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# Rapid Induction of Thymic Lymphomas by Isopropyl Methanesulfonate: A Preliminary Report (42397)

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*Abstract.* The direct-acting $S_N1$ alkylating agent isopropyl methanesulfonate (IMS) was carcinogenic by subcutaneous injection in female Hsd:(ICR)BR mice, causing thymic lymphoid neoplasms within 7 months in at least 20 of 32 treated mice. No such neoplasms were observed in mice treated with the direct-acting $S_N2$ methyl homolog, methyl methanesulfonate (MMS). Both the IMS-treated mice and the MMS-treated mice initially received 20 μmole of the respective compounds by sc injection once weekly; however, because of toxic effects the dose of IMS was reduced to 10 μmole per injection on the 63rd day and further reduced to 5 μmole per injection on the 120th day, after which this dose was maintained until the 202nd day when the last surviving IMS-treated mouse became moribund and was sacrificed. In 2 of the MMS-treated mice, 93% of which were alive at 288 days, tumors were observed at the site of injection, one being a papilloma and the other a subcutaneous sarcoma. IMS has not previously been implicated as a carcinogen, to our knowledge. Its induction of thymic lymphomas may conceivably be related to its ability to alklyate exocyclic oxygen atoms in the DNA of hemopoietic cells. 

Methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS) are direct-acting alkylating agents (1) which are carcinogenic in rodents (1). MMS was first shown to be carcinogenic in male RF mice following administration in drinking water for life (2). Thymic lymphomas developed in 14.9% of the treated mice versus 3.7% of the controls. The neoplasms originated in the thymus, infiltrated locally into the lungs, and then spread throughout the body, reducing the mean survival time to 18.4 months in treated mice as compared with 20.5 months in controls. Lung tumors also were observed in treated mice. In female C57BL mice injected ip with MMS, EMS, *N*-methyl-*N*-nitrosourea (NMU), and *N*-ethyl-*N*-nitrosourea (NEU), the incidence of thymic lymphomas was increased greatly by NMU and NEU, slightly by EMS, and not by MMS (3). Since MMS and EMS react with nucleophiles mainy by an $S_N2$ (bimolecular) mechanism, while NMU and NEU react mainly by an $S_N1$ (unimolecular) mechanism (1), thymic lymphoma induction was apparently correlated with the ability of the compounds to react through the $S_N1$ mechanism and thus to alkylate the O-6 exocyclic oxygen atom of guanine in DNA (3). In general, the more electrophilic ($S_N1$) a reagent is, the greater its tendency to react with weaker nucleophiles, such as exocyclic oxygen atoms in DNA (1). This lower sensitivity of $S_N1$ compounds to nucleophilicity is due to the high reactivity of the carbonium ion intermediate they are postulated to form (4). These properties can be expressed quantitatively by the Swain and Scott substrate parameter *S* (5). Thus the values of *S* for MMS, EMS, NMU, and NEU are 0.83, 0.67, 0.42, and 0.26 respectively (1).

Isopropyl methanesulfonate (IMS) has an *S* value of 0.29 (1), which is comparable to that of NMU and NEU and it reacts with nucleophiles mainly via the $S_N1$ mechanism (1). Studies on the *in vitro* reactions of IMS, EMS, and MMS with salmon sperm DNA at 37°C and pH 7.1 revealed the ratios of O-6/N-7 alkylation of DNA guanine to be 0.30, 0.03, and 0.004, respectively (6), which are consistent with the reported *S* values of the compounds

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and their mechanisms of reactivity with nucleophiles. Although IMS is a direct-acting mutagen in *Escherichia coli* Sd-4 and *S. typhimurium* TA 1530 (7), there has been no report incriminating it as a carcinogen, to our knowledge. The goal of these experiments was to determine the carcinogenicity of IMS upon sc injection in female Hsd:(ICR)BR mice, as compared to that of MMS. In this paper we report that sc injection of IMS results in rapid induction of thymic lymphomas.

**Materials and Methods.** *Chemicals.* IMS was purchased from Kodak Laboratory Chemicals (Rochester, N.Y.), MMS from the Aldrich Chemical Company (Milwaukee, Wisc.), and Tricaprylin (trioctanoin) from United States Biochemical Corporation (Cleveland, Ohio).

