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# The chemotherapeutic potential of glycol alkyl ethers: structure-activity studies of nine compounds in a Fischer-rat leukemia transplant model

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**Summary.** Structure-activity studies with nine glycol alkyl ethers were conducted with a cellular leukemia transplant model in male Fischer rats. This in vivo assay measures the effects of chemical treatment on neoplastic progression in transplant recipients. Chemicals were given ad libitum in the drinking water simultaneously with the transplants and continued throughout the study. In all, 20 million leukemic cells were injected s.c. into syngeneic rats, which after 60 days resulted in a 10-fold increase in relative spleen weights, a 100-fold increase in white blood cell counts, and a 50% reduction in red blood cell (RBC) indices and platelet counts. At this interval, ethylene glycol monomethyl ether (2-ME) given at a dose of 2.5 mg/ml in the drinking water completely eliminated all clinical, morphological, and histopathological evidence of leukemia, whereas the same dose of ethylene glycol monoethyl ether (2-EE) reduced these responses by about 50%. Seven of the glycol ethers were ineffective as anti-leukemic agents, including ethylene glycol, the monopropyl, monobutyl, and monophenyl ethylene glycol ethers, diethylene glycol, and the monomethyl and monoethyl diethylene glycol ethers. 2-ME more than doubled the latency period of leukemia expression and extended survival for at least 210 days. A minimal effective dose for a 50% reduction in the leukemic responses was 0.25 mg/ml 2-ME in the drinking water (15 mg/kg body weight), whereas a 10-fold higher dose of 2-EE was required for equivalent anti-leukemic activity. In addition, the in vitro exposure of a leukemic spleen mononuclear cell culture to 2-ME caused a dose- and time-dependent reduction in the number of leukemia cells after a single exposure to 1–100 μM concentrations, whereas the 2-ME metabolite, 2-methoxyacetic acid, was only half as effective. The two glycol alkyl ethers with demonstrable anti-leukemic activity, 2-ME and 2-EE, also exhibited a favorable efficacy-to-toxicity ratio and should be considered for further development as chemotherapeutic agents.

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## Introduction

Ethylene glycol alkyl ethers have been studied intensively since the observations in the late 1970s that these compounds caused embryotoxic [29], teratogenic [39], and spermatotoxic [28] responses. The toxic effects of the glycol ethers were the subject of a review in 1983 containing 36 papers on more than 15 different compounds from this class of chemicals [17]. There were a variety of adverse responses identified in multiple target organs, which included the blood, liver, kidney, brain, testes, and embryonic tissue, with a sensitivity that was dependent primarily on the dose, but also on the route and the specific chemical given [10, 11, 28, 30, 34].

Since the glycol ethers were specifically toxic to rapidly dividing cell populations in the fetus [29] and testis [30] at relatively low concentrations, it was hypothesized that some of these compounds might also be effective as anti-leukemic agents. Evidence in support of this hypothesis was provided in the study by Houchens et al. [22], which showed that administration of ethylene glycol monomethyl ether (2-ME) and ethylene glycol monoethyl ether (2-EE) prevented mortality in mice challenged with L1210 leukemia cells in an allogeneic tumor model. Furthermore, a recent study conducted by the National Toxicology Program demonstrated that administration of 2-EE in a 2-year gavage study completely prevented the occurrence of spontaneous leukemia in male and female rats (NIH, unpublished report). This was a unique observation among more than 350 2-year studies [19], in which the incidence of spontaneous leukemia averaged about 25% in F344/N rats [18] and could range up to 70% in individual studies [9].

Recently we began to validate an in vivo leukemia assay that measures the positive or negative effects of chemical treatment on neoplastic progression in Fischer 344/N rats transplanted with mononuclear leukemic cells [8]. We demonstrated that the leukemia transplant assay was valid for chemicals that either exacerbate or inhibit the progression of leukemia, using two compounds, 2,4,6-trichlorophenol and 2-EE, whose positive and negative

**Table 1.** Structure-activity relationships of glycol ethers

| Name | Chemical structure | Response |
|---|---|---|
| Ethylene glycol (EG) | $H\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |
| EG-monomethyl ether (2-ME) | $CH_3\text{-}O\text{-}CH_2\text{-}CH_2OH$ | Inhibited |
| EG-monoethyl ether (2-EE) | $CH_3\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | Inhibited |
| EG-monopropyl ether | $CH_3\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |
| EG-monobutyl ether | $CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |
| EG-monophenyl ether |  | No effect |
| Diethylene glycol (DEG) | $H\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |
| DEG-monomethyl ether | $CH_3\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |
| DEG-monoethyl ether | $CH_3\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2OH$ | No effect |

tumor effects were established in 2-year rodent carcinogen bioassays. We found that 4-hexylresorcinol [4] also inhibited the progression of neoplasia in the transplant model, but at lower concentrations the glycol alkyl ether was more effective, extending the latency period for the expression of leukemia by 30 days beyond that in non-chemically treated transplant recipients [8].

