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Subchronic oral toxicity studies of Se-methylselenocysteine, an organoselenium compound for breast cancer prevention

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Abstract

Se-methylselenocysteine (MSC) is an organoselenium compound being developed for breast cancer chemoprevention. To characterize MSC toxicity, CD rats received daily gavage doses of 0, 0.5, 1.0, or 2.0 mg/kg/day (0, 3, 6, or 12 mg/m²/day), and beagle dogs received daily gavage doses of 0, 0.15, 0.3, or 0.6 mg/kg/day (0, 3, 6, or 12 mg/m²/day) for 28 days. In rats, MSC induced dose-related hepatomegaly in both sexes; mild anemia, thrombocytopenia, and elevated liver enzymes were observed in high dose females only. Microscopic pathology included hepatocellular degeneration (high dose males, all doses in females); arrested spermatogenesis (high dose males); and atrophy of corpora lutea (middle and high dose females). In dogs, MSC induced mild anemia in middle and high dose males, and in high dose females. Toxicologically significant microscopic lesions in dogs were seen only in the liver (peliosis and vacuolar degeneration in high dose males, midzonal necrosis in males in all dose groups). Based on liver pathology seen in female rats in all dose groups, the No Observed Adverse Effect Level [NOAEL] for MSC in rats is < 0.5 mg/kg/day. Based on alterations in hematology parameters and liver morphology in male dogs in all dose groups, the NOAEL for MSC in dogs is < 0.15 mg/kg/day.

Keywords

Se-Methylselenocysteine; Selenium

1. Introduction

The potential activity of selenium (Se) as a cancer preventive agent has been of interest since its original identification as a component of the glutathione peroxidase system. Through this role, Se may protect the cell against oxidative damage, presumably acting through mechanisms that involve catalyzed reduction of lipid hydroperoxides (Hoekstra, 1975). Since these early observations, a large number of additional mechanisms have been proposed through which Se may inhibit carcinogenesis in the breast and other tissues (Ip et al., 2002; El-Bayoumy and Sinha, 2004). These mechanisms include, but are not limited to, alterations in Phase I and Phase II enzyme activities, thereby resulting in decreased production and/or enhanced detoxification of carcinogens (Ip and Lisk, 1997; Sohn et al., 1999); antiproliferative effects, perhaps

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mediated via inhibition of DNA synthesis, protein kinase C, and/or kinase signaling pathways (Sinha et al., 1996; Sinha et al., 1999; Ip et al., 2000; Unni et al., 2004; Hwang et al., 2006); prevention of clonal expansion of preneoplastic lesions (Ip and Dong, 2001; Ip et al., 2002); caspase activation and the induction of apoptosis (Unni et al., 2001; Yeo et al., 2002; Unni et al., 2004; Goel et al., 2006); and inhibition of angiogenesis (Jiang et al., 1999; Corcoran et al., 2004; Mousa et al., 2006).

In consideration of the broad range of potential anticarcinogenic activities of Se compounds, a large number of epidemiology studies have been conducted to investigate the hypothesis that Se status is inversely associated with human cancer risk. These epidemiology studies have generated both positive and negative results, but do provide substantial support for the hypothesis that Se compounds have cancer preventive activity (Combs, 2005). In recent years, numerous investigators have reported inverse associations between Se biomarkers (toenail Se or serum Se levels) and the risk of neoplasia in several sites, including the prostate (van den Brandt et al., 2003; Vogt et al., 2003; Li et al., 2004), esophagus and gastric cardia (Wei et al., 2004), liver (Sakoda et al., 2005), colon (Ghadirian et al., 2000; Jacobs et al., 2004; Peters et al., 2006), lung (van den Brandt et al., 1993; Zhuo et al., 2004), skin (Burke et al., 1992), and urinary bladder (Zeegers et al., 2002). It should be noted, however, other studies have found no association between Se status and the risk of cancer of the breast (Dorgan et al., 1998; Ghadirian et al., 2000; Männistö et al., 2000), prostate (Ghadirian et al., 2000; Allen et al., 2004), ovary (Pan et al., 2004), non-cardiac stomach (Wei et al., 2004), urinary bladder (Michaud et al., 2002), or lung (Mahabir et al., 2006). Intervention trials have reported similarly mixed data, as individuals receiving Se supplementation as part of the Nutritional Prevention of Cancer Trial demonstrated significant decreases in prostate cancer risk (Clark et al., 1996), but had increased risks of developing melanoma or non-melanoma skin cancer (Duffield-Lillico et al., 2003).

