Oral and Topical L-Selenomethionine Protection from Ultraviolet-Induced Sunburn, Tanning and Skin Cancer

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Abstract

This study demonstrates that both oral and topical selenium (Se) supplementation can reduce the incidence of acute and chronic damage to the skin (i.e., sunburn and tanning or pigmentation and skin cancer) induced by ultraviolet (UV) irradiation without giving any signs or symptoms of toxicity. Groups of mice were treated with 1) vehicle lotion, 2) 0.02% L-selenomethionine (SeMet) lotion, or 3) vehicle lotion and 1.5 ppm SeMet in the drinking water. Within each group, most mice were given UV irradiation three times per week while others served as controls without UV exposure. Measurement of the animals' weights and food intakes and clinical evaluation demonstrated that mice treated with Se showed no signs of toxicity. The Se concentrations of skin and liver showed that both means of delivery increased the level of Se in the skin and the liver, with the skin Se concentrations higher in areas where the lotion was directly applied, UV irradiation caused significantly less damage to the skin of the mice treated with Se. No animals given either topical or oral Se developed any blistering typical of sunburn as did the non-Se-treated animals. Scoring of skin pigmentation demonstrated reduced tanning (a measure of free radical damage to the skin) in the Se supplemented mice. Furthermore, weekly counts of the total number of clinically detectable skin tumors demonstrated that mice treated with Se had a delayed onset and a markedly lesser incidence of skin cancer induced by the UV irradiation.

Abbreviations

Se, selenium; SeMet, L-selenomethionine; SeGSHpx, selenium-dependent glulathionine peroxidase; SOD, superoxide dismutase.

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Selenium (Se) is an essential trace element in humans and in animals. Selenium levels are usually maintained in the body through food. Good sources include whole grain cereals, seafood, garlic, liver and eggs. Foods from animal sources are generally richer than those from vegetable sources, so vegetarians should supplement their diet with Se to fulfill the requirement. Unfortunately all foods lose Se in processing — for example brown rice has 15 times the Se content of white rice, and whole-wheat bread contains twice as much Se as white bread.

Se is unevenly distributed throughout the world. Se rich soils are thought to result from ancient volcanic eruptions and subsequent leeching to ancient inland seas long since evaporated. Wind and rain may remove Se from the soil into the sea, thus causing a deficiency in locally grown plants and animal feed. Areas which were glaciated in the Ice Age have had all of the Se removed by snow-melting glaciers. Certain areas of the United States (such as South Dakota) and areas of China have such high levels that ruminant animals such as cattle can sometimes develop metabolic toxicity.

Oral Se is known to protect against cancer, primarily because it is an essential component of the antioxidant enzyme glutathione peroxidase,[1] the only known Se-containing enzyme in the body. However Se has been shown to have other protective effects that may not involve Se-dependent glutathione peroxidase (SeGSHpx) activity, such as repairing DNA,[23] reducing the DNA binding of carcinogens,[4] inhibiting neoplastic transformation,[5] and suppressing gene mutations at the lysine and histidine loci.[6]

Extensive proof that Se reduces cancer has been demonstrated in animal tumor models. Moderate Se supplementation at levels above the dietary requirements has been shown to

decrease the number of tumors induced by several chemical carcinogens[7-13] and viruses[14] and to reduce the incidence of spontaneous mammary tumors.[15] In addition, Se supplements have been shown to inhibit the growth of transplanted tumors in mice[1416] and to decrease the mutagenic activity of several known carcinogens.[17-19] In tissue culture, Se has been shown to reduce the metabolic activation of certain carcinogens by altering the patterns of their degradation to produce less toxic metabolites.[20"24]

The effect of Se on decreasing the incidence of various types of cancer in humans has been investigated. Epidemiological studies[2526] have demonstrated in areas where soil Se levels are high, there is decreased death due to cancer, and where Se soil levels are lower, there is increased death from cancer. Several retrospective case studies have detected significant inverse correlations of the incidence of internal neoplasms with blood Se concentrations.[27-31] However, such data are difficult to interpret because of the possibilities that the cancer can affect the general nutritional status and, therefore, the Se status of patients and that the neoplastic tissue may sequester Se. One perspective study done by hypertension detection and follow-up groups in six US medical centers also detected an inverse correlation between blood Se and cancer incidence.[32] Furthermore, a study of 240 skin cancer patients in good general heath demonstrated a significantly lower mean plasma Se concentration than control subjects without skin cancer.[33] In fact, those patients whose blood concentrations were in the lower decile had 4.4 times the incidence of skin cancer as those in the highest decile.[33]

The effect of Se specifically on skin cancer has been investigated prior to the research reported here. Two previous studies conducted with hairless hr/hr mice demonstrated that oral administration of sodium Selenite can inhibit acute reactions such as inflammation , pigmentation, hyperkeratosis, and ulceration[34] as well as skin carcinogenesis induced by ultraviolet (UV) irradiation.[35] In the latter study, the protective effect of Se against skin cancer was dose dependent. However, the oral doses used in each of these studies appear to have been toxic, inasmuch as they produced moderate inhibitions in growth rates.