These studies have now been extended to examine the structure-activity relationships between a series of nine different glycol alkyl ethers and their potential antileukemic activity. The chemicals that were tested in the in vivo leukemia transplant model included ethylene glycol, the one- to four-carbon ethers of ethylene glycol, a phenyl glycol ether, diethylene glycol ether, and the one- and two-carbon diethylene glycol ethers. We found that two of these chemicals, 2-ME and 2-EE, showed promise as antineoplastic agents, and then confirmed their efficacy in replicate experiments conducted by separate laboratories.

## Materials and methods

A leukemia cell line was maintained in vivo by serial transplantation as a slow growing, heterogeneous neoplasm with a latency period of about 60 days [7]. The neoplastic cells were obtained from the spleens of three leukemic donors at each transplant interval and were transferred as an aliquot of pooled mononuclear cells into healthy, syngeneic recipients at 60 to 90-day intervals. The methods for the separation of the leukemic mononuclear cells and their morphological and biochemical characterization have previously been published [6].

For the in vitro studies, the leukemic mononuclear spleen cells were cultured in RPMI medium 1640 supplemented with 10% horse serum containing 20 μg/ml gentamicin. Cells were seeded in 5 ml medium in 25-$cm^2$ tissue-culture flasks (Corning, N.Y.) and maintained without shaking at 37°C in a humidified incubator with 5% $CO_2$ in air. Test chemicals were added in medium to the final concentrations indicated on day 1 after the cultures had been started. For determination of cell numbers, duplicate aliquots of each flask were counted daily using a hemocytometer. Trypan blue dye exclusion was used to determine cell viability. Each assay was conducted in duplicate and the experiment was replicated with similar results. The data presented represent the results from one experimental trial.

For the in vivo studies, male F344/N rats were obtained as 6-week-old weanlings from NIH production contracts (Charles River Breeding Laboratories, Wilmington, Mass.) and were housed in filter-top polycarbonate cages in groups of four. Rats were fed NIH-07 diet and water ad libitum. Room temperature was maintained at 21°–22°C and humidity, at 50%–55%, with a 12-h: 12-h light cycle. After about 2 weeks acclimation, rats were offered the chemicals ad libitum in the drinking water; at the start of each experiment one-half of the rats in each treatment group were given leukemia transplants. Untreated controls and non-chemically treated leukemia-transplant recipients were also included in each experimental series. The sample size consisted of 8–10 rats for each treatment group in each experiment.

Ethylene glycol, ethylene glycol monopropyl ether, ethylene glycol monobutyl ether, ethylene glycol monophenyl ether, diethylene glycol, and diethylene glycol monoethyl ether were mixed in the drinking water at doses of 2.5, 5.0, and 10.0 mg/ml; ethylene glycol monobutyl ether was tested at doses of 3.0 and 6.0 mg/ml. 2-EE and diethylene glycol monomethyl ether were tested only at doses of 2.5 and 5.0 mg/ml. 2-ME was tested at doses of 0.25, 1.0, 2.5, 3.0, 5.0, 6.0, and 10.0 mg/ml in the drinking water. In each experiment, estimates of the combined water consumption of four rats per cage were measured at two to three separate intervals. The chemical structures for the glycol alkyl ethers are depicted in Table 1.

Each of the chemicals was obtained from the archival samples of material from the 2-year NTP carcinogenicity studies. Their identity was verified, and the stability of each dose preparation was determined. Dose preparations were mixed at appropriate intervals, and the concentrations were verified with standard curves using flame ionization gas chromatography.

All of the experiments were terminated at 55–65 days post-transplant, when non-chemically treated transplant recipients expressed clinical signs of leukemia but before mortality ensued. Animals were anesthetized with carbon dioxide and bled from the inferior cava and their body and organ weights were obtained. Red blood cell (RBC) counts, packed cell volume, hemoglobin concentration, and platelet counts were determined with a hematology analyzer, and blood mononuclear cells from the 2-ME experiment were separated on Sepracell tubes, counted, and frozen at –70°C for later enzyme analyses. Spleen weights were recorded, and samples of blood, spleen, and liver were stained with hematoxylin and eosin for histological examination. The slides were coded such that they could be examined without prior knowledge of treatment groups. Standardized criteria were used to stage mononuclear cell leukemia in the spleen and liver sections, with grades assigned from 0–6. The grades described a range in progression of the leukemia, as previously described [8]. Dunnet's multiple comparison test was used for statistical analysis of the data, with a level of acceptance of probability of $P < 0.05$.