A much more consistent picture emerges from experimental studies of Se chemoprevention. Numerous studies have demonstrated that Se supplementation can inhibit carcinogenesis in animal models for human cancer (reviewed in Ip et al., 2002; El-Bayoumy and Sinha, 2004); target tissues in which Se protects against carcinogenesis include the mammary gland, liver, colon, skin, and lung. The chemopreventive activity of Se compounds has been demonstrated most convincingly in animal models for breast cancer; both inorganic (selenite, selenate) and organic (selenomethionine [SeMet], selenocysteine, *Se*-methylselenocysteine [MSC]) forms of Se demonstrate useful preventive activity against cancer induction in the rodent mammary gland. It should be noted, however, that although chemoprevention studies in many animal tumor systems demonstrate inhibition of carcinogenesis by Se compounds, a few have shown no effect (e.g., Lijinsky et al., 1989; McCormick and Rao, 1999), and at least one study demonstrated an enhancement of carcinogenesis by Se supplementation (Birt et al., 1988).

It is clear that both the dose level and the molecular form of Se are important components of chemopreventive activity. In studies to compare the relative chemopreventive efficacy of inorganic and organic forms of Se, sodium selenite was more effective in reducing mammary tumor incidence than was the same amount of Se administered as SeMet (Thompson et al., 1984). Treatment with SeMet was not only less effective in cancer prevention than was selenite, but was also more toxic, as it induced severe liver damage. Similarly, in studies using the DMBA model, Ip and Hayes (1989) found that SeMet was less active than selenite over a graded dose range corresponding to 1 to 5 ppm Se. Interestingly, however, Se concentrations in blood, liver, kidney, and skeletal muscle, and the half-life of glutathione peroxidase were higher in rats treated with SeMet than in animals fed equivalent doses of selenite (Ip and Hayes, 1989), suggesting that the nutritional and anticarcinogenic actions of Se compounds may be separable. In this regard, Ip et al. (1991) have proposed that selenite and SeMet may require

further metabolism to elicit anticarcinogenic activity, and suggest that monomethylated or partially methylated forms of Se may be the active chemopreventive species.

Se-methylselenocysteine (MSC) is a monomethylated selenoamino acid that is being developed for breast cancer prevention (Medina et al., 2001). MSC confers significant protection against breast cancer induction in rodent model systems (Ip et al., 1999; Medina et al., 2001; Unni et al., 2004), and preliminary toxicology data suggest that MSC may induce less systemic toxicity than do either inorganic Se or other organic Se compounds such as SeMet (El-Bayoumy and Sinha, 2004). MSC is rapidly metabolized to methylselenol, and recent studies suggest that methylselenol may be a critical mediator of the anticarcinogenic activity of MSC and other organoselenium compounds (reviewed in Ip et al., 2002).

A critical consideration for the use of Se compounds in cancer chemoprevention is their significant toxicity. The results of dose-response studies for chemopreventive efficacy and toxicity suggest that the margin of safety (ratio of minimum toxic dose to minimum effective dose) for most Se compounds is quite narrow. Most chemoprevention studies using pure selenium compounds have been conducted using Se dose levels of 2 to 5 ppm in the diet or in drinking water. Significant toxicity is encountered at Se dose levels only marginally greater than 5 ppm. In general, organic Se compounds such as selenoamino acids are less toxic (per μ mole Se) than are inorganic forms of Se; among organic Se compounds, preliminary data have been presented by Ip and colleagues suggesting that MSC may be less toxic than are other organic Se compounds such as SeMet.

The present studies were conducted to provide a systematic evaluation of the toxicity of MSC in rats and dogs, to identify sensitive target tissues for the toxicity of MSC in the two species, and to support the identification of appropriate starting dose levels of MSC for use in initial clinical trials with this agent.

2. Materials and methods

Prior to the initiation of *in vivo* experimentation, study protocols were reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All aspects of the program involving animal care, use, and welfare were performed in compliance with United States Department of Agriculture regulations and the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Both studies were conducted in full compliance with the Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).

2.1. Subchronic oral toxicity study in rats

Male and female CD rats (CrI:CD®[SD]IGSBR) were received at approximately five to six weeks of age from virus-free colonies maintained at Charles River Laboratories (Portage, MI). Rats were housed individually in suspended stainless steel cages in a temperature-controlled room maintained on a 12 h light/dark cycle, and were held in quarantine for two weeks prior to the initiation of drug administration. With the exception of scheduled fasting periods, rats were allowed free access to Certified Rodent Diet 5002 (PMI Nutrition International, Inc., Brentwood, MO). City of Chicago drinking water was supplied to rats *ad libitum* using an automatic watering system.

