

Interruption of Cell-Cell Communication in Chinese Hamster V79 Cells by Various Alkyl Glycol Ethers: Implications for Teratogenicity

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Intercellular communication most likely plays a significant coordinating role in morphogenesis. Blockage of a specific type of intercellular communication, that mediated by gap junctions, has been proposed as a mechanism of action of some teratogens. Several glycol ethers have recently been shown to be teratogenic in laboratory animals. Because these compounds are negative in genotoxic assays, it is suggested that they may act by nongenetic, perhaps membrane-mediated mechanisms.

In the present study several structurally related alkyl glycol ethers were examined for their ability to block junction-mediated intercellular communication. Interruption of intercellular communication was measured *in vitro* by an assay that depends on the transfer of metabolites via gap junctions, i.e., metabolic cooperation. All compounds tested—ethylene glycol (EG), ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), ethylene glycol monopropyl ether (EGPE), and ethylene glycol monobutyl ether (EGBE)—were able to block metabolic cooperation *in vitro*. The potencies of the compounds were inversely related to the length of the aliphatic chain, the dose required for maximum blockage increasing as the aliphatic chain shortened. Some differences in the maximum amount of blockage were detected, but these were not consistent and hence were not considered significant. Cytotoxicity, as measured by cell survival, was also related to the structure of the compound, generally increasing with increased length of the aliphatic chain. There were structurally related differences in the concentration ranges over which the compounds were effective. The noncytotoxic ranges over which communication was blocked became reduced as the length of the aliphatic chain increased. EG was effective over the broadest range, followed by EGME. The remaining compounds had very narrow effective ranges relative to EG and EGME. Because they are effective over such broad noncytotoxic ranges, blockage of intercellular communication may be most significant as a teratogenic mechanism for EG and EGME. Although the other compounds in the series also blocked communication, they were more cytotoxic and they interrupted communication in a relatively narrow window of concentrations. Consequently, interrupted intercellular communication may be mixed with cytotoxicity in the embryo and the mother, and thus be less specific as a mechanism of teratogenesis for EGEE, EGPE and EGBE.

Introduction

Embryogenesis is a highly complicated phenomenon in which intercellular communication is likely to play an important coordinating role. Diffusible morphogens and hormones are the most frequently considered methods of intercellular communication in the embryo (1). Another type of intercellular communication that has received less attention involves direct transfer between adjoining cells through junctional structures. The gap junction is the structure most commonly believed to mediate this form of intercellular communication (2).

Intercellular communication via gap junctions is a proposed mechanism for controlled transmission of

information. Cells linked by gap junctions rapidly pass electrical currents and can form functional syncytiums in excitatory tissues such as the heart (3) and the myometrium during labor (4). Pools of small metabolites (1000 to 1500 daltons, depending upon configuration) are also shared among cells joined by gap junctions (2).

Chemicals shared among junctionally coupled cells can serve as signals for growth and differentiation, and can relay information about cell position and cell number (2). Junctional communication is thus a potential means of coordinating development. Several examples are known in which changes in junctional communication occur coincident with developmental changes (5). Recently, Lo and Gilula (6,7) chronicled progressive compartmentalization of junctional communication among cells of the early developing mouse embryo. While these data are encouraging, there is currently no direct evidence that junctional communication is involved in embryogenesis.

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One manifestation of the transfer of metabolites between coupled cells is metabolic cooperation, in which metabolites are passed from wild-type, metabolically competent cells to cells that are deficient in a specific enzymatic pathway. Metabolic cooperation is measured using radiolabeled compounds and autoradiography (8), or by cocultivation of wild-type and metabolically deficient cells in the presence of a toxic precursor (9).

Based on observations of Yotti et al. (10), Trosko and his associates (11-13) have developed an assay that measures interruption of metabolic cooperation *in vitro*. The basis of the assay is the transfer of the phosphorylated metabolite of 6-thioguanine (6-TG) from wild-type Chinese hamster V79 cells that contain hypoxanthine-guanine phosphoribosyltransferase (HGPRT) to mutant V79 cells that lack HGPRT. The mutant V79 cells (6-TG^r cells) that lack HGPRT are usually resistant to 6-TG because they cannot metabolize it to its toxic form. Alternatively, the wild-type cells (6-TG^s cells) are sensitive to 6-TG because they contain HGPRT. When the 6-TG^s and 6-TG^r cells are cocultivated in the presence of 6-TG, the phosphorylated metabolite of 6-TG is transferred, presumably by gap junctions, from the 6-TG^s cells to the 6-TG^r cells. All of the 6-TG^s cells and any junctionally coupled 6-TG^r cells will not be able to divide in the presence of 6-TG. If, however, metabolic cooperation is blocked, then more 6-TG^r cells will survive and form colonies because they do not receive the toxic metabolite from the 6-TG^s cells.