*Animals.* Female Hsd:(ICR)BR mice (formerly designated ICR/Ha) were obtained from Harlan Sprague–Dawley (Indianapolis, Ind.) and were started on the experiment at 6 weeks of age. Housing and feeding of mice were as described previously (8).

*Bioassay.* The protocol for these experiments called for the injection of equimolar doses of IMS or MMS contained in 0.05 ml trioctanoin by the sc route into the left flank 1×/week for 52 weeks (52 injections). Surviving mice were then to be allowed to live an additional 85 days (total 450 days) and observed for induction of cancer. At 450 days, surviving mice were to be killed by cervical disolocation. Mice were randomly divided into four groups. Group 1, 30 mice, received 0.05 ml trioctanoin 1×/week by sc injection in the left flank for 288 days. Group 2, 32 mice, received 2.8 mg (20 μmole) of IMS in 0.05 ml trioctanoin 1×/week by sc injection in the left flank for 63 days, then 1.4 mg (10 μmole) in 0.05 ml trioctanoin for 57 days, and then 0.7 mg (5 μmole) in 0.05 ml trioctanoin for 82 days, when the experiment was terminated (the total time of application of IMS was 202 days, 25 injections). Group 3, 30 mice, received 2.2 mg (20 μmole) of MMS in 0.05 ml trioctanoin 1×/week by sc injection in the left flank for 288 days. Group 4, 60 mice, were untreated controls observed for 288 days. Mice which became moribund were killed by cervical dislocation. Mice which died or were sacrificed were necropsied after death. Histopathology was performed as described previously (9). This paper summarizes results observed at 288 days. Experiments with Group 1, 3, and 4 mice are continuing, and their final results will be the subject of a future publication.

*Short-term toxicity tests.* Groups of five mice received 40, 20, and 4 μmole of IMS or MMS in 0.05 ml trioctanoin 1×/week by sc injection in the left flank for 9 weeks (nine injections) to determine the maximum tolerated dose (i.e., that which caused no mortality or local damage at the injection site and did not depress weight gain) for use in the long-term experiments.

**Results.** By the 63rd day, 7 of the 32 IMS-treated mice had died or become moribund (Fig. 1), 2 of which were observed to have developed lymphoid neoplasms. Hence the dose of IMS was reduced from 20 to 10 μmole per injection in an attempt to salvage the experiment. In spite of this reduction in dosage, an additional 15 mice died or were sacrificed due to morbidity during the following 57 days, so that on the 120th day the dose of IMS was again reduced by one-half, to 5 μmole per injection. Of those 15 mice, 10 were found to have lymphoid neoplasms. The latter dosage was maintained until the 202nd day, when the last surviving IMS-treated mouse became moribund and was sacrificed. Altogether, 20 of the 32 IMS-treated mice developed lym-

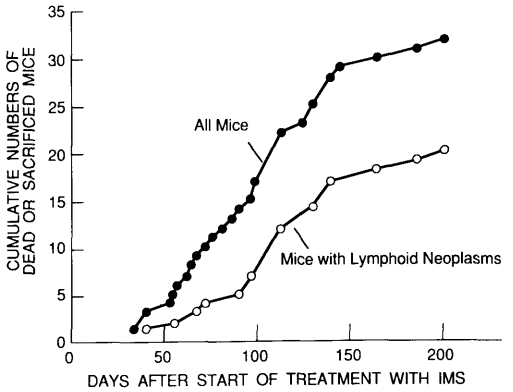


FIG. 1. Time distribution of mortality and lymphoid neoplasms in 32 IMS-treated mice. Mice were injected with IMS in 0.05 ml trioctanoin, 1×/week sc in the left flank, 20 μmole per injection for 63 days, then 10 μmole per injection until the 120th day, and then 5 μmole per injection until the last surviving mouse became moribund and was sacrificed on the 202nd day.