After release from quarantine, animals were assigned to experimental groups using a computer-based randomization procedure that blocks for body weights. Groups of 20 rats/sex received daily oral (gavage) exposure to MSC at doses of 0.5, 1.0, or 2.0 mg/kg/day (0, 3, 6, or 12 mg/m²/day; approximate selenium doses of 0.2, 0.4, and 0.8 mg/kg/day) in a vehicle of purified water (5 ml/kg/day) for 28 days, or to purified water only (5 ml/kg/day) for the same period.

MSC was supplied by the National Cancer Institute. MSC dose levels used in the 28-day toxicity study were selected on the basis of a preliminary 14-day range-finding study; in the range-finding study, significant suppressions of animal body weight were observed within the first week of exposure in groups receiving MSC doses of 1.4 mg/kg/day or greater (data not shown). On a mg/m² basis, doses of MSC used in the 28-day toxicity study in rats provide multiples of 20 to 200-fold over presumed human doses resulting from self-medication with commercially available capsules that contain from 100 to 250 µg of MSC.

Throughout the study, rats were observed a minimum of twice daily to monitor their general health status; detailed clinical examinations and measurements of body weight and food consumption were performed weekly. Indirect funduscopy ophthalmic examinations were performed on all animals during the quarantine period (pre-test) and during the final week of the treatment period. Blood samples for clinical chemistry, hematology, and coagulation evaluations were collected from fasted rats at the terminal necropsy (day 29). Clinical pathology assays were performed using automated instruments (Synchron CX5 Clinical Chemistry Analyzer [Beckman Instruments, Brea, CA]; Advia System 120 Hematology Analyzer [Bayer Corp., Tarrytown, NY]; MLA Electra 900 Automatic Coagulation Timer [Hemoliance, Raritan, NJ]). Non-tissue binding of MSC was not examined.

On study day 29, all surviving rats were euthanized by CO₂ overdose and underwent a complete gross necropsy with tissue collection. At necropsy, weights of the adrenals, brain, heart, kidneys, liver, ovary/testes, spleen, thyroids, and uterus were collected. All gross lesions plus approximately 45 tissues per rat were collected and fixed in 10% neutral buffered formalin. Histologic processing and histopathologic evaluations were performed on all tissues from all rats in the high dose and vehicle control groups; histologic processing and histopathologic evaluation of tissues from animals in the middle and low dose groups were limited to gross lesions and identified target tissues.

2.2. Subchronic oral toxicity study in dogs

Male and female purebred beagle dogs were received at approximately five to six months of age from CRP, Inc. (Kalamazoo, MI), and were held in quarantine for three weeks prior to randomization into experimental groups. Dogs were housed individually in stainless steel cages in a temperature-controlled room maintained on a 12-h light/dark cycle. Dogs were provided with 400 g of Certified Canine Diet 5007 (PMI Nutrition International, Inc.) for a minimum of 2 h each day, and were permitted free access to City of Chicago drinking water supplied via an automatic watering system. Each dog received a supervised daily exercise period outside of its cage.

After release from quarantine, dogs were assigned to experimental groups using a computerized randomization program that blocks for body weight. Groups of four dogs/sex received daily oral (gavage) exposure to MSC at doses of 0.15, 0.30, or 0.60 mg/kg/day (0, 3, 6, or 12 mg/m²/day) in a vehicle of purified water (2 ml/kg/day) for a minimum of 28 consecutive days. These MSC doses equate to approximate selenium doses of 0.06, 0.12, and 0.24 mg/kg/day, respectively, and provide multiples of presumed human doses (administered by self-medication rather than in a controlled clinical setting) that are comparable to those reported for the rat study. Vehicle controls received daily gavage exposure to purified water (2 ml/kg/day) only. MSC dose levels used in the 28-day toxicity study were selected on the basis of a preliminary 14-day range-finding study; in the range-finding study, mortality was observed in dogs receiving MSC at a dose of 2.0 mg/kg/day (data not shown).

Throughout the study, dogs were observed a minimum of twice daily to monitor their general health status. Detailed clinical examinations and body weight measurements were performed weekly and food consumption was quantitated daily. Indirect funduscopy ophthalmic

examinations and electrocardiographic (ECG) evaluations were performed on all dogs during quarantine (pre-test) and during the final week of the treatment period. ECGs were evaluated for heart rate and rhythm, amplitude of the P wave and QRS complex, and duration of the P wave, PR, QRS, and QT intervals.