## Results

Ethylene glycol and 2-ME were more toxic than the other seven glycol ethers. Mortality occurred in rats dosed with 10.0 mg/ml ethylene glycol and in those given 6.0 or 10.0 mg/ml 2-ME. These treatment groups were killed early and no data were collected from these groups or from

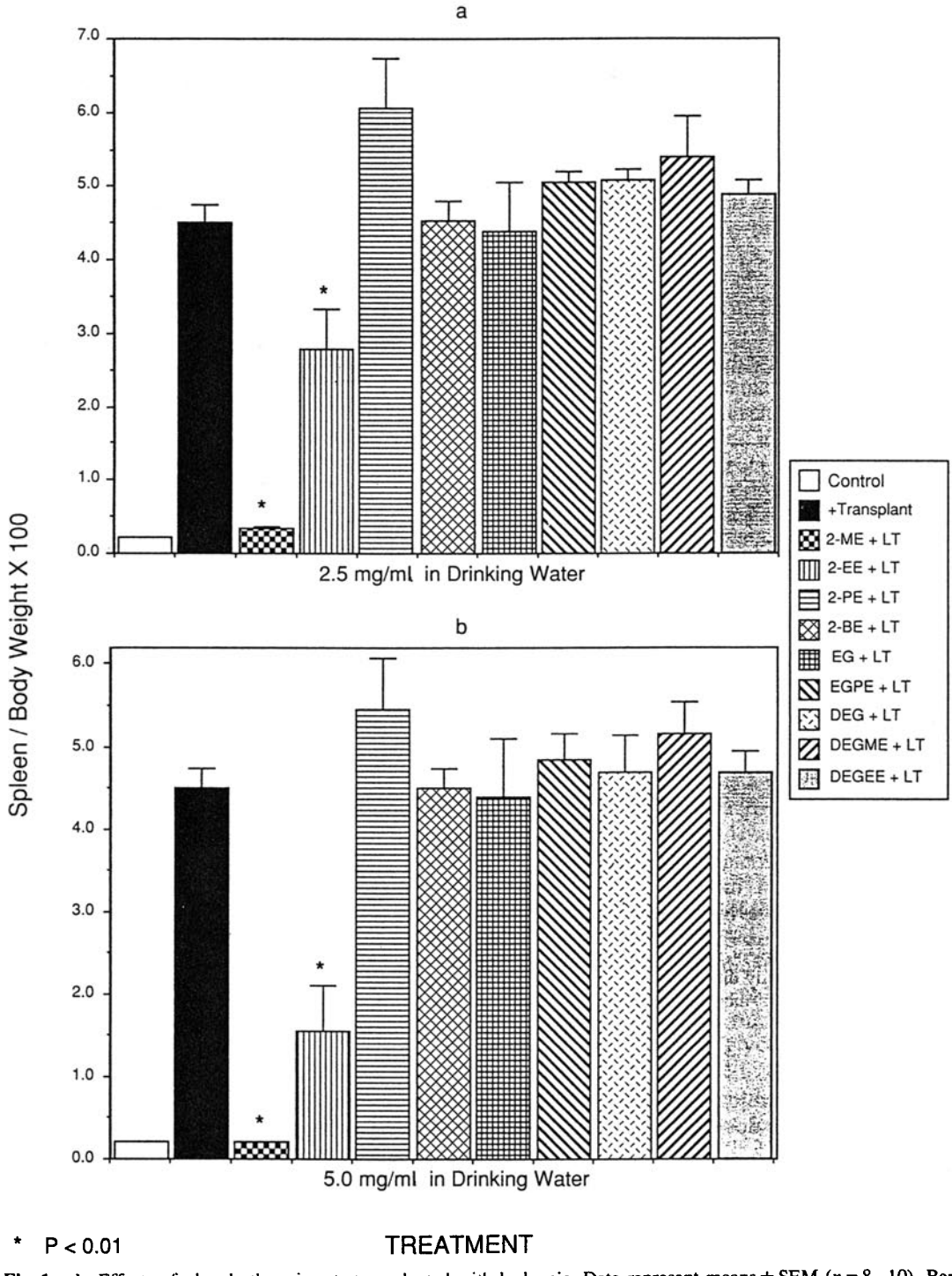


**Fig. 1 a, b.** Effects of glycol ethers in rats transplanted with leukemia. Data represent means ± SEM ($n = 8-10$). Bar 1, untreated controls; bar 2, non-chemically treated transplant recipients; bars 3–11, chemically treated transplant recipients; *, significantly different from non-chemically treated transplant recipients ($P < 0.01$). *LT*, leukemia transplant; *2-ME*, ethylene glycol monomethyl ether; *2-EE*, ethylene glycol monoethyl ether; *2-PE*, ethylene glycol monopropyl ether; *2-BE*, ethylene glycol monobutyl ether; *EG*, ethylene glycol; *EGPE*, ethylene glycol monophenyl ether; *DEG*, diethylene glycol; *DEGME*, diethylene glycol monomethyl ether; *DEGEE*, diethylene glycol monoethyl ether

the remainder of the 10-mg/ml dose groups. Decreases in body weight occurred in rats dosed with 2-ME at ≥ 3.0 mg/ml and in those given ethylene glycol monobutyl ether at ≥ 5.0 mg/ml. The no-effect level for the toxicity of 2-ME was the 2.5 mg/ml dose in non-transplanted rats; 3.0 mg/ml caused some non-specific toxicity, as judged by body weight loss, depressed RBC indices, and platelet counts. 2-EE did not cause toxicity at the doses given (2.5 and 5.0 mg/ml), except for small and variable decreases in RBC indices. Ethylene glycol monobutyl ether was hematotoxic in non-transplanted rats, causing dose-related decreases in all of the RBC indices at 3.0 and 6.0 mg/ml. None of these three glycol alkyl ethers affected spleen weight in non-transplanted rats at either the high or

