that received 0.9 NaCl instead of the drug. The animals received the same dose of the drug for seven days. The last dose of selenomethionine was applicated on the 7th day after hepatectomy, one hour before sacrificing. Sham operated animals (the control group) received 0.9 NaCl. Sham operation consisted of laparatomy with removal of a small piece of omental fat.

The animals were killed by bleeding through abdominal aorta. The kidneys were removed quickly, the excess of blood was removed by blotting, renal fascia discarded and kidneys have been frozen at -70oC for up to one week, until they were analyzed. The renal tissue was cut in small pieces and homogenized in ice-cold water. The water homogenate (10 w/v) was used for the estimation of PAO and DAO activities, determination of MDA and protein amounts.

For PAO and DAO activity determination the homogenates (10 w/v) were centrifuged at 1500 x g for 10 min at 4oC. PAO (spermine-tetra hydrochloride obtained from "Sigma", was used as a substrate) and DAO (putrescine-dihydrochloride "Sigma" was used as a substrate). The enzyme activities were measured in the resulting supernatants by spectrophotometric method of Bachrach and Reaches [8], modified by Quash et al. [9]. One unit of enzyme activity was defined as an increase in optical density of 0.100 at 660 nm. The amount of MDA was determined by spectrophotometric method, using thiobarbithuric acid (TBA) purchased from "Sigma"[10]. Proteins were determined according to the method of Lowry and collaborators with bovine serum albumin as a standard [11].

The obtained results were statistically analyzed using Student's T-test.

RESULTS

In renal tissue of hepatectomized animals PAO activity increases compared to the controls ($p<0.001$), while the supplementation of experimental animals with Se-Met causes the decrease of this enzyme activity ($p<0.001$) (Fig. 1).

Figure 1

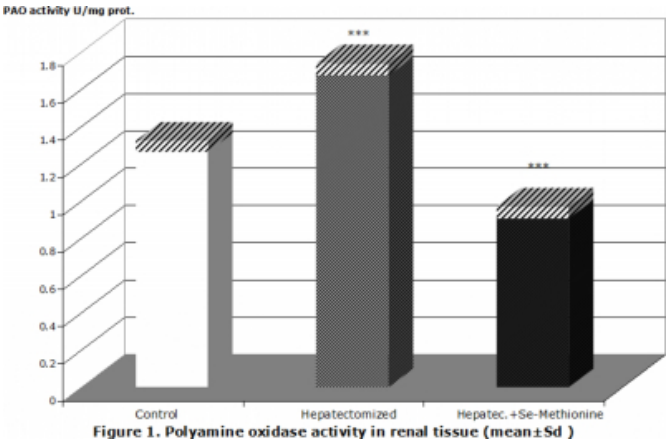

Figure 1. Polyamine oxidase activity in renal tissue (mean±Sd)

Figure 2

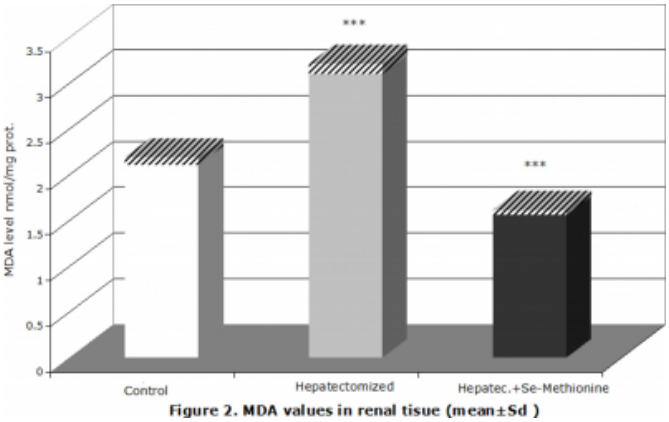

Figure 2. MDA values in renal tisue (mean±Sd)

The amount of MDA follows PAO activity changes, increasing in renal tissue of hepatectomized animals at the 7th day after operation and getting reduced upon the supplementation with Se-Met (Fig. 2).

The activity of DAO increases in renal tissue 7th day after partial hepatectomy ($p<0.001$).

Figure 3

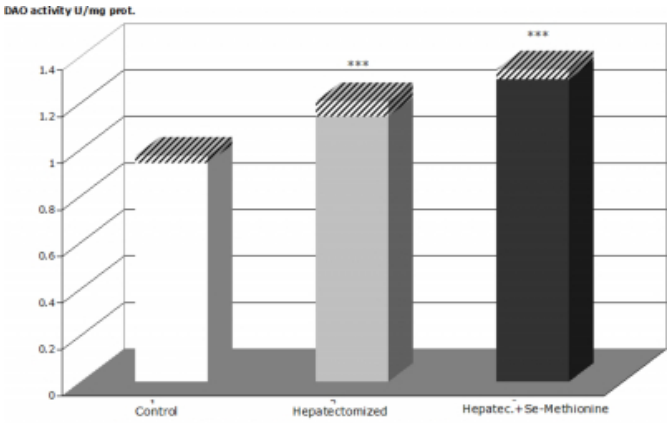

Figure 3. Diamine oxidase activity in renal tissue (mean±Sd)

The supplementation of hepatectomized animals with SeMet significantly increases DAO activity in renal tissue in comparison with the control and hepatectomized animals (Fig. 3).

DISCUSSION

Selenium is a trace element in human nutrition and plays several important roles in the forms of selenoenzymes. In adition to its role as an essential trace nutritient, selenium is thought to be associated with cancer prevention [1213]. The latest studies prove that selenium may be of great significance in disease prevention, in which pathogenesis oxidative processes play an important part [14].