Blood samples for clinical chemistry, hematology and coagulation evaluations were collected from fasted dogs at pre-test and at the terminal necropsy. Clinical pathology assays were performed using automated instruments, as described for the 28-day toxicity study in rats. Non-tissue binding of SMC was not examined. Urine samples were collected from fasted dogs at pre-test and during the final week of dosing, and were analyzed by dipstick and microscopy.

At necropsy, all gross lesions and approximately 45 tissues were collected from each animal and fixed in 10% neutral buffered formalin for histopathologic evaluation. All tissues collected from all study dogs were processed by routine histologic methods and evaluated histopathologically.

2.3. Statistical Analyses

Statistical evaluation of continuous data from the rodent and canine toxicology studies was performed by analysis of variance (ANOVA), with *post-hoc* analyses performed using Dunnett's test. Incidence data were compared by χ^2 analysis or Fischer's Exact Test. A minimum significance level of $p < 0.05$ was used in all comparisons.

3. Results

3.1. Subchronic oral toxicity study in rats

Daily gavage administration of MSC to rats for 28 days at doses of up to 2 mg/kg/day (12 mg/m²/day) induced no mortality in either sex. No evidence of treatment-related gross toxicity was identified during clinical observations of male rats exposed to any dose of MSC used in the study. In females, agent-related clinical observations were limited to dose-related alopecia in the middle (4/20 rats) and high (10/20 rats) dose groups.

MSC induced dose-related body weight loss and/or suppression of body weight gain in both sexes. All groups of male rats gained body weight throughout the dosing period (Fig. 1). However, mean terminal body weight in male rats receiving daily oral exposure to the high dose of MSC was reduced by approximately 12% versus sex-matched vehicle controls ($p < 0.05$). Throughout the study, group mean body weights in male rats receiving the middle or low doses of MSC were slightly, but not significantly, reduced from those seen in male rats in the vehicle control group.

MSC had a much greater effect on body weight in female rats than in males. During the first week of exposure, dose-related body weight loss was seen in groups receiving the middle and high doses of MSC ($p < 0.05$ versus vehicle control; Fig. 2). At the end of the second week of MSC exposure, mean body weight in female rats in the high dose group was still below their initial (Day 0) weight; body weight in the high dose group remained significantly below the mean body weight of female vehicle controls throughout the dosing period. Body weights in female rats in the middle dose group were also significantly below sex-matched vehicle controls at all times in the exposure period ($p < 0.05$; Fig. 2). Group mean body weights in female rats receiving the low dose of MSC were not significantly different from control.

The effects of MSC on animal body weight were clearly associated with reductions in food consumption. As seen with body weights, significant reductions in mean food consumption in male rats were seen only in the high dose group (Fig. 3). By contrast, statistically significant, dose-related reductions in mean food consumption were observed in female rats in both the

middle and high dose MSC groups during all four weeks of the dosing period, and in the low dose group during the first week of exposure (Fig. 4).

Clinical pathology evaluations performed at the terminal necropsy also demonstrated that MSC induced greater toxicity in female rats than in male rats. In male rats, statistically significant alterations in clinical pathology parameters were limited to very small (< 4%) reductions in mean RBC volume and mean cell hemoglobin in the high dose group (Table 1); all other hematologic, clinical chemistry, and coagulation parameters in male rats exposed to MSC were within normal limits and did not differ from sex-matched vehicle controls.

By contrast to the minimal hematologic effects in male rats, MSC induced mild, dose-related anemia and thrombocytopenia in female rats. Statistically significant, dose-related reductions in hematocrit, hemoglobin, mean RBC volume, and platelet count were seen in female rats exposed to the middle and high doses of MSC (Table 2). Furthermore, significant reductions in RBC count and mean cell hemoglobin content, coupled with an apparent compensatory increase in reticulocyte count, were seen in high dose females only. High dose female rats also demonstrated a statistically significant increase in prothrombin time (Table 2).

Statistically significant increases in alkaline phosphatase (152%), alanine transaminase (81%), aspartate transaminase (60%), and γ -glutamyltranspeptidase (200%) were seen in females in the high dose group only; these changes were not seen in females in the middle or low dose groups, or in males exposed to MSC at any dose level. Small but statistically significant increases in mean cholesterol (38 to 56%) and triglyceride (48 to 81%) levels were seen in females (but not in males) in all MSC dose groups.