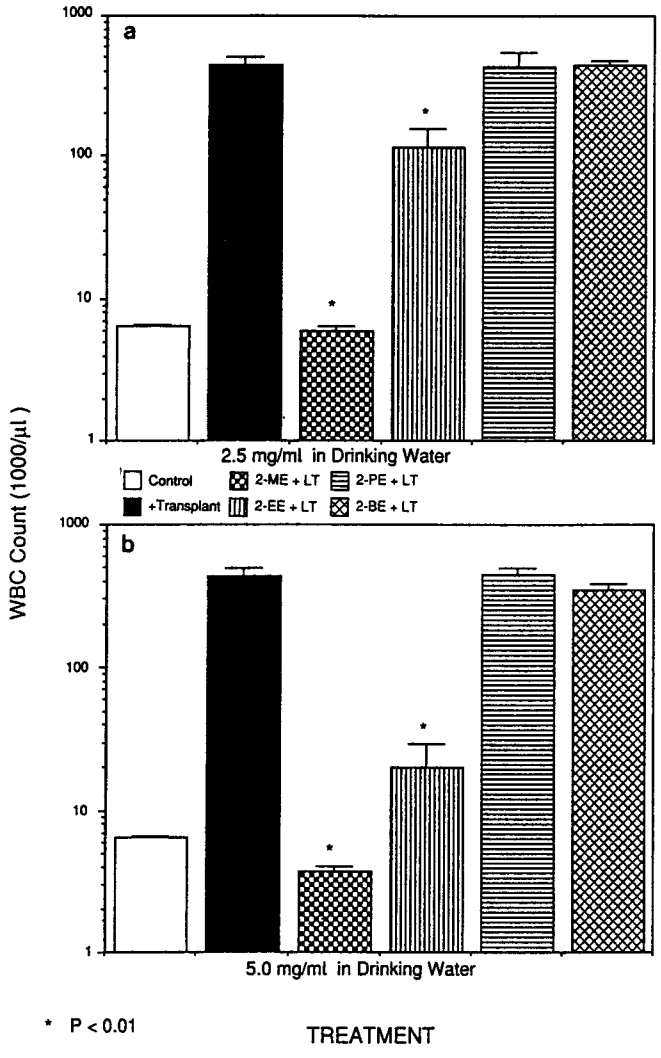


Fig. 2 a, b. Effects of glycol alkyl ethers in rats transplanted with leukemia. Data represent the means ± SEM ($n = 8-10$). Bar 1, untreated controls; bar 2, non-chemically treated transplant recipients; bars 3–6, chemically treated transplant recipients; *, significantly different from non-chemically treated transplant recipients ($P < 0.01$). Abbreviations as in Fig. 1

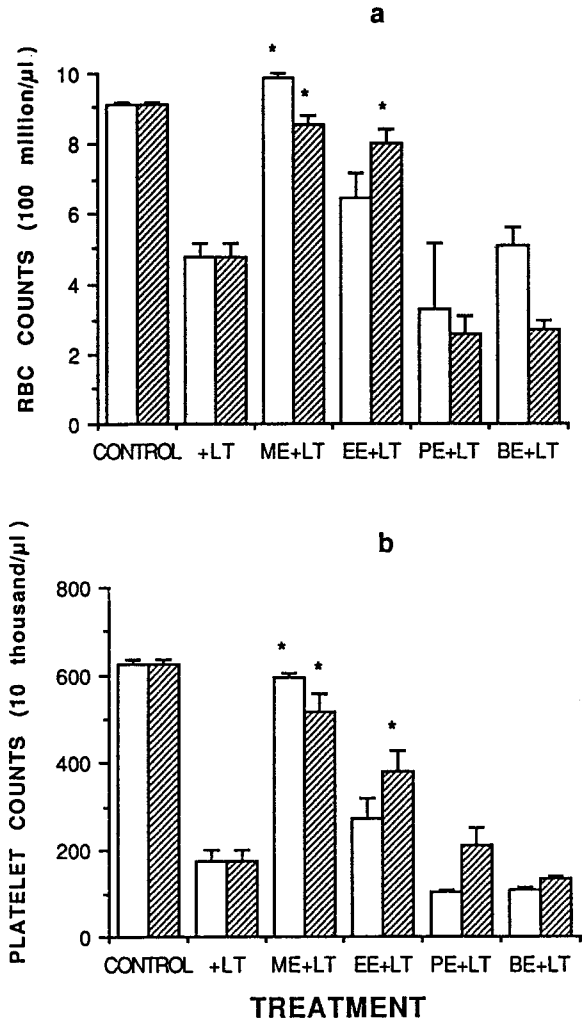


Fig. 3 a, b. Effects of 2-ME in rats with transplanted leukemia. Data represent the means ± SEM ($n = 8-10$). Bar 1, 2.5 mg/ml dose group; bar 2, 5.0 mg/ml dose group; *, significantly different from non-chemically treated transplant recipients ($P < 0.01$). Abbreviations as in Fig. 1

the low dose, whereas each of these chemicals caused minor reductions in WBC counts at the high dose (data not presented).