The study presented here[36] was conducted to determine whether 1) oral Se in the form of L-selenomethionine (SeMet) protects against pigmentation and skin cancer induced by UV irradiation and 2) topical SeMet gives similar protection.

The present study employed Se in the form of SeMet, which is known to be absorbed transdermally[37] and is shown in the studies presented here to give increased liver levels of Se in mice.

Se has been used for years in topical preparations primarily because of its antifungal properties. Shampoos and lotions containing Se sulfide (2.0% as over-the-counter preparations in the former or 2.5% by prescription in the latter) effectively treat tinea versicolor, a common superficial fungal infection of the skin.[38] Other lotions and shampoos containing the same chemical form of Se (1.0-2.0%) are effective in the treatment of seborrheic dermatitis and dandruff.[39] However, the Se from these preparations is apparently not absorbed by the skin,[40] so that the effect of these formulations is truly only on the outer layers of human skin.

Materials and Methods

Animals and Treatments

Groups of 38 BALBx female mice (shaved weekly) or 16 Skh:2 hairless, pigmented, dark-eyed, female mice were treated with 1) lotion vehicle, 2) 0.02% SeMet lotion (100 *k* per application), or 3) vehicle lotion and 1.5 ppm SeMet in the drinking water. All animals were fed a nutritionally adequate diet (Purina 5001) which was found on actual analysis to contain 0.171 ppm of Se. Throughout the studies, the weight of the mice and the feed and water intakes were monitored weekly.

The mice were given Se supplementation beginning one week prior to UV exposure for the rest of their lifetimes. During the period of irradiation, the lotion was applied three times per week at least 30 minutes before each UV-irradiation exposure. Each application of skin lotion appeared to penetrate the skin within 10 minutes.

To check that the cutaneous and oral supplementation gave increased tissue (i.e., skin and liver) levels of Se, 15 weeks after the topical application of the lotions, six irradiated and four non-irradiated BALB:c mice were killed by cervical dislocation. The surviving BALB:c mice were killed at 39 weeks

and the surviving Skh:2 mice, at 49 weeks. These termination times were determined by the requirements for statistical analysis constrained by the high mortality of the mice not supplemented with Se.

Lotion and Se Supplementation

The lotion was a standard oil-in-water form of cosmetic base.[36]

SeMet (Nutrition 21, San Diego, CA) was mixed into the lotion carrier at a concentration of 0.02% Se. Those animals supplemented orally were given water containing 1.5 ppm SeMet. The amount of SeMet provided by topical application was about equal to that provided by oral supplementation; i.e., about 6 ng Se per mouse per week provided by either route.

Assays for Se and Antioxidant Enzymes

To test the correct level of Se in the skin, no topical Se was applied to the mice for six days before they were killed, although oral supplementation was continued until they were killed. Se was measured in tissues and feed samples by the fluorimetric method of Olsen and coworkers.[41] The activity of SeGSHpx in tissue homogenates was measured by the method of Paglia and Valentine[42] as modified by Lawrence and Burk,[43] with 0.25 M H_20_2 as substrate and the activity of superoxide dismutase (SOD) was measured by the method of Misra and Fridovich.[44] Protein was determined by the method of Lowry;[45] enzyme activities were expressed per unit protein.

UV Irradiation

The irradiation was given three times per week using four Westinghouse FS40 sunlamp bulbs. The irradiation was initiated at approximately 75% of the average minimal erythema and increased incrementally until the maintenance exposure times (50 minutes and 15 minutes per session for the BALBx and the Skh:2 mice, respectively) were attained. This irradiation continued for 28 weeks for the BALB:c mice and for 24 weeks for the Skh:2 mice. This dose of UV irradiation has been shown to induce pigmentation and skin cancers in a similar breed of mouse.[34,35]

Evaluation of Skin Damage Induced by UV Irradiation

All animals were examined weekly to determine the degree of short-term sun damage, i.e., sunburn which is inflammation as indicated clinically by erythema and blistering in both breeds and pigmentation (or tanning) in the Skh:2 mice. Skin pigmentation of the Skh:2 animals was graded biweekly until 16 weeks, at which time maximal pigmentation was observed. To assess the degree of skin pigmentation, scoring was done by two independent observers "blind" (i.e., without knowing the animals' supplementation group): 0 = no pigmentation and 4 = maximal darkening. Also the numbers and sizes of tumors on each animal were noted weekly. Tumors >3 mm and <5 mm and those >5 mm were counted separately. Occasionally in the Skh:2 mice, small tumors enlarged to co-join to appear as one large tumor; in those cases, the tumor count remained two. The diagnosis of tumor was confirmed by biopsy and histological examinations of clinically representative tumors from each animal.

Evaluation of Eye Damage

The eyes of all animals were examined at 15 and 38 weeks after dilation of the pupils with 1.0% tropicamide with a slit-lamp biomicroscope to evaluate possible corneal and lenticular damage. When the six UV-exposed BALBx mice from each group and four non-UV-irradiated BALBx mice were killed at 15 weeks, the eyes were preserved in formalin and analyzed histologically.