This assay has been widely applied to identify tumor promoters (11,12) and was recently proposed as an assay for teratogens (13). The assay was employed in the present study of ethylene glycol (EG) and some structurally related monoalkyl glycol ethers since some of these compounds have recently been found to be teratogenic in laboratory animals (14-17). Two alkyl glycol ethers have been tested in a battery of mutagenesis assays (18,19). They were not mutagenic in the Ames assay, and they failed to induce unscheduled DNA synthesis, sister chromatid exchanges or chromosomal damage. Such results suggest that the teratogenicity of the glycol ethers may be due to nongenetic mechanisms. We therefore hypothesized that EG and monoalkyl ethers act as teratogens by interrupting junction-mediated intercellular communication.

Materials and Methods

Cell Culture

Wild-type Chinese hamster V79 cells (6-TG^s cells) and a mutant line of V79 cells that lack HGPRT (6-TG^r cells) were used in these experiments. The cells were grown in modified Eagle's medium (20) (Eagle's balanced salt solution with a 50% increase of all essential amino acids and vitamins except glutamine), supplemented with a 100% increase of nonessential amino acids, 1 mM sodium pyruvate and 3% fetal calf serum.

Stock cell cultures were maintained in medium without antibiotics but gentamicin (50 µg/mL) was added to the medium during experiments. The cells were incubated in a humidified air atmosphere with 5% CO₂ at 37°C. Both cell lines were routinely checked for the presence of mycoplasma using Chen's technique (21) and were found to be free of contamination.

Measurement of Intercellular Communication

Interruption of intercellular communication was measured using an assay described by Trosko et al. (11,12). To measure blockage of communication, a mixture of 100 6-TG^r cells were plated with 4×10^5 6-TG^s cells in 35 mm² dishes. Cytotoxicity was monitored by plating 100 6-TG^r cells in separate dishes. Four hours after seeding the cells, the test compounds were added in 1 mL of medium. One-half hour later 50 µL of 6-TG were added to each dish. The compounds were tested at six doses each and there were 10 plates per dose. The doses were selected from pilot studies and spanned the noncytotoxic range of effective blockage of communication. Background was determined by plating 100 6-TG^r cells with 4×10^5 6-TG^s cells in the presence of 6-TG without adding any other compound. For a positive control, one group was exposed to TPA at 1 ng/mL. The 6-TG^s cell population was checked for spontaneous mutants by plating 4×10^5 6-TG^s cells per plate in the presence of 6-TG. Every 3 days the plates were changed to fresh medium containing 10 ng/mL 6-TG. After 8 days of incubation the plates were stained with Crystal Violet and the number of colonies per plate were scored. Each colony represents the survival and proliferation of a 6-TG^r cell. The increased number of recovered colonies over background reflects blockage of junction-mediated intercellular communication. The recovery of 6-TG^r mutants was expressed as the percent plating efficiency. All compounds were tested simultaneously, except that ethylene glycol was not included in the replicate experiment.

Chemicals

The glycol ether compounds were kindly supplied by Bryan Hardin of NIOSH. The source of ethylene glycol (EG) was Aldrich Chemical Co., Inc. The source of ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE) and ethylene glycol monobutyl ether (EGBE) was Fisher Scientific Co. Ethylene glycol monopropyl ether (EGPE) was obtained from Tennessee Eastman Co.

Results

Each of the alkyl glycol ethers as well as EG were effective blockers of intercellular communication at noncytotoxic doses in the V79 cell assay. As seen in Figures 1-5, the recovery of 6-TG^r cells was greatly

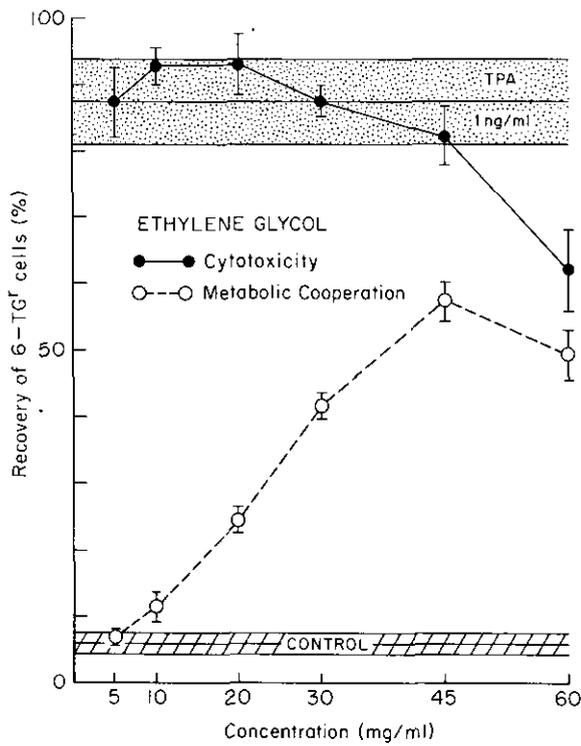


FIGURE 1. Effect of ethylene glycol on survival of 6-TG^r cells cultured alone (cytotoxicity) and in the presence of 6-TG^s cells (metabolic cooperation).