There was no significant change in water consumption between the rats treated with the different glycol ethers at the 2.5- or 5.0 mg/ml dose levels. Water consumption of the four rats per cage varied between 12 and 15 ml/rat daily; these data served as the basis for subsequent calculations to estimate the chemical dose for each treatment group.

The effects of chemical treatment on the progression of the leukemia transplant were determined by comparison with the responses in untreated rats given the transplant. The experiments were terminated at about 60 days post-transplant, and the expression of leukemia was quantified by measurement of relative spleen weight, WBC count, RBC indices, and platelet counts. The magnitude of these changes in untreated transplant recipients amounted to 10-fold increases in relative spleen weights, 100-fold increases in WBC counts, and 50% reductions in RBC indices and platelet counts. The leukemic responses were also quantified histopathologically in the spleen and liver by assignment of grades of severity. There was very close correlation between all of the measurements of disease indices.

2-ME and, to a lesser extent, 2-EE treatment delayed the progression of leukemia in transplant recipients, as determined by the degree of splenomegaly (Fig. 1). None of the other seven glycol ethers prevented the increases in relative spleen weight. Whereas the reduction in splenomegaly in transplant recipients treated with 2-EE was dose-related, an effective threshold level for this response was attained at the 2.5 mg/ml dose of 2-ME; in addition, splenomegaly was completely prevented at this interval by 2-ME treatment, whereas 2-EE treatment was only partially effective.

The inhibition of neoplastic growth in the transplant recipients by treatment with the one- and two-carbon ethers of ethylene glycol was even more dramatic as reflected by the WBC counts (Fig. 2). In rats that expressed

**Table 2.** Histopathological diagnosis of leukemia in spleens of chemically treated rats at 55–65 days post-transplant

| Treatment groups | Average grade[a]: | |
|---|---|---|
| | Incidence | Spleen |
| Controls | 0/30 | None |
| Chemicals alone | 0/80 | None |
| 2-ME: | | |
| Leukemia transplant only | 10/10 | 4.9 |
| 2.5 mg/ml 2-ME+transplant | 5/ 8 | 1.9 |
| 3.0 mg/ml 2-ME+transplant | 0/10 | None |
| 5.0 mg/ml 2-ME+transplant | 0/ 7 | None |
| 2-EE: | | |
| Leukemia transplant only | 0/10 | 4.9 |
| 2.5 mg/ml 2-EE+transplant | 10/10 | 4.7 |
| 5.0 mg/ml 2-EE+transplant | 7/10 | 3.1 |
| Ethylene glycol monopropyl ether (2-PE): | | |
| Leukemia transplant only | 12/12 | 4.7 |
| 2.5 mg/ml 2-PE+transplant | 3/3 | 5.0 |
| 5.0 mg/ml 2-PE+transplant | 4/ 4 | 5.0 |
| Ethylene glycol monobutyl ether (2-BE): | | |
| Leukemia transplant only | 10/10 | 4.6 |
| 3.0 mg/ml 2-BE+transplant | 10/10 | 5.3 |
| 6.0 mg/ml 2-BE+transplant | 10/10 | 4.9 |

[a] Grades: 0–6; 0, none; 2, trace; 3, mild; 4, moderate; 5, severe; 6, massive

**Table 3.** Effects of 2-ME in rats with transplanted leukemia

| Treatment[a] | Spleen[b] | WBC[b] | RBC[b] | Platelets[b] |
|---|---|---|---|---|
| Controls | 0.20 ± .003 | 8.36 ± .26 | 9.90 ± .09 | 715 ± 27 |
| +LT | 3.58 ± .25 | 323 ± 47 | 5.51 ± .44 | 223 ± 27 |
| 0.25 mg/ml 2-ME+LT[c] | 1.75 ± .44 | 114 ± 49 | 8.28 ± .64 | 477 ± 65 |
| 1.00 mg/ml 2-ME+LT[c] | 1.49 ± .57 | 90 ± 57 | 8.93 ± .63 | 521 ± 74 |
| 2.50 mg/ml 2-ME+LT[c] | 0.50 ± .16 | 10.6 ± 3.6 | 9.46 ± .34 | 637 ± 60 |
| 5.00 mg/ml 2-ME+LT[c] | 0.21 ± .009 | 2.7 ± .32 | 8.53 ± .31 | 514 ± 45 |

[a] Dose given in drinking water for 55–65 days post-transplant
[b] Spleen/body weight × 100; WBC count, 1,000/μl; RBC count, 10 million/μl; platelet count, 10,000/μl
[c] All values significantly different from non-chemically treated transplant recipients
Data represent the means ± SEM ($n$ = 8–10). LT, transplanted leukemia

manifestations of transplanted leukemia, almost 100% of the WBCs were identified as mononuclear leukemic cells. Once again, either the low or the high dose of 2-ME delayed the progression of leukemia in the transplant recipients such that there were no increases in WBC counts. Hematology was carried out in only three other experimental series. Although 2-EE treatment caused a significant reduction in the WBC count compared with that in non-chemically treated transplant recipients, neither dose level completely abrogated the response. Treatment of transplant recipients with ethylene glycol monopropyl or the monobutyl ethers did not prevent the increase in elevated WBC counts.