Autopsy

Because previous experiments on hr/hr hairless mice had demonstrated an increased incidence of leukemia and malignant lymphoma (approx. 3.3%) in mice exposed to UV irradiation,[46] as evidenced by enlarged mediastinal and peripheral lymph nodes and a varying degree of perivascular infiltrate of the liver and kidneys, complete autopsies were done on three mice from each UV-irradiated group after death or after they were killed.

Statistical Analysis

For most parameters, treatment effects were evaluated by analyses of variances by the Cox-Mantel (log-rank) test[47] and regression analysis according to the Cox proportional hazards model.[48] Furthermore, the time to develop a specific number of tumors was analyzed.[49]

Results

Mouse Growth Rate and Water and Food Intake

The average body weight of the BALB:C mice exposed to UV irradiation (21.8 ± 0.2g) was slightly decreased compared with that of non-irradiated controls (24.2 ± 0.5g), despite a slightly increased food intake and a comparable water intake . The body weight and the water and food intake of the Skh:2 mice were not affected by exposure to UV irradiation . The application of SeMet by either route had little effect on the food or water intake or on the body weights. In all cases, both treated and untreated mice thrived.

Levels of Se and Antioxidant Enzyme

The topical application of the SeMet substantially enhanced Se concentration in the skin of both the BALB:c and the Skh:2 mice, with a preferential increase in Se levels in the dorsal skin, the localized area actually treated. Liver Se levels were also increased after both topical application and oral administration. It is interesting to note that with both oral and topical Se-supplementation during the period of acute UV damage (15 weeks), the Se contents of the skin and liver were increased more than after the UV exposure was terminated. No significant changes in the activities of the hepatic enzymes selenium-dependent glula-thionine peroxidase (SeGSHpx) or superoxide dismutase (SOD) were observed.

Skin Damage Induced by UV Irradiation

Despite the fact that the initial exposure to UV was equal to only about 75% of the minimal erythema dose (MED) for these animals and the increase in UV exposure was gradual, two-thirds of the BALB:c mice and all of the Skh:2 mice not given oral or topical SeMet developed at least one blister within the second or third week of irradiation. None of the topically or orally supplemented animals developed any blisters typical of acute sunburn.

By 12 weeks, the Skh:2 mice had developed maximal tanning. Both oral and topical SeMet effectively reduced the UV-induced skin pigmentation. The oral delivery was slightly more protective than the topical initially but with continued UV-irradiation (by 12 weeks), both forms were equally effective in their protection, as shown below:

Pigmentation of Skh:2 Mice After Exposure to UV-Irradiation

Week	Control	Topical Se	Oral Se
7	3.3 ±0.4	2.3 ±0.3	1.7 ± 0.2
12	3.5 ± 0.3	2.7 ± 0.2	2.5 ± 0.2
16	3.6 ± 0.2	2.6 ± 0.3	2.6 ± 0.3

(Values are ± standard deviation on a scale of 1 to 4, with 0 = no pigmentation and 4 = maximal pigmentation.)

Skin tumors were induced in the animals exposed to UV-irradiation; none of the control mice (not exposed to UV-irradiation) developed any tumors. The UV-exposed BALB:c mice characteristically had one or two nodular squamous-cell carcinomas per animal, confirmed by biopsy and histologic analysis. The Skh:2 mice characteristically developed multiple tumors; some Skh:2 mice were riddled with tumors, while others had only one or two large tumors. The tumors were either clinically and histologically similar to those in the BALBx mice, or they were keratoacanthoma-like. All tumors biopsied were carcinomas varying from well differentiated to poorly differentiated; none were benign.

Figure 1 (see figures pages 92-94) shows the number of tumors >3 mm in size observed in the three irradiated groups of BALB:c mice. Figure 2 shows the number of animals having tumors >3 mm in size. These graphs clearly show that both topical and oral selenium supplementation lead to comparable protection against skin cancers. Note also that both topical and oral SeMet retarded the onset of skin cancers by about five to six weeks. The non-irradiated control groups had no tumors during the experimental period.

Figure 3 shows the number of tumors >3 mm in size observed in the three irradiated groups of Skh:2 mice. The Skh:2 mice were more susceptible to skin tumors induced by UV-irradiation than the BALBx mice because not only did they begin to show clinically apparent tumors earlier with smaller doses of irradiation, but also they developed more tumors per animal. Figure 4 shows the number of animals having tumors >3 mm in size. By the termination of the experiment at 49 weeks, almost all mice had at least one tumor >3 mm. These figures clearly show that

topical and oral SeMet administration protected against skin cancers induced by UV-irradiation in the Skh:2 mice. In observing the number of tumors induced, the oral SeMet appears to be somewhat more effective than the topical for the Skh:2 mice. As with the BALBx mice, both topical and oral SeMet also effectively retarded the onset of clinically apparent tumors by nine and five weeks, respectively. The non-irradiated control group had no tumors observed during the experimental period.

