Selenomethionine or Methylseleninic Acid Inhibits Mutagenesis of a Reporter Gene in Mouse Bone Marrow

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Abstract. Recent laboratory and clinical studies have utilized selenium in the form of pure seleno-L-methionine (SeMet) in combination with DNA-damaging cancer chemotherapy drugs. In mice, the selenium protected bone marrow and other tissues from dose-limiting toxicity. In fact, because of the protection from dose-limiting toxicity, a doubling or even tripling of the maximum tolerated dose (MTD) was enabled. Previously we showed that SeMet protects bone marrow by a DNA repair mechanism that requires the XPC DNA repair protein. XPC is rate-limiting and is required for repair of cisplatin or carboplatin adducts. Herein we used a mouse strain that carries a lambda phage reporter gene in the genome that serves as a mutagenesis target. SeMet protects from carboplatin mutagenesis in mouse bone marrow. Methylseleninic acid (MSA), a metabolite of SeMet, also protected against mutagenesis in mouse bone marrow.

Agents which enhance the selectivity of cancer chemotherapy have been eagerly sought. Typically, dose-limiting toxicity to bone marrow and other tissues is an impediment to successful chemotherapy. Numerous approaches have included agents that protect bone marrow, or agents that sensitize cancer cells. An agent that did both, protect bone marrow and sensitize cancer cells would be highly valuable. Studies in mice have shown that seleno-L-methionine (SeMet) holds promise as such an agent. Firstly, it is well-tolerated and does not cause toxicity by itself. It is now a subject of human clinical trials (1). Secondly, in mice, the maximum tolerated dose (MTD) of chemotherapy drugs was doubled and even tripled when combined with SeMet (2). Thirdly, distinct anticancer effects are attributed to SeMet either alone or in combination with chemotherapy drugs in both human and mouse studies (1-4).

Cells possess a baseline capacity to repair DNA damage such as that caused by cisplatin or carboplatin, important chemotherapy drugs used in the treatment of many types of cancer. In each of the experimental conditions referred to above, selenium pretreatment was required in order to modulate the effects of subsequent chemotherapy drugs. We reported previously that SeMet enhanced DNA repair of carboplatin-induced DNA damage in mouse bone marrow and fibroblasts. SeMet did not enhance DNA repair in cancer cell lines. Thus, the SeMet promoted a selective DNA repair response that favored cell survival in bone marrow (5, 6).

Here, we used a mouse strain that carries a lambda phage reporter gene in the genome. One can treat cells with a DNA-damaging agent, prepare genomic DNA, then rescue wild-type or mutant lambda phage with the use of in vitro phage packaging extracts. The frequency of mutant phage can be used to calculate mutant frequency. Pretreatment with SeMet or MSA decreased the mutant frequency in mouse bone marrow cultures.

Materials and Methods

Abbreviations: XPC, protein encoded by the xeroderma pigmentosum Xpc gene; NER, nucleotide excision DNA repair.

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Selenium and carboplatin treatments. In mice and in human patients receiving SeMet, a serum selenium concentration of 15 μM was obtained (1). A fraction of SeMet is however metabolized to methylselenol, that fraction being variable among tissues (2). Serum selenium concentrations do not allow the identification of biochemical form after SeMet metabolism. Therefore, we used either 15 μM SeMet, to parallel the in vivo studies, or 1 μM MSA, to mimic the metabolite. Both selenium compounds are water soluble and were kept frozen as 15 mM stock solutions in sterile water. Cell cultures were treated 15 h with SeMet or MSA. For some experiments as indicated, SeMet or MSA treatment was followed by carboplatin to induce DNA damage, at concentrations and duration indicated.

