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Comparative excretion and tissue distribution of selenium in mice and rats following treatment with the chemopreventive agent 1,4-phenylenebis(methylene)selenocyanate

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Abstract

In a previous preliminary investigation, we reported on the excretion, tissue disposition and metabolism of the chemopreventive agent 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) in the rat, but similar studies in the mouse have not been explored. Following the oral administration of *p*-XSC (50 μmol/kg body weight), selenium excretion in feces was comparable to that in urine in mice, but in rats, feces was the major route of excretion. Tetraselenocyclophane (TSC) was the major metabolite detected in mouse and rat feces. In both species, levels of selenium in exhaled air were negligible. At termination, in the mouse, the stomach had the highest selenium content followed by liver and blood, but lung and kidney contained negligible levels of selenium; in the rat, the selenium level in liver was the highest followed by kidney, stomach, blood and lung. The identification of TSC as a fecal metabolite in both species let us to postulate the following metabolic pathway: *p*-XSC → glutathione conjugate (*p*-XSeSG) → a selenol (*p*-XSeH) → TSC. Since the glutathione conjugate appears to be the proximal precursor for the selenol metabolite that may be an important intermediate in cancer chemoprevention, we report for the first time the synthesis of *p*-XSeSG and its other potential metabolites, namely the cysteine- and *N*-acetylcysteine-conjugates of *p*-XSC. HPLC analysis of the urine and bile showed a few metabolites of *p*-XSC; none of which eluted with the synthetic standards described above. When we examined the conversion of *p*-XSC and *p*-XSeSG in vitro using rat cecal microflora, TSC was formed from *p*-XSeSG but not from *p*-XSC. The formation of TSC from *p*-XSC in vivo but not in vitro suggests that *p*-XSC needs to be metabolized to *p*-XSeSG or an intermediate derived from its further metabolism. Thus, *p*-XSeSG was given orally to rats and the results showed that the pattern of selenium excretion after *p*-XSeSG treatment was similar to that of *p*-XSC; TSC was also identified as a fecal

Abbreviations: *p*-XSC, 1,4-phenylenebis(methylene)selenocyanate; TSC, tetraselenocyclophane; *p*-XSeSG, glutathione conjugate of *p*-XSC; *p*-XSe(NAc)Cys, *N*-acetylcysteine conjugate of *p*-XSC; *p*-XSeCys, cysteine conjugate of *p*-XSC; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrum

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metabolite of *p*-XSeSG. It may be that the conversion of *p*-XSeSG to TSC is too facile, or the mere conjugation of *p*-XSC with glutathione does not occur in rats and mice.

Keywords: Selenium; *p*-XSC; Glutathione conjugate of *p*-XSC; Excretion; Tissue distribution; Rats; Mice

1. Introduction

We have developed several synthetic organoselenium compounds that are effective cancer chemopreventive agents, and are less toxic than either inorganic selenium or certain naturally occurring selenoamino acids [1,2]. Bioassays using rats showed that one of the most effective of these compounds, 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC), inhibits tumors in the mammary glands, colon and tongue [3–5]. Furthermore, we demonstrated that diet supplementation with *p*-XSC inhibits the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumors in A/J mice [6], reduces pulmonary metastasis of B16BL6 melanoma cells and inhibits the growth of these metastatic tumors in mouse lung, in part, by inducing apoptosis [7]. Thus, in addition to its remarkable chemopreventive efficacy for the inhibition of tumor development, *p*-XSC may also be valuable in preventing metastatic disease.

Metabolism is important in determining the chemopreventive efficacy and toxicity of selenium compounds [8], as shown, for instance, in the case of sodium selenite [8,9]. After absorption, selenite is reduced by glutathione, through selenodiglutathione, to H_2Se, which is a key metabolite in both selenite chemoprevention and toxicity. H_2Se is methylated to mono-, di-, and tri-methylated derivatives, before excretion; methylselenol appears to be an important intermediate in cancer chemoprevention [9,10]. Clearly, excretion, tissue distribution and metabolism studies of selenium compounds can provide important insight into the mechanism of action.

In a previous study, we examined the excretion profile of *p*-[^{14}C]XSC in female CD rats by monitoring radioactivity and selenium content [11]. On the basis of radioactivity, ∼24% of the dose was excreted in the urine and 75% in the feces over 7 days. According to selenium measurements, <1% of the dose was present in the exhaled air. In the feces, approximately 15% of the dose was extractable with ethyl acetate and was identified as tetraselenocyclophane (TSC). The identification of TSC as in vivo metabolite of *p*-XSC led us to postulate the following metabolic pathway: *p*-XSC → glutathione conjugate (*p*-XSeSG) → a selenol (*p*-XSeH) → TSC. In this pathway, the formation of *p*-XSeH may be a critical step, since the selenol moiety is considered an important entity in cancer chemoprevention by selenium compounds. If the glutathione conjugate were the proximal precursor for the selenol metabolite, it would be expected that it would be more effective in chemoprevention than *p*-XSC. It is relevant in this respect that Rao et al. [12] demonstrated that the chemopreventive efficacy of the conjugate is better than that of the parent *p*-XSC in the azoxymethane-induced colon carcinogenesis in the F344 rat model. However, both agents were equally effective in the DMBA-induced mammary carcinogenesis in the CD rat model [13]. Collectively, these results suggest that *p*-XSeSG is an important putative metabolite in cancer chemoprevention by *p*-XSC in the rat mammary and colon tumorigenesis model systems.

The excretion pattern, tissue distribution, and metabolism of *p*-XSC in A/J mice have never been investigated. Furthermore, the form of selenium that is responsible for the chemoprevention of *p*-XSC against the development of NNK-induced lung tumorigenesis in A/J mice is not known [6]. Therefore, we examined the excretion pattern and tissue distribution of selenium in mice treated with *p*-XSC by gavage. For comparison, a parallel study was conducted in CD rats using *p*-XSC. In addition, we report for the first time a full description of the synthesis of *p*-XSeSG and related compounds that might represent its further metabolites, i.e. cysteine- and *N*-acetylcysteine-conjugates of *p*-XSC (Fig. 1).