Organ weight measurements performed at the terminal necropsy identified a number of significant effects of MSC. Increases in absolute and/or relative liver weights were observed in all dose groups in both sexes. In rats exposed to the high dose of MSC, absolute liver weights were increased by 12% in males and 18% in females, while relative liver weights were increased by 27% in males and 43% in females.

High dose male rats also demonstrated small but statistically significant reductions in the absolute weights of the adrenals, heart, testes, and thymus. In female rats, statistically significant reductions in absolute weights of the adrenals, heart, and ovaries were seen in the middle and high dose groups, while reductions in the absolute weights of the brain, thymus, and thyroids were present in females in the high dose group. However, with the exception of liver weights, none of the changes in absolute organ weights in MSC-treated rats remained significant when organ weights were normalized to terminal fasted body weights. These data suggest that observed reductions in absolute organ weights (other than the liver) reflect reductions in body weight induced by MSC, and do not demonstrate organ-specific toxic effects of the organoselenium compound.

Gross pathology in male rats at the terminal necropsy provided no evidence of MSC toxicity. In females, abnormal pigmentation and rough, irregular surface of the liver were observed in > 50% of rats in the high dose group, providing further corroborative evidence that the liver is primary a target organ for MSC toxicity. Gross pathology in other organs in female rats was unremarkable.

Histopathologic evaluation of tissues identified the liver as the primary site of MSC toxicity in both sexes of rats (Table 3). In females, hepatocellular degeneration was identified in 3/20 females in the low dose group, 7/20 females in the middle dose group, and 18/20 females in the high dose group; lesion severity also increased with increasing MSC dose (Fig. 5). Female rats in the high dose group also demonstrated peliosis hepatis (15/20 rats; Fig. 6) and hepatic subacute inflammation (9/20 rats); these changes were not identified in female rats in the middle

or low dose groups. In males, microscopic changes in the liver were limited to hepatocellular degeneration (6/20 male rats in the high dose group; no changes at lower doses); peliosis hepatic and inflammation were not seen. In addition to being identified in lower incidence, degenerative liver lesions seen in male rats in the high dose group were less severe than were comparable lesions seen in high dose females.

In male rats, testicular degeneration and arrested spermatogenesis were seen in 11/20 and 10/20 rats in the high dose group, respectively. These changes were not seen in the middle or low dose group, but did correlate with oligospermia observed in the epididymis of 11/20 high dose rats.

In female rats, MSC induced atrophy of the corpora lutea in 10/20 high dose and 7/20 middle dose rats; no ovarian changes were seen in female rats treated with the low dose of MSC. In the hair follicle, MSC induced atrophy (8/20 high dose females) and epithelial necrosis (4/20 high dose females); these microscopic changes were not seen in male rats, and were correlated to gross clinical observations of alopecia in females (only) in the high dose group. Microscopic changes in the hair follicle were not seen in female rats exposed to the middle or low doses of MSC.

Histopathologic evaluations demonstrated remaining tissues to be within normal limits in all dose groups in both sexes.

3.2. Subchronic oral toxicity study in dogs

Daily oral (gavage) administration of MSC to beagle dogs for 28 days at doses of 0.15, 0.3, or 0.6 mg/kg/day (3, 6, or 12 mg/m²/day) induced no mortality in either sex. Clinical signs were unremarkable in all groups of female dogs and in male dogs treated with the low or middle doses of MSC. One male dog in the high dose group was observed to be hypoactive at several days during the second half of the four-week dosing period.

Oral administration of MSC to dogs induced no statistically significant effects on group mean body weight, body weight gain, or mean daily food consumption in either sex in any dose group (data not shown). Group mean body weights and body weight gains in male dogs receiving the middle or low doses of MSC, and in female dogs exposed to all doses of MSC were comparable to those of sex-matched vehicle controls at all times during the study. In comparison to a mean four-week weight gain of 0.69 kg in male dogs in the vehicle control group, male dogs exposed to the high dose of MSC lost 0.02 kg during the four-week dosing period; however, this reduction in body weight gain was not statistically significant.

MSC induced a mild, but dose-related and statistically significant anemia in both sexes of beagle dogs; the effects of MSC were slightly more severe in males than in females. In male dogs, statistically significant reductions in RBC count and hematocrit were observed at the middle and high doses (Table 4), while hemoglobin concentration was reduced at the high dose only. Female dogs in the high dose group demonstrated significant decreases in hematocrit and hemoglobin concentration; no changes in these parameters were seen in female dogs exposed to the middle or low doses of MSC. Other hematology evaluations, as well as the results of clinical chemistry, coagulation, and urinalysis tests failed to identify any additional effects of MSC exposure in either sex.