Figure 5 and 6 show the mortality rate of UV-irradiated BALBx and Skh:2 mice. The non-irradiated control mice had no deaths during the experimental period. These figures suggest a shortening of lifespan caused by exposure to UV irradiation. Supplementation with Se may protect against the shortening of lifespan induced by UV irradiation, though a definitive conclusion cannot be made based upon data from this limited number of animals.

Two methods of statistical analysis of the tumor data (i.e., the Cox Mantel [log rank] test[47] and the Cox proportional hazards model of regression analysis[48]) demonstrated that for the BALB:c mice, both oral and topical SeMet were equally effective in reducing the risk of skin cancer. Statistical analysis of the two treatments applied to Skh:2 mice showed oral SeMet to be somewhat more effective than topical in reducing the UV-induced skin cancers.

Eye Damage Induced by UV Irradiation

High doses of sodium Selenite administered intraperitoneally or subcutaneously at certain stages of development are known to cause nuclear cataracts in some animals.[50,51] Furthermore, UV irradiation has been shown to be cataractogenic in animals.[52] Peroxidation of lentricular plasma membrane lipids is one of the molecular mechanisms involved in cataract formation in humans,[53] and the activity of SeGSHpx is decreased in both animal and human cataractous lenses (K.C. Bhuyan, unpublished observation). Therefore, the dilated eyes of all BALB:c mice were examined with a slit-lamp ophthalmoscope at weeks 15 and 38 to evaluate potential adverse reactions or possible therapeutic effects of Se on corneal vascularization and opacity and cataract induced by UV irradiation. There was no significant effect of Se treatment in protecting against UV-induced vascularization and opacity of the cornea or cataract in the UV-irradiated mice at either week 15 or 38. Histologic examination demonstrated that there were no specific nuclear cataracts[50,51] which would have indicated an adverse reaction to the Se administered.

Autopsy

No case of leukemia or malignant lymphoma was detected in the limited number of cases autopsied.

Discussion

Selenium was first recommended for cancer therapy more than 70 years ago[54] and has never been proven to be carcinogenic in man. Although one early claim exists in the scientific literature that the trace mineral selenium may be carcinogenic in rats fed with a low protein and extremely high selenium diet,[55] that study was discredited since the "tumors" noted were most probably hyperplasia of cirrhotic livers. Many subsequent studies have never corroborated that research, and many recent studies demonstrate significant anticarcinogenic effects for many types of cancers.[8,25-33]

Clearly, these experiments demonstrate that SeMet, administered either topically or orally, is effective in protecting against skin cancer induced by UV irradiation both by retarding the onset and reducing the number of lesions. Statistical analysis showed topical and oral administration to be equally effective in BALBx mice, whereas oral administration was more effective in the Skh:2 mice.

This study further demonstrates that both oral and topical SeMet were effective in reducing the acute damage induced by UV irradiation-inflammation (sunburn), blistering, and pigmentation (tanning). None of the mice treated with SeMet by either route developed blisters during the early weeks of UV irradiation, but about two-thirds of the BALB:c mice and all the Skh:2 mice exposed to the UV irradiation with no Se supplementation had at least one observable blister. A direct antiinflammatory effect in the Se has been studied previously: e.g., sodium Selenite was found to have and anti-inflammatory effect in the Selye granuloma induction assay in rats.[56] Also, inflammatory reactions may be medi-

ated via membrane damage. Because Se is an essential component of SeGSHpx, the anti-inflammatory actions noted in those experiments might have been due to the decreased oxidative damage to skin cell membranes.

Both oral and topical SeMet were effective in reducing the pigmentation of the UV-ex-posed Skh:2 mice. UV-induced tanning is caused by some combination of several mechanisms — division of melanocytes, activation of pigment formation in amelanogenic melanocytes, migration of dermal melanocytes into the epidermis, and increased transfer of melanosomes to keratinocytes.[57]

Mice have been extensively used in studies of photocarcinogenesis because tumors can be so readily induced.[58] Obviously, the hairless mouse has been used more because shaving the hair is not required. The vulnerability to skin cancer may be a result of a limited capacity to repair UV-induced pyrimidine dimers,[59,60] the thin stratum corneum,[61] and, in the case of the BALBx mice, the inability to form pigment. This model is ideal for evaluation the efficacy of SeMet against UV-induced damage to the skin.

Two previous studies on hairless hr/hr mice indicated that oral administration of sodium Selenite via the drinking water at doses of 8, 4, and 2 ppm (comparable to 4.4, 2.2, and 1.1 ppm Se, respectively) could inhibit the acute inflammation and pigmentation[34] as well as the number of tumors[35] induced by UV irradiation. However in those experiments, mice experienced a moderate inhibition of growth rate as evidenced by lower body weight. In contrast, in this experiment the SeMet supplementation of 1.5 ppm in the drinking water (i.e., to 0.6 ppm Se) with comparable doses given topically gave no inhibition of growth rate.