Selenomethionine, an organic selenium compound, has been demonstrated to have significant chemopreventive activity. However, the mechanism of Se-Met action has yet to be identified. Previously, it was found that the treatment of cells with Se-Met induced apoptosis and altered the cell cycle [15].

Selenium compounds exert their biological effects either directly or by being incorporated into enzymes and other bio-active proteins. In Se-Met, the main organic form of Se, the selenium atom is present in the position occupied by a sulfur atom in methionine. Glutathione peroxidase and thioredoxin reductase are two natural antioxidant enzymes that contain selenium and depend upon selenium activity for their antioxidant functionality [1617]. Both selenite and Se-Met supplementation have been shown to reduce significantly oxidative DNA damage (8–OHdG formation) due to ultraviolet radiation [17].

The cancer chemopreventive effect of selenium supplementation is well known. The dietary selenium levels of which Se-Met was the primary constituent, significantly reduced the incidence of lung, colon and prostate cancers [1819].

The liver has a remarkable capacity to regenerate after injury. In rats after partial hepatectomy of 70%, the original liver mass is completely restored within 7–10 d. Liver regeneration triggered by two-third partial hepatectomy is a well-established model system in rodents for studying the molecular mechanisms of rapidly growing tissue [2021], in which the increased polyamine synthesis is well documented. Regenerating hepatocytes produce growth factors that can function as mitogens for these cells. A mitogenic signal or signals from hepatocytes appear in blood during liver regeneration. Some of the major and well-studied mitogen players, that act together in this process include: hepatocyte growth factor (HGF), growth hormon (GH), TNF-alpha, interleukin-6, epidermal growth factor, norepinephrine, insulin and many others [2223]. Some of them influence polyamine metabolism [2425].

HGF, also known as scatter factor (SF), was first identified as blood-born hepatic mitogen arising during liver regeneration. Several studies have shown that HGF is dramatically increased following partial hepatectomy, leading hepatocytes into DNA synthesis [2627] HGF is growth factor with multiple cellular effects that are important for the development and tissue regeneration. However, HGF mitogenic responses are found only in liver and kidney after partrial hepatectomy, although HGF is an active mitogen for many different cell types. Although it was first described as a growth factor for hepatocytes, HGF has been shown to be an important factor in renal organogenesis and in renal injury. HGF stimulates the growth of renal epithelial cells (mitogen), enhances the motility of epithelial cells (motogen) and induces renal epithelial tubule formation (morphogen), by acting on the single trans-membrane tyrosine kinase receptor c-met. HGF prevents acute renal failure and accelerates renal regeneration in mice [262829].

The experimental results show that HGF afects polyamine metabolism. When human HGF was intravenously injected into normal rats and rats after 70% hepatectomy, the hepatic content of putrescine and activities of catalytic enzymes of putrescine biosynthesis were significantly increased [30].

In hepatocyte culture HGF increases simultaneously ornithine decarboxylase and S-adenosylmethionine decarboxylase activities, polyamine concentration and DNA synthesis [31]. Spermidine and spermine are essential for HGF-induced DNA synthesis in primary cultured rat hepatocytes [32]. An increase in polyamine levels has been

found in HGF-induced apoptosis [33]. Growth hormone (GH) afects polyamine biosynthesis in rat liver increasing ODC activity [343536]. Constitutively elevated levels of circulating GH increase and considerably change the activity of ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) in liver and kidney [37]. Human insulin-like growth factor I (IGF-I) is known to be the mediator of growth hormone dependent proliferation. In IGF-I-mediated growth in various organ systems in rats polyamines obviously play an important role [38].

Our results confirmed that during the regenerative period of liver tissue polyamine metabolism in renal tissue modifies and that Se-meth supplementation to hepatectomized animals caused the decrease of renal PAO activity compared to hepatectomy. PAO catalyzes the oxidative deamination of polyamines spermine and spermidine, producing corresponding amino aldehyde, H_2O_2, NH_3 and malondialdehyde (MDA), potentially toxic agents, involved in induction of apoptosis in mammalian cells. The amount of MDA follows PAO activity; it increases after operation in kidney tissue and decreases upon Se-Met treatment.

The obtained results support the literature evidence that selenium affects the uptake, degradation and inhibition of free oxygen radicals, thus protecting the body cells against premature ageing, cell damage and metabolic disturbances which lead to oxidative stress and malignancy (20). Selenium leads to MDA concentration decrease in the serum of rabbits or rats with experimental hypercholesterolaemia [39]. Taken in mind PAO activity and MDA levels in our results, we may conclude that supplementation of rats with SeMeth has a protective effect to kidney tissue of hepatectomized animals.

There are obscure literature data about Se-Met influence on putrescine metabolism. Injection of Se-Met to female rats, results in significant increases in liver selenium inducing ODC and AdoMet DC activities. This induction does not result in concomitant increases in putrescine, spermidine and spermine [40]. However, the previous investigations point out that selenium application in the form of selenite leads to putrescine level decrease in malignant-proliferating tissue [12]. In our study, the increased DAO activity in animals treated with Se-Met could be the reason for the mentioned decrease of putrescine amount. We have represented the similar effects of Se-Met to DAO activity in our earlier paper [6].

On the basis of literature evidence and our recently published results, it could be suggested that the relevance of these findings to elucidation of the biological activities attributable to Se-Met, considering polyamine metabolism, need further investigation.

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