The results of ophthalmic examinations and electrocardiographic evaluations provided no evidence of MSC toxicity in any treated animal. Similarly, gross pathology at the terminal necropsy was unremarkable.

As was the case in the rat study, histopathologic evaluation of tissues from the canine toxicity study identified the liver as a primary site of MSC toxicity (Table 5). It is of interest, however that the sex distribution of hepatic lesions in dogs differs from that observed in rats: whereas hepatic changes induced by MSC in rats were more severe in females than in males, microscopic alterations in liver morphology were seen in males only. In male dogs, midzonal necrosis was identified in 4/4 dogs in the high dose group, 4/4 dogs in the middle dose group, and 3/4 dogs in the low dose group; this change was present in 0/4 vehicle controls. Other hepatic changes (peliosis hepatis and midzonal degeneration; Figure 7) were identified in 1/4 male dogs in the high dose group only, and were not seen in groups exposed to the middle or low dose of MSC. No microscopic evidence of MSC toxicity was identified in the liver of any female dog in the study.

Also consistent with the findings of the rat toxicity study was the observation of an increased incidence of degenerate spermatocytes in the epididymis of male dogs.

Microscopic alterations were also identified in several other tissues of MSC-treated dogs (Table 5); however, these lesions were interpreted as being of less toxicologic significance than the liver lesions described above. Increased incidences of acute intestinal inflammation and crypt dilatation were seen in dogs of both sexes exposed to MSC. Similarly, thymic atrophy and depletion/necrosis of gut-associated lymphoid tissue were also observed in higher incidences in both male and female dogs exposed to MSC than in animals in sex-matched vehicle control groups. The results of histopathologic evaluations demonstrated that all other tissues from dogs in all dose groups in both sexes were within normal limits.

4. Discussion

The results of the present studies clearly identify the liver and the hematopoietic system as the primary targets for the toxicity of MSC in both rats and dogs. Although the relative sensitivity of males and females to MSC toxicity differs in the two species, consistent patterns of alterations in hepatic morphology and changes in hematology parameters were identified in both rodent and non-rodent test systems.

The liver appears to be the most sensitive target tissue for MSC toxicity in both rats and dogs. More than 75% of female rats exposed to the high dose of MSC demonstrated peliosis hepatis and hepatocellular degeneration; these microscopic changes were associated with statistically significant elevations in plasma alkaline phosphatase, transaminases, and γ -glutamyltranspeptidase. Peliosis hepatis was seen only in female rats in the high dose group, and was not identified in either male rats or in female rats exposed to the low or middle doses of MSC. By contrast, a statistically significant incidence of hepatocellular degeneration was identified in female rats in the middle dose group, and a non-significant ($0.05 < p < 0.10$) increase in the incidence of hepatocellular degeneration (versus sex-matched vehicle controls) was seen in female rats exposed to the low dose of MSC. Hepatocellular degeneration in female rats in the middle and low dose groups was not associated with alterations in plasma levels of transaminases or other enzymes that are commonly used as markers of liver toxicity.

The liver was also a sensitive target for MSC toxicity in dogs; however, in contrast to the results of the rat study, male dogs were identified as the more sensitive sex. Histopathologic changes in the liver were identified in male dogs only, and consisted of peliosis hepatis and vacuolar degeneration in one male dog in the high dose group, and midzonal necrosis in 3 of 4 dogs in the low dose group and 4 of 4 dogs in groups receiving the middle and high doses of MSC. Liver morphology in female dogs was within normal limits in all animals, regardless of exposure to MSC. Treatment-related effects on liver morphology in male dogs were not

associated with statistically significant alterations in any plasma enzymes that are commonly used as markers of hepatic toxicity.

MSC also had significant impact on hematologic parameters in both species. Although agent effects on animal hematology were quantitatively modest, both the middle and high doses of MSC induced statistically significant alterations in several RBC-related parameters in female rats. MSC also induced a mild thrombocytopenia in female rats, which was associated with a statistically significant increase in prothrombin time. Changes in hematology parameters in male rats were seen only in the high dose group and were of minimal severity; as such, these changes are not considered to be toxicologically significant.

MSC induced mild anemia in both sexes in dogs. In male dogs, statistically significant reductions in RBC counts and hematocrit were observed at both the high and middle dose levels; hemoglobin concentration was also significantly reduced in male dogs in the high dose group. Similarly, statistically significant reductions in hematocrit and hemoglobin concentration were observed in female dogs in the high dose group only.