Although there was no evidence of any adverse affects in these experimental mice due to Se supplementation, very high levels of selenium (orders of magnitude greater than those which were shown here to be effective in protecting against UV-induced damage) have been reported in animals and humans to have adverse effects. In humans Se toxicity is rare. With a few exceptions, documented cases of acute Se toxicity have involved the occupational exposure of workers in copper smelting or Se rectifier plants with inhalation of Se fumes from fires or heated metals.[62-66]

Chronic Se toxicity has been reported in humans as the result of high intake of oral supplements. The consumption of 1 mg per day of Na_2Se0_3 (equal to five times the maximal recommended daily dose) appears to produce no toxic signs or symptoms.[67] Although there were no signs of Se toxicity in patients with neuronal ceroid lipofuscinosis given 1.6 mg per day,[68] one 62-year-old man given 0.9 mg/day of Na_2Se0_3 for two years developed garlic breath odor and thickened, fragile nails, both signs of selenosis.[69] These symptoms subsided as soon as he discontinued taking the Se supplement. It is important to note that the blood levels of Se were not monitored in these individuals. Therefore, based on the experiments presented herein and documented reports in medical literature, it is highly unlikely that the small amount of Se demonstrated to be effective in protecting against UV damage could cause any systemic or local adverse reaction.

Clearly the level of Se was increased in the skin and the liver after both topical and oral administration of SeMet. With the topical application, the level in the dorsal skin at the site of application was higher that in ventral skin. Interestingly, the levels measured in the skin of the BALB:c mice at 15 weeks (when the UV damage was acute) were higher than at 39 weeks (after the UV-irradiation had been terminated for 11 weeks). Possibly the inflammation caused by the acute UV damage caused increased percutaneous absorption. Also, the level of Se in the skin of BALBx animals was higher with topical application than with oral administration, whereas in the Skh:2 mice the levels were comparable with both means of supplementation. This result might be attributed to greater percutaneous absorption of the topical formulation in the BALB:c mice because of slight exfoliation of the skin caused by the weekly shaving.

The fact that Se is absorbed percutaneously by topical application of SeMet was further substantiated by measurement of the short term (i.e., 15 and 20 hours) uptake of topical ^{75}SeMet in rats fitted with plastic "Elizabethan" collars to prevent self-licking. Indeed an increase in the ^{75}Se content of plasma and liver was measured (G.F. Combs, et al, manuscript in preparation).

Because the diet contained a nutritionally adequate amount of Se, it was not expected

that extra Se supplementation would affect the activity of SeGSHpx,[70] as was observed. In further experiments the activity of SeGSHpx in the skin of Skh:2 mice was not elevated in areas of increased Se after the application of topical SeMet (K.E. Burke, C. Keen, R.N. Nakamura, manuscript in preparation. These results suggest that the protective effect of SeMet against UV-induced skin cancer did not involve this protective antioxidant enzyme.

Since the obvious antioxidant parameters were not altered by Se supplementation, this author is currently investigating other potential mechanisms, including identifying the precise cellular location of the Se within the tumors by electron microscopy (K.E. Burke and V. Garnys, manuscript in preparation).

There is no doubt that UV-induced skin cancer formation is a cumulative process that begins with initial exposure.[71] In fact, it has been estimated that one blistering sunburn in a child doubles the potential to develop skin cancer as an adult.[71] If mouse tumorigenesis, with its short latent period, can be inhibited effectively with topical or oral SeMet as the experiments presented here demonstrate, a similar effect might be expected for humans with their long latent period. Indeed, regular use of a sunscreen with a sun protection factor (SPF) of 15 during the first 18 years of life may reduce by 78% the lifetime incidence of non-melanoma skin cancer.[72]

Therefore, the protection which might be provided by either topically or orally administered SeMet may be of great significance to individual health. This is of special importance especially today when because of the decrease in the protective filtering of UV-irradiation by the ozone layer and increased outdoor leisure, the number of skin cancers has increased more than any other form of cancer, and second only to lung cancer, the deaths due to skin cancer have increased more than deaths due to any other form of cancer. The author recommends that every adult take 100 ug/day of SeMet, particularly during summer months. Any individual who has had a personal history of cancer of any kind should take 200 jig/day.