The RBC count, packed cell volume, hemoglobin concentration, and platelet counts in non-chemically treated rats were severely depressed at the 60-day post-transplant interval. Figure 3 illustrates the changes in two of these parameters; the others were correlated and are not presented. Ethylene glycol monopropyl and monobutyl ether treatment of transplant recipients did not prevent the decreases in RBC or platelet counts; 2-EE was partially effective but there was no clear dose response. There were no alterations in the erythrocytic indices in rats given leukemia transplants and treated with 2.5 or 5.0 mg/ml 2-ME, which again shows that progression of leukemia was markedly delayed in the transplant recipients.

The leukemia transplant experiments with 2-ME, 2-EE, and ethylene glycol monobutyl ether were repeated in a separate laboratory, with slightly different doses for two of the chemicals (3.0 and 6.0 mg/ml for 2-ME and the ethylene glycol monobutyl ether) but with almost identical results (data not presented). At 55 days post-transplant, drinking-water doses of 3.0 mg/ml 2-ME completely prevented the expression of leukemia in transplant recipients, as determined by relative spleen weights, WBC counts, and measurements of RBC indices. 2-EE was partially effective at about twice the 2-ME dose (5.0 mg/ml), and ethylene glycol monobutyl ether was ineffective.

Histopathological examination of the spleen and liver extended and supported the observations that 2-ME treatment could markedly delay neoplastic progression in transplant recipients (Table 2). There was no leukemia in rats treated with ≥ 3.0 mg/ml 2-ME. There was no leukemia in 3/8 rats given 2.5 mg/ml 2-ME, and the response was graded between none and trace in 5 of them. There were no lesions indicative of leukemia in the livers of any of these rats. 2-EE reduced the severity of leukemia in 7/10 rats given 5.0 mg/ml and eliminated leukemia in 3 of them. Ethylene glycol monopropyl and monobutyl ethers were ineffective in reducing the incidence or severity of leukemia in transplant recipients.

Further leukemia-transplant experiments were conducted with 2-ME to determine the nature of the dose-response curve for anti-neoplastic activity and to gain insight into the efficacy/toxicity ratio for this chemical. Table 3 illustrates that a dose of 0.25 mg/ml 2-ME in the drinking water could reduce splenomegaly and leukoblastosis to 50% of the levels observed in transplant recipients without chemical treatment. These responses described an inverse dose response between 0.25 and 2.5 mg/ml 2-ME, the latter of which was about the minimal effective dose for completely eliminating leukemic manifestations in transplant recipients. Table 3 illustrates similar anti-neoplastic responses as reflected by the RBC indices and platelet counts.

In vitro studies with spleen mononuclear leukemic cells grown in culture from the Fischer rat leukemia-transplant model showed that there was a dose-dependent and progressive reduction in the number of leukemic cells over a 5-day period after a single exposure to 1–100 μM con-