The results of histopathologic evaluations suggest that the testis is a secondary target of MSC toxicity in males of both species. Additional studies will be required to determine whether the arrested spermatogenesis observed in male rats exposed to the high dose of MSC, and the increased incidence of degenerate spermatocytes observed in male dogs exposed to MSC will translate to a functional deficit in fertility.

The design of the rat and dog toxicity studies involved identical dose levels of MSC (on a mg/m² basis); as such, quantitative interspecies comparisons of agent toxicity can be made. In rats, gross (hepatomegaly) and microscopic (degeneration) liver pathology provided a sensitive indicator of MSC toxicity in both sexes, with females being more sensitive than males. Because hepatocellular degeneration was observed in female rats in all dose groups, the NOAEL for subchronic oral administration of MSC to rats was identified as being < 0.5 mg/kg/day (< 3 mg/m²/day).

Although female rats were more sensitive to MSC toxicity than were male rats, male and female dogs demonstrated the opposite pattern. Although no alterations in hepatic morphology were observed in female dogs, midzonal hepatic necrosis was seen in male dogs exposed to all dose levels of MSC. On the basis of microscopic hepatic pathology in male dogs, the NOAEL for subchronic oral administration of MSC to dogs is below 0.15 mg/kg/day (< 3 mg/m²/day).

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Abbreviations

ANOVA	analysis of variance
ECG	electrocardiogram or electrocardiographic
NOAEL	No Observed Adverse Effect Level
MSC	<i>Se</i> -methylselenocysteine

Se

selenium

SeMet

d,l-selenomethionine

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Fig. 1.
Group mean body weights in male rats receiving daily oral exposure to MSC.

Fig. 2.
Group mean body weights in female rats receiving daily oral exposure to MSC.

Fig. 3.
Group mean food consumption in male rats receiving daily oral exposure to MSC.

Fig. 4.
Group mean food consumption in female rats receiving daily oral exposure to MSC.

Table 1

Selected hematology parameters in male rats exposed to MSC

	Male RBC (10 ⁶ /μL)	Male HCT (%)	Male HGB (g/ dL)	Male MCV (fl)	Male MCH (pg)	Male reticulocyte (%)	Male platelet (10 ³ /μL)	Prothrombin time (sec)
Vehicle control	7.5 ± 0.4	44.4 ± 2.0	15.5 ± 0.6	59.3 ± 1.6	20.8 ± 0.8	3.4 ± 0.6	1093 ± 114	15.6 ± 0.9
MSC–0.15 mg/ kg	7.5 ± 0.4	43.8 ± 2.0	15.2 ± 0.5	58.2 ± 1.6	20.3 ± 0.8	3.6 ± 0.6	1072 ± 139	16.5 ± 2.7
MSC–0.30 mg/ kg	7.7 ± 0.3	44.7 ± 1.8	15.6 ± 0.6	58.4 ± 1.6	20.4 ± 0.7	3.7 ± 0.7	1131 ± 77	15.5 ± 0.7
MSC–0.60 mg/ kg	7.7 ± 0.5	44.2 ± 2.4	15.4 ± 0.9	57.8 ± 1.6 ^b	20.1 ± 0.7 ^a	3.6 ± 0.7	1146 ± 128	15.8 ± 1.0

All values are Mean ± Standard Deviation.

^a $p < 0.05$ versus sex-matched vehicle control group.

^b $p < 0.01$ versus sex-matched vehicle control group.

Table 2

Selected hematology parameters in female rats exposed to MSC

	Female RBC (10 ⁶ /μL)	Female HCT (%)	Female HGB (g/ dL)	Female MCV (fl)	Female MCH (pg)	Female reticulocyte (%)	Female platelet (10 ³ /μL)	Prothrombin time (sec)
Vehicle control	7.8 ± 0.3	47.1 ± 1.7	15.8 ± 0.4	60.2 ± 1.6	20.2 ± 0.6	4.2 ± 0.9	1198 ± 133	15.2 ± 0.5
MSC – 0.15 mg/kg	7.9 ± 0.4	47.4 ± 2.1	15.9 ± 0.7	60.5 ± 1.7	20.3 ± 0.6	3.9 ± 0.7	1194 ± 102	15.7 ± 0.5
MSC – 0.30 mg/kg	7.8 ± 0.3	45.6 ± 1.9 ^a	15.3 ± 0.7 ^a	58.6 ± 1.8 ^b	19.7 ± 0.6 ^a	4.3 ± 0.8	1067 ± 111 ^a	16.1 ± 0.5
MSC – 0.60 mg/kg	7.4 ± 0.4 ^b	43.2 ± 1.8 ^b	14.8 ± 0.6 ^b	58.3 ± 1.6 ^b	20.0 ± 0.5	5.3 ± 1.2 ^b	880 ± 183 ^b	17.4 ± 2.4 ^b

All values are Mean ± Standard Deviation.