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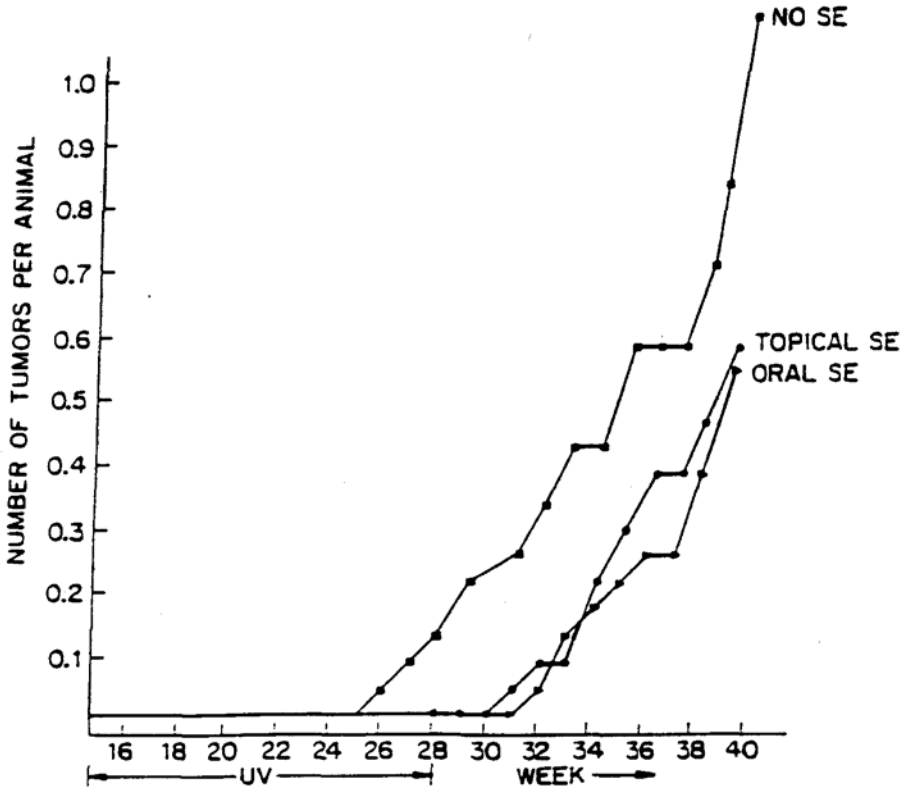

Figure 1. The effect of L-selenomethionine in reducing the number of skin tumors (>3m) per animal in UV-irradiated BALB:c mice.

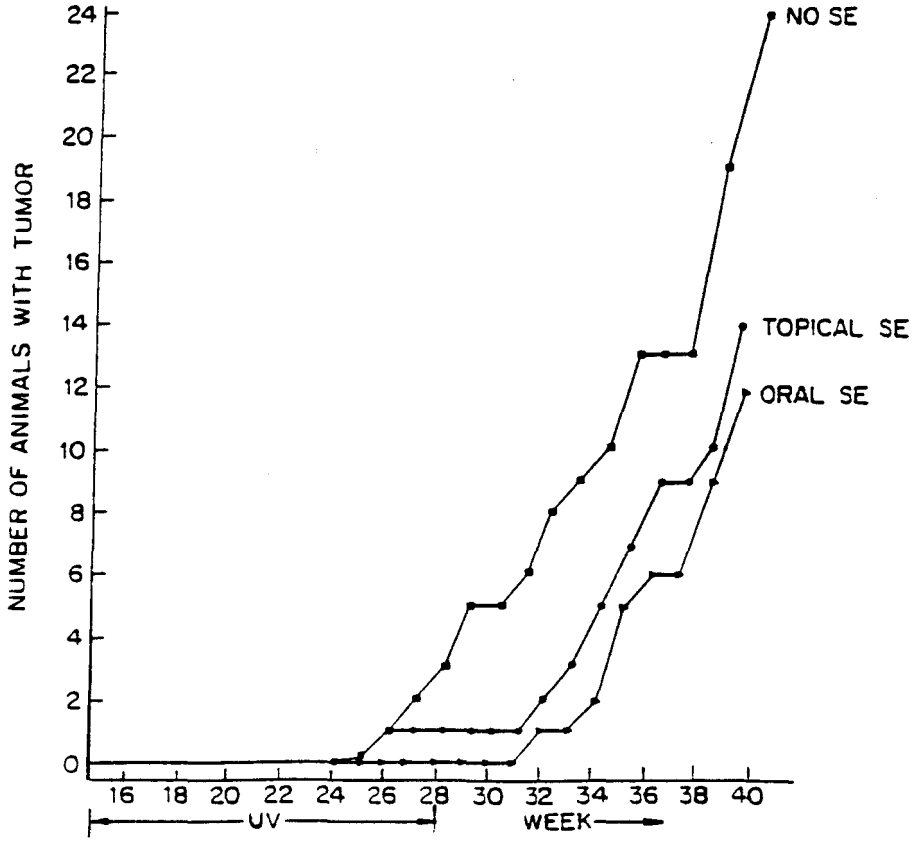

Figure 2. The effect of L-selenomethionine in reducing the number of BALB:c mice with skin tumors >3 mm after UV-irradiation.