phoid neoplasms, the first as early as 40 days after the start of treatment and the last on the 202nd day (Fig. 1); the mean survival time of the tumor-bearing mice was 118 days. The neoplasms typically appeared to originate in the thymus, invade neighboring tissues, and ultimately spread via the blood to distant organs. The thymic lymphomas of the affected mice increased rapidly in size, filling the chest cavity and causing respiratory failure as noted earlier by others (3). Microscopically, the neoplastic cells were morphologically indistinguishable from lymphoblasts. Two of the mice with lymphoid neoplasms were observed to have second primary tumors at the site of injection; one was a subcutaneous hemangioma in a mouse sacrificed at 68 days, and the other was an epidermal papilloma in a mouse sacrificed at 99 days. Five IMS-treated mice which died or were sacrificed in a moribund state on the 33rd, 40th, 62nd, 64th, and 97th days showed no gross or microscopic evidence of neoplasia, but neither the thymus nor the bone marrow in these animals was examined histologically; hence the possibility that these mice also might have had early thymic lymphoid neoplasms cannot be excluded. The tissues of 7 IMS-treated mice, which died on Days 53, 58, 76, 81, 87, 125, and 145 were too autolyzed to be examined histologically. While the animals were under observation, 3 of the 15 animals used in the preceding short-term toxicity study were found upon microscopic examination to have developed lymphoid neoplasms.

In contrast to the high rate of neoplasia in the IMS-treated mice, no neoplasms were observed in any of the solvent-treated controls, 100% of which survived throughout the 288-day observation period.

In the MMS-treated mice, 93% of which were alive at 288 days, no lymphoid neoplasms were observed; however, one of the mice was observed to have a papilloma at the site of injection on the 140th day, and another was observed to have a subcutaneous sarcoma at the site of injection on the 232nd day. No other neoplasms were detected in the mice of this group.

**Discussion.** The goal of this experiment was to determine whether the direct-acting $S_N1$ alkylating agent IMS is carcinogenic on sc injection into mice and to compare its effects with those of the direct-acting $S_N2$ methyl homolog, MMS.

Our results parallel those reported previously for MMS, EMS, NMU, and NEU administered in a single ip injection to female C57BL mice (1, 3). MMS, EMS, NMU, and NEU administered in doses of 1.0, 2.4, 0.8, and 2.1 mmole/kg, respectively, yielded thymic lymphomas in 0, 2, 83, and 38% of the mice, respectively, within 250 days after injection. Thus the most potent of these direct-acting alkylating agents was NMU, in spite of the fact that NEU is the more reactive electrophile. The presumed reason for the difference in potency is that the extent of alkylation of DNA *in vivo* per unit dose of the ethyl homolog is only about one-fifth that of NMU. The dose of NEU was limited by toxicity. The lower binding of NEU, as compared to NMU, was attributed to its high reactivity, with concomitantly rapid hydrolysis (1, 3).

In our experiments, MMS was injected sc at a dose of 0.72 μmole/kg/mouse/week, so that by 288 days after the initial injection (when 28 of 30 treated mice were still alive), each surviving mouse had received approximately 30 mmole/kg of MMS, yet none had developed thymic lymphomas. Conversely, at the time when the first lymphoma was observed after the same dosage of IMS (40 days), the affected mouse had received only about 4.3 mmole/kg of IMS; the mean survival time of tumor-bearing IMS-treated mice was only 118 days. These induction times compare with a previously reported minimal latent period for thymic lymphoma of 65–70 days (and an average latent period of 100–140 days) in mice injected ip with EMS, NMU, and NEU (1, 3).

IMS, NMU, and NEU react with nucleophiles mainly by the $S_N1$ mechanism, and these compounds have similar half-lives in $H_2O$ (15–20 min) (1). NMU and NEU in C57BL mice (3) and IMS in Hsd:(ICR)BR mice have been observed to induce thymic lymphomas rather than neoplasms at the site of injection. The induction of thymic lymphomas by these agents thus may be related to their ability to alkylate exocyclic oxygens in the DNA of hemopoietic cells. From experiments on the induction of thymic lymphoma by ionizing radiation it may be pos-

tulated that severe damage to hemopoietic cells in the bone marrow, as well as damage to cells in the thymus, is involved (10).

MMS and EMS, on the other hand, react with nucleophiles by the $S_N2$ mechanism and have half-lives in $H_2O$ of 9.5 and 11.5 hr, respectively (1). In *in vitro* studies, MMS has been observed to alkylate bases in DNA to a sevenfold greater extent than IMS, presumably because of its lower reactivity with $H_2O$, but to cause eight times less O-6 alkylation of guanine (6). Similar differences between these carcinogens would be expected *in vivo*.

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