^a p < 0.05 versus sex-matched vehicle control group.^b p < 0.01 versus sex-matched vehicle control group.

Table 3

Exposure-related microscopic lesions in rats exposed to MSC

MSC dose (mg/kg/day)	Incidence in males (No. with lesion/No. evaluated)				Incidence in females (No. with lesion/No. evaluated)			
	0	0.5	1.0	2.0	0	0.5	1.0	2.0
Liver								
-- Peliosis hepatis	0/20	0/20	0/20	3/20	0/20	0/20	0/20	15/20 ^b
-- Hepatocellular degeneration	0/20	0/20	0/20	6/20 ^b	0/20	3/20	7/20 ^b	18/20 ^b
-- Subacute inflammation	0/20	0/20	0/20	0/20	0/20	1/20	3/20	9/20 ^b
Skin								
-- Hair follicle atrophy	0/20	--	--	0/20	0/20	0/20	0/20	8/20 ^b
-- Hair follicle epithelial necrosis	0/20	--	--	0/20	0/20	0/20	0/20	4/20 ^a
Testes								
-- Arrested spermatogenesis	0/20	0/20	0/20	10/20 ^b	--	--	--	--
-- Degeneration	1/20	0/20	1/20	11/20 ^b	--	--	--	--
Epididymis								
-- Oligospermia	0/20	0/20	0/20	11/20 ^b	--	--	--	--
Ovary								
-- Atrophy of corpora lutea	--	--	--	--	0/20	0/20	7/20 ^b	10/20 ^b

^a p < 0.05 versus sex-matched vehicle control group.

^b p < 0.01 versus sex-matched vehicle control group.

Table 4

Selected hematology parameters in dogs exposed to MSC

	Male RBC (10 ⁶ / μL)	Male HCT (%)	Male HGB (g/dL)	Female RBC (10 ⁶ /μL)	Female HCT (%)	Female HGB (g/dL)
Vehicle control	7.3 ± 0.4	45.7 ± 1.4	15.7 ± 0.5	7.1 ± 0.3	46.0 ± 1.3	15.7 ± 0.6
MSC – 0.15 mg/kg	7.0 ± 0.3	43.7 ± 1.8	14.9 ± 0.7	7.3 ± 0.5	46.8 ± 1.6	15.9 ± 0.4
MSC – 0.30 mg/kg	6.6 ± 0.4 ^a	42.3 ± 1.9 ^a	14.6 ± 0.8	6.9 ± 0.2	45.1 ± 0.9	15.5 ± 0.4
MSC – 0.60 mg/kg	6.2 ± 0.3 ^a	39.0 ± 1.7 ^a	13.2 ± 0.8 ^a	6.7 ± 0.5	42.9 ± 1.6 ^a	14.6 ± 0.6 ^a

All values are Mean ± Standard Deviation.

^a p < 0.05 versus sex-matched vehicle control group.

Table 5

Exposure-related microscopic lesions in dogs exposed to MSC

MSC Dose (mg/kg/day)	Incidence in males (No. with lesion/No. evaluated)				Incidence in females (No. with lesion/No. evaluated)			
	0	0.15	0.30	0.60	0	0.15	0.30	0.60
Liver								
-- Peliosis hepatis	0/4	0/4	0/4	1/4	0/4	--	--	0/4
-- Vacuolar degeneration	0/4	0/4	0/4	1/4	0/4	--	--	0/4
-- Midzonal necrosis	0/4	3/4	4/4	4/4	0/4	--	--	0/4
Epidymis								
-- Degenerate spermatocytes	1/4	2/4	4/4	4/4				
Small Intestine								
-- Crypt dilatation	0/4	2/4	2/4	2/4	0/4	1/4	3/4	3/4
-- Crypt inflammation - acute	0/4	2/4	1/4	1/4	0/4	1/4	3/4	3/4
-- GALT - depletion (ileum)	0/4	0/4	0/4	3/4	0/4	1/4	0/4	1/4
-- GALT - lymphoid necrosis	2/4	2/4	3/4	4/4	1/4	2/4	1/4	3/4
Thymus								
-- Atrophy	2/4	1/4	2/4	4/4	1/4	2/4	4/4	4/4