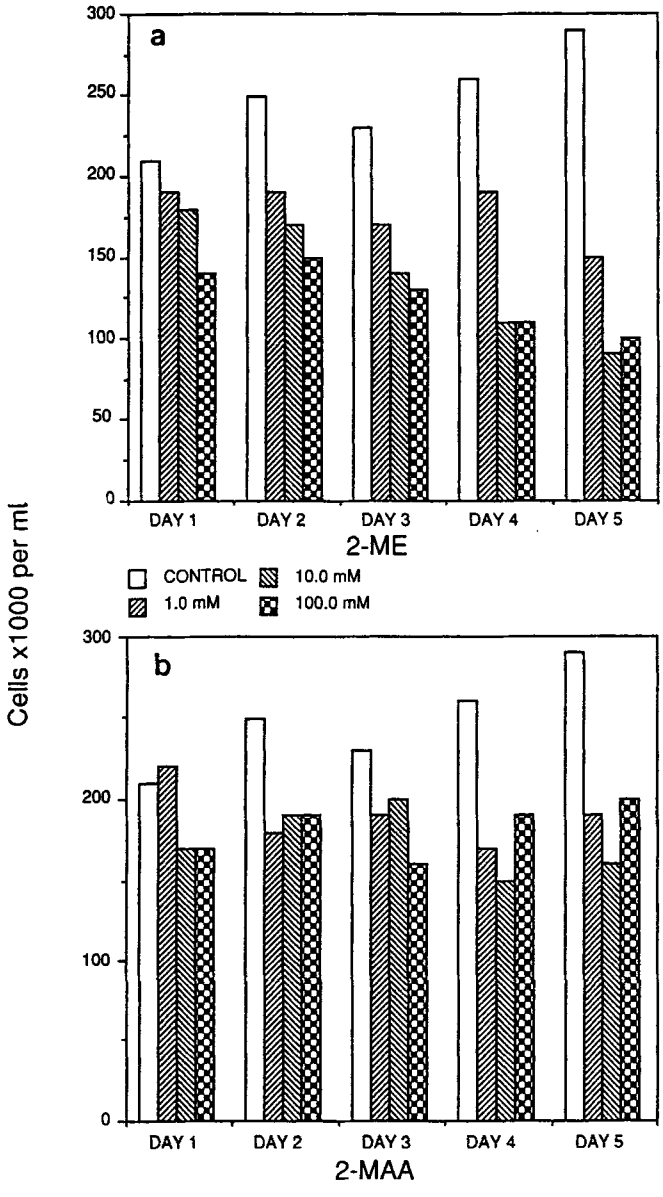


**Fig. 4a, b.** Effects of 2-ME or ethylene glycol methoxyacetic acid (2-MAA) on leukemia cell counts on days 1–5 after a single exposure on day zero. Each assay was conducted in duplicate and replicated once; cell counts represent the average from one experiment

centrations of 2-ME (Fig. 4a). Exposure of the leukemic cells to the same concentrations of the metabolite of this glycol ether, ethylene glycol methoxyacetic acid (2-MAA), was about half as effective in reducing the number of leukemic cells, and the changes were not related to dose or time (Fig. 4b).

## Discussion

The glycol alkyl ethers exhibited a spectrum of toxicity that was dependent mainly on the dose, but also on the carbon chain length, the route of exposure, and the species studied. An extensive review of their toxicity was published in 1984, mainly using rats, mice, rabbits, and dogs exposed by oral and inhalation routes to a variety of mono- and di-glycol alkyl ethers [17]. The majority of the experiments were conducted with 2-ME or 2-EE, since these are high-production compounds that have been shown to exert general toxicity at very high doses, inducing lesions in the liver, kidney, and CNS of rats and other species [31, 33]. Cases of human overdose exposure that caused similar target-organ toxicity were also reported [5, 32].

At reduced concentrations, the lower-molecular-weight glycol alkyl ethers exhibited a more specific toxicity, which was restricted to the rapidly dividing cell populations in the hematopoietic system [16], reproductive system [30], and developing embryo [29].

The lowest effect level for the toxicity of the glycol ethers occurred in the bone-marrow stem-cell compartment and in the circulating erythrocytes and leukocytes. Two recent studies demonstrated that 2-ME inhibited stem-cell proliferation in mice given 400 mg/kg for 4 days [21]; data from mice given the same dose indicated that residual marrow stem-cell injury persisted at 15 and 21 weeks, when ability to recover from whole-body irradiation was measured [20]. Previous studies with 2-ME and ethylene glycol monobutyl ether in rats showed that at equivalent doses of 500 mg/kg given p.o. for 4 days, the monobutyl ether was more toxic to the erythroid elements, whereas 2-ME was more toxic to the WBCs [16]; the 100-mg/kg dose of 2-ME initially caused a small decrease in lymphocyte count and a continual decrement in thymus weight, which persisted for 8 days after treatment.

We also observed lymphocytopenia and a reduction in thymus weight in rats dosed with 100 or 350 mg/kg 2-ME for 14 or 60 days (data not presented). 2-EE was slightly less toxic than 2-ME, since it had less effect on RBC indices and did not cause lymphocytopenia. The common theme for the toxicity of both of these short-chain glycol ethers was their well-known effect on rapidly dividing cell populations. These observations suggested that some of the glycol alkyl ethers might be effective as anti-neoplastic agents. If the potential therapeutic effects of the glycol ethers occurred at doses below those that caused toxic responses in healthy cells, these chemicals could be exploited as chemotherapeutic agents.

2-ME and 2-EE were both effective at delaying neoplastic progression in leukemia-transplant recipients, but the one-carbon moiety was about twice as potent. In dose-response studies, the anti-leukemic activity of 2-ME, as determined by the prevention of splenomegaly, leukoblastosis, and anemia and the absence of histopathological lesions in transplant recipients, reached an effective threshold at the 2.5 mg/ml concentration given ad libitum in the drinking water. Based on measurements of water consumption, this corresponded to a 2-ME dose of about 100 mg/kg. Dose levels >2-fold greater than this were required before 2-ME caused any significant reproductive toxicity in rats [3, 13, 14]. Acute treatment of rats with 100 mg/kg 2-ME for 4 days followed by 22 days' recovery did not affect the hematopoietic system, except for minor and variable reductions in leukocyte counts [16]. Treatment with 500 mg/kg 2-ME initially caused mild anemia, marked leukopenia, hemorrhagic bone marrow, and testicular atrophy, but only the gonadal change persisted. In the present studies some non-specific toxicity, expressed as decrements in body weight, RBC indices, and platelet

counts, began to occur in rats dosed with 350–400 mg/kg 2-ME (3.0- and 5.0-mg/ml dose groups), but more severe toxicity and mortality did not commence until about 650 mg/kg 2-ME (6.0-mg/ml dose groups) had been ingested.