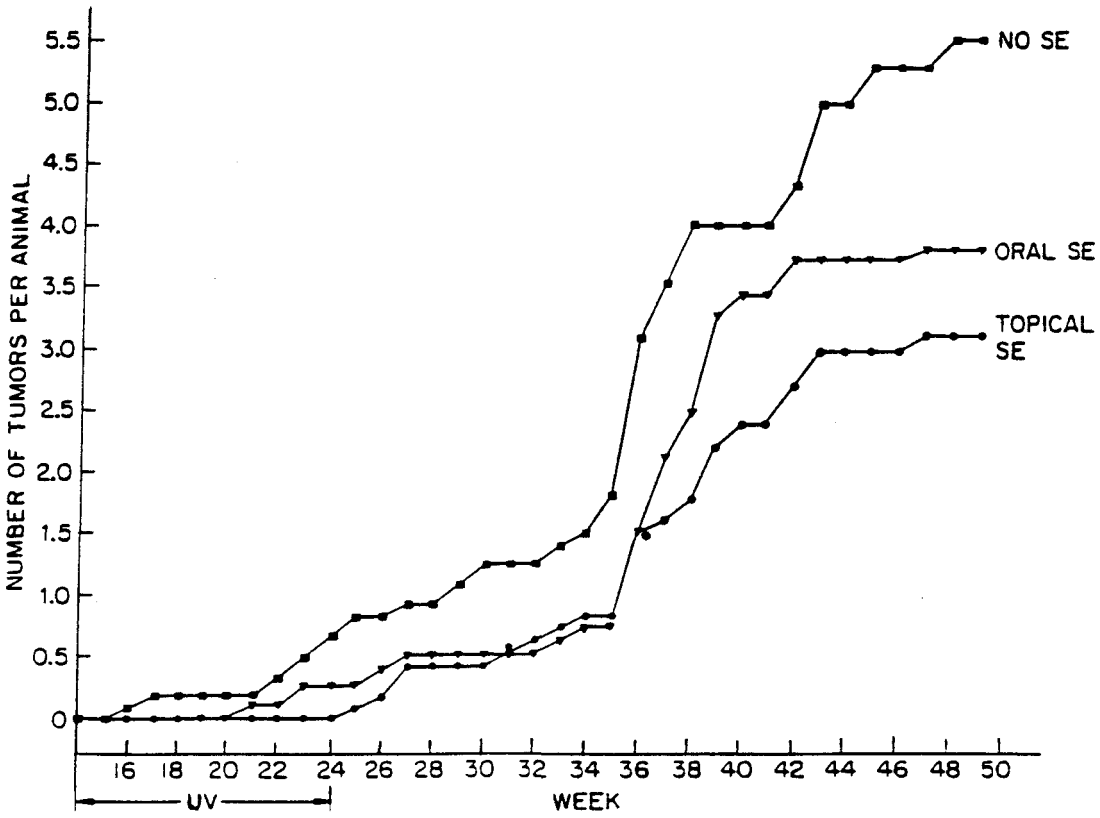

Figure 3. The effect of L-selenomethionine in reducing the number of skin tumors (>3mm) per animal in UV-irradiated Skh:2 mice.

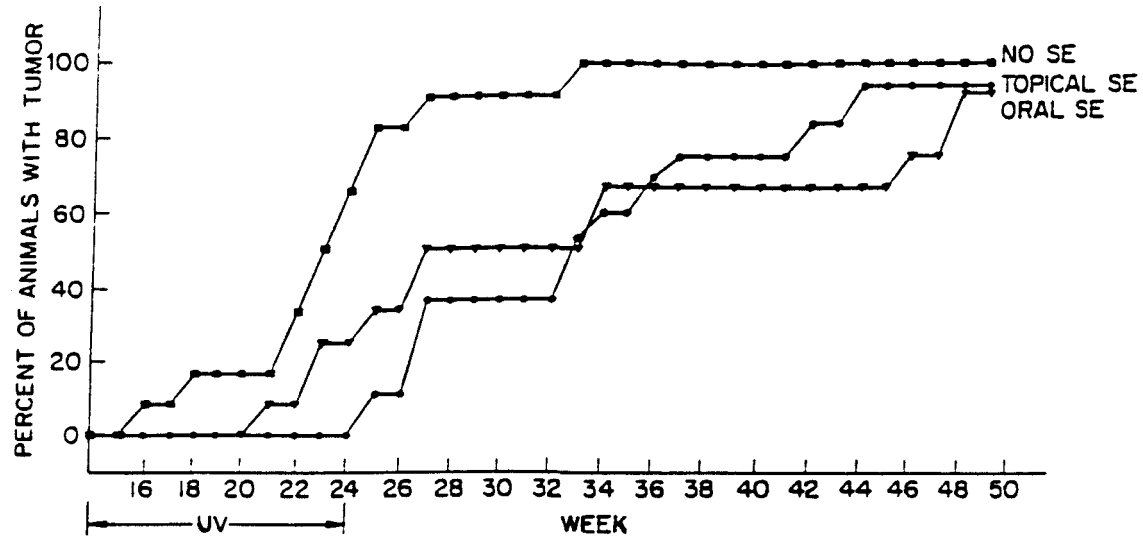

Figure 4. The effect of L-selenomethionine in reducing the number of Skh:2 mice with skin tumors >3 mm after UV-irradiation.