Big Blue mutagenesis assays. The Big Blue mouse carries a lambda LIZ phage reporter gene integrated in the genome. After treatments of Big Blue cell cultures, genomic DNA was prepared using Qiagen columns with reagents supplied by the manufacturer (Qiagen, Valencia, CA, USA) and packaged into lambda phage using Stratagene Gigapack packaging extracts. Plaques were visible on a lawn of E Coli G1250 (Stratagene) after culture at 37˚C overnight. The lambda LIZ system carries a temperature-sensitive allele of the lambda cII gene. As such, all plaque-forming units can form plaques at the permissive temperature of 37˚C. However, only mutant plaques can grow at the non-permissive temperature of 24˚C. Therefore, the ratio of the number of plaques at 24˚C to the number of plaques at 37˚C provides a measure of mutant frequency of the cII gene as a target. Plaques were counted manually using a colony counter.

Results

The DNA repair protein XPC is relevant to the protective mechanism. SeMet treatment of bone marrow cells of wild-type mice resulted in increased cell survival after carboplatin treatment (Figure 1). The relevance of XPC was shown by the use of bone marrow derived from Xpc<sup>−/−</sup> mice (6). Xpc<sup>−/−</sup> bone marrow was sensitive to carboplatin and was not rescued by SeMet, even when the carboplatin dose was corrected to be approximately equitoxic in wild-type and Xpc<sup>−/−</sup> bone marrow (Figure 1). Thus, Figure 1 shows that XPC is important to the SeMet protective mechanism. Xpc<sup>−/−</sup> bone marrow lacked the protective response.

A prediction that follows is that if a bona fide DNA repair response is induced, then SeMet or its metabolite MSA would inhibit mutagenesis. As our interest is in protecting bone marrow, we tested if SeMet or MSA would inhibit mutagenesis in bone marrow, using carboplatin as a DNA-damaging agent. As is shown in Figure 2, SeMet (15 μM, 15-h treatment) or MSA (1 μM, 15-h treatment) significantly inhibited mutagenesis in bone marrow (Figure 2).

Discussion

In mice, SeMet has been shown to protect bone marrow and gut epithelium from cancer chemotherapy drugs. In fact, the MTD for chemotherapy drugs was increased by 2-3-fold in SeMet-treated mice (2). At the same time, xenograft tumors were cured in SeMet-treated mice, tumors that did not grow back even months post-treatment.
While the molecular basis for the observed selectivity is probably complex, we have focused on a selective DNA repair response involving XPC.

We showed previously that p53 and a p53-regulated DNA repair protein XPC can contribute to the observed selectivity. Specifically, bone marrow, gut epithelium, and other dose-limiting tissues have wild-type p53 genes and SeMet treatment results in elevated XPC protein and elevated DNA repair of cisplatin or carboplatin DNA damage (5). Conversely, the majority of cancer cells carry somatically-acquired p53 mutations, rendering p53, and the elevation of XPC protein inactive. Thus, SeMet enhanced DNA repair in bone marrow and gut, but did not enhance DNA repair in cancer cells (5).

The importance of the XPC protein in protecting bone marrow is evident by our experiments utilizing Xpc−/− mice. In wild-type mice, the bone marrow is protected by SeMet (Figure 1), but Xpc−/− bone marrow cannot be rescued by SeMet and is in fact quite sensitive to carboplatin irrespective of whether SeMet is used (6). Thus, cancer cells that carry alterations in Xpc genes might, like the Xpc−/− mouse, exhibit marked sensitivity to carboplatin.

Surprisingly, this study is the first to test the hypothesis that selenium would inhibit mutagenesis. The literature is complicated by the use of different selenium compounds, but we used SeMet because that is the form widely used in clinical trials for both cancer prevention and in combination with chemotherapy (1, 3, 7). SeMet decreased mutation frequency in the lambda phage plaque-forming assay (Figure 2). MSA, a metabolite of SeMet, likewise decreased mutation frequency. Thus the mechanism is shared between the two compounds.

The mechanisms we have shown pertain to XPC protein and global nucleotide excision DNA repair specifically. We would caution against extrapolation to other classes of DNA damage. Our data pertain to cisplatin, carboplatin, nitrogen mustards including melphan, and possibly oxaliplatin. Although other authors have shown selenium protective effects in bone marrow against, for example ionizing radiation (8), the mechanism we show here may not be applicable to those findings.

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Conflict of Interest Statement

The Authors declare that there are no conflicts of interest.

References