The lowest dose of 2-ME that we tested for anti-leukemic activity was 15 mg/kg (0.25-mg/kg dose group), which reduced the severity of the leukemic responses by about 50%. Other treatment modes were used to reduce the manifestations of leukemia in a myeloid leukemia transplant model with the brown Norway rat, including nitrous oxide given alone [12, 23] or in combination with the folate inhibitors methotrexate [25] or 5-fluorouracil [24]. In all, 10 days' therapy with nitrous oxide alone resulted in a 50% reduction in leukocytosis but was ineffective in reversing the 90% reduction in platelet count in leukemic rats. Splenomegaly in those leukemic rats was reduced by 33%, from 4.2 to 2.8 g [12]. Combined treatment with nitrous oxide and the folate inhibitors yielded therapeutic responses that were better than additive; we could completely prevent the splenomegaly, leukoblastosis, and reduction in RBC indices and platelet counts in leukemic rats by treatment with 100 mg/kg 2-ME (2.5-mg/ml dose).

The animals given leukemia transplants in the present studies expressed clinical signs of the disease at 55–65 days post-transplant and, based on previous observations, would become moribund and die about 2 weeks later, e.g., at 65–75 days post-transplant. We did not collect sufficient survival data for statistical analysis of rats that were given leukemia transplants and then treated with either 2-ME or 2-EE. However, in parts of other studies we observed that continual dosing with 2-ME at 2.5 mg/ml in the drinking water extended the survival of four rats to at least 210 days post-transplant, when they were killed and showed no evidence of splenomegaly or leukoblastosis. In another example, nine rats that were given leukemia transplants and then continually dosed with 5.0 mg/ml 2-EE survived about twice as long as undosed rats that underwent leukemia transplants. Similar observations were reported in allogeneic mice dosed with either 300–1,200 mg/kg 2-ME or 600–2,400 mg/kg 2-EE and given an L1210 leukemia transplant, in which the mean survival was increased from 9 to >43 days [22].

Since toxicity to non-leukemic cells attributable to 2-ME was not observed until the 350-mg/kg dose and 50% inhibition of leukemic responses occurred at 15 mg/kg, an efficacy/toxicity ratio of 0.04 could be calculated. 2-EE displayed lower efficacy as an anti-proliferative agent (initial neoplastic repression occurred at 2.5 mg/ml or about 100 mg/kg). Since toxicity to non-leukemic cells attributable to this compound was first observed at the 5.0-mg/ml dose, or 400 mg/kg, the calculated efficacy/toxicity ratio of 0.22 was less favorable than that for 2-ME.

In previous validation studies of the leukemia transplant model [8], we reported a greater anti-leukemic efficacy for 2-EE than that found in the present experiments. During successive transfers of the leukemia cell line the latency period decreased. More vigorous neoplastic growth probably obscured a clear demonstration of chemical intervention at the experimental interval selected for termination in the present experimental series. Nevertheless, the potential for the chemotherapeutic activity of 2-EE was again demonstrable, as shown by the dose-related responses for the anti-leukemic activity of this chemical. In addition, previous 2-year chronic studies showed that high doses of 2-EE (500 and 1,000 mg/kg by gavage administration for 5 days/week) reduced the incidence of splenomegaly [26] and reduced the incidence of leukemia to zero in both male and female rats (NIH, unpublished report). These data indicate that further studies should be conducted to investigate the potential of the various glycol ethers, especially 2-ME and 2-EE and their metabolites, for the treatment of leukemia.

Since there are only relatively minor structural differences between the nine glycol alkyl ethers tested, and since only two of them were effective in the in vivo assay, one mode of action of 2-ME or its spermatotoxic [27] and teratotoxic [35] metabolite, 2-methoxyacetic acid, or of 2-EE and its metabolites, could be direct cytotoxicity. Previously it was shown that glycol ethers incorporated into alkyl lysophospholipids, such as ET-18-OCH3, exerted selective cytotoxicity to murine ascites tumors [36] and to four different human leukemic cell lines [1]. Subsequently, several investigators demonstrated that alkyl lysophospholipid analogues with ether groups incorporated into them were more effective in vitro as anti-leukemic agents [2, 15, 37]. We evaluated the selective cytotoxicity of the more potent anti-leukemic agent, 2-ME, and its metabolite, 2-methoxyacetic acid (2-MAA), by the in vitro addition of micromolar doses of these compounds to rodent leukemia cell suspensions. There were dose- and time-related reductions in the number of suspended rat leukemia cells for up to 5 days after a single exposure to concentrations of 1–100 μM 2-ME. Unlike the results of the teratogenic or spermatotoxic experiments, 2-MAA was much less effective than the parent compound in reducing the number of rat leukemic cells in culture. These observations suggest that cytotoxicity was not solely responsible for the anti-proliferative activity of 2-ME.

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