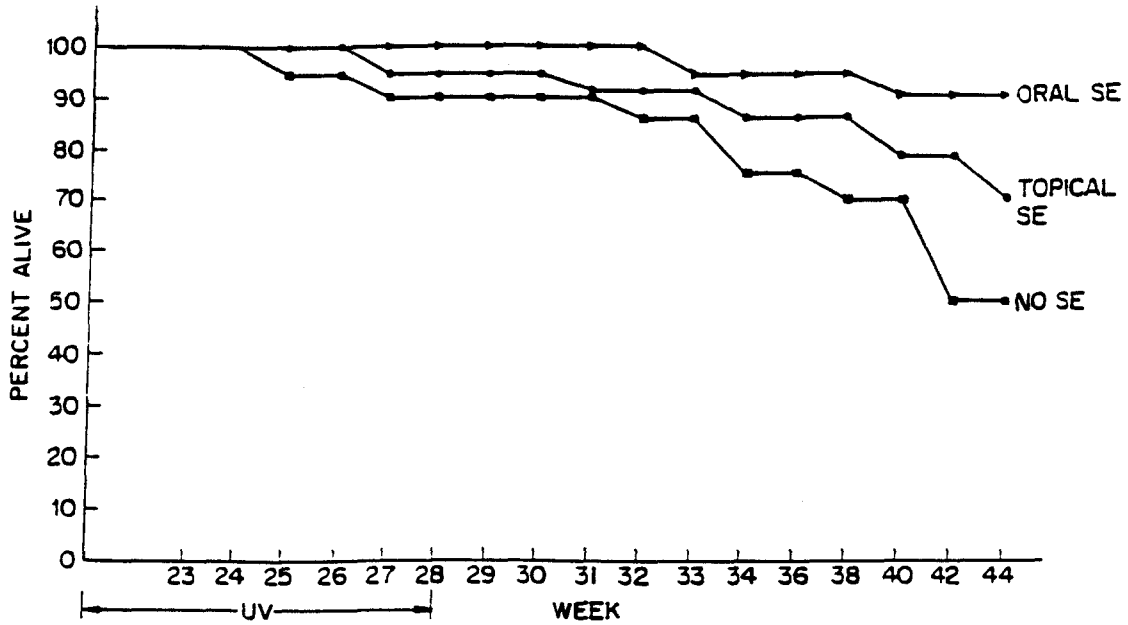

Figure 5. The effect of L-selenomethionine in protecting against increased mortality of BALB:c mice after UV-exposure.

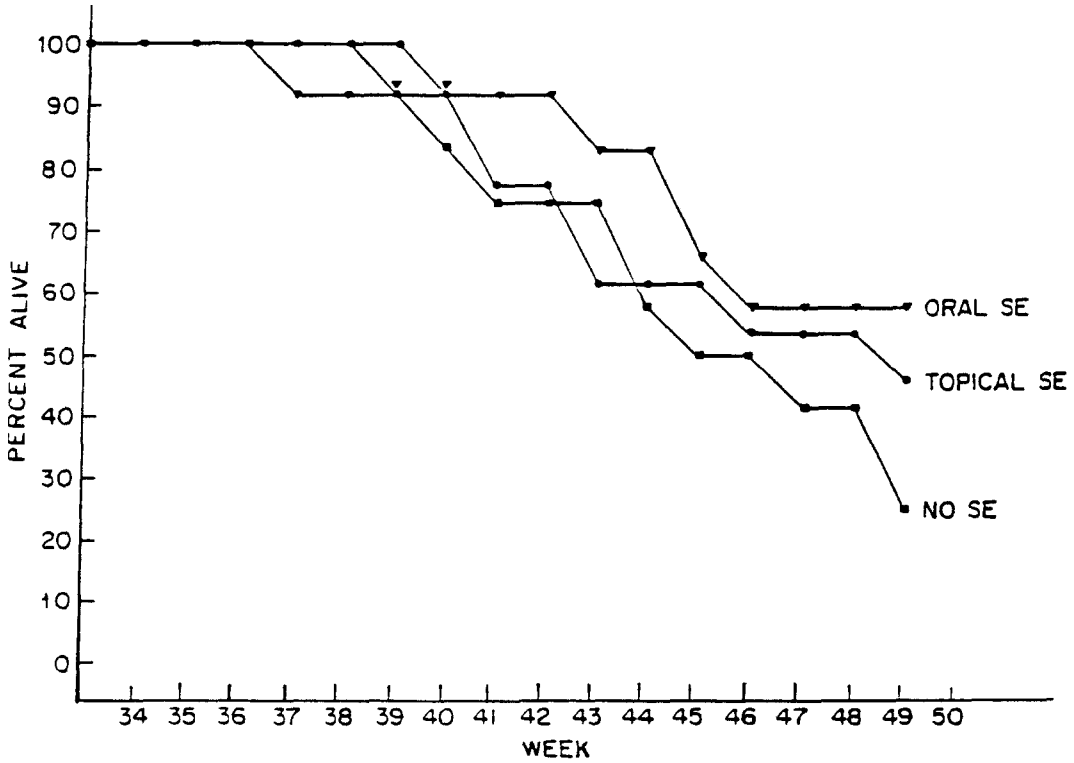

Figure 6. The effect of L-selenomethionine in protecting against increased mortality of Skh:2 mice after UV-exposure.