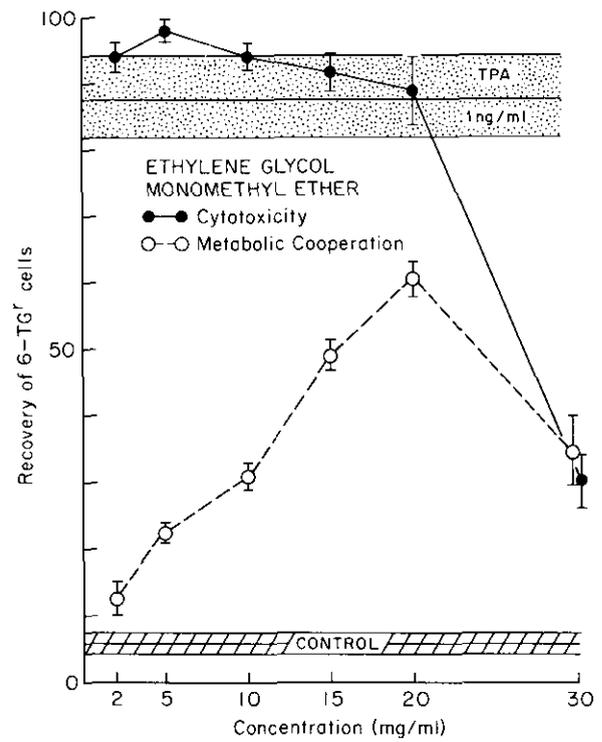


FIGURE 3. Effect of ethylene glycol monomethyl ether on survival of 6-TG^r cells cultured alone (cytotoxicity) and in the presence of 6-TG^s cells (metabolic cooperation).

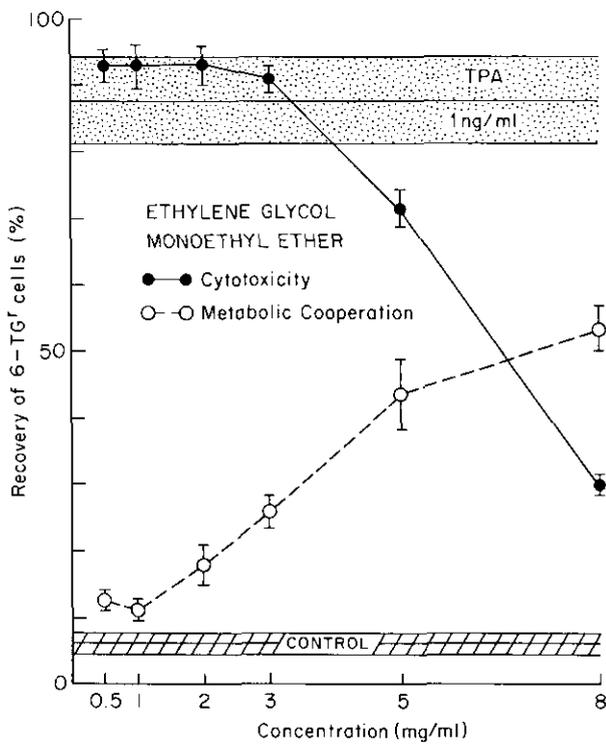


FIGURE 2. Effect of ethylene glycol monomethyl ether on survival of 6-TG^r cells cultured alone (cytotoxicity) and in the presence of 6-TG^s cells (metabolic cooperation).

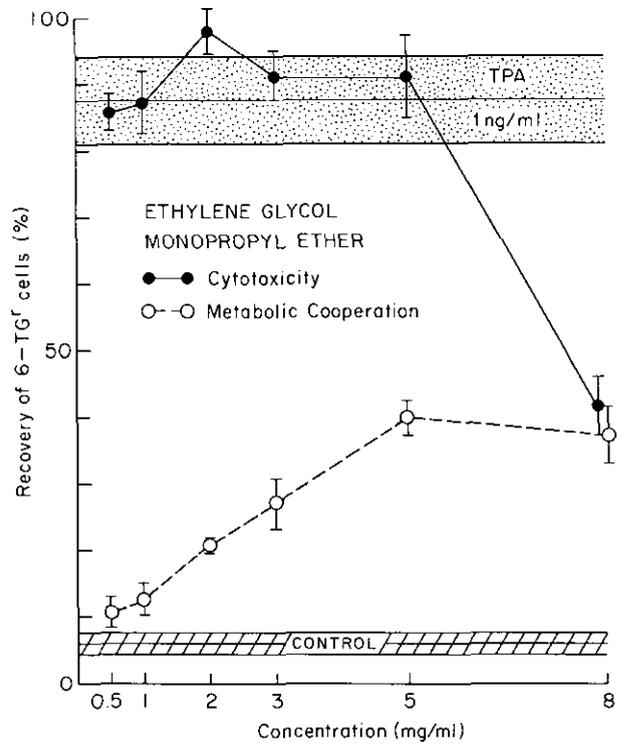


FIGURE 4. Effect of ethylene glycol monopropyl ether on survival of 6-TG^r cells cultured alone (cytotoxicity) and in the presence of 6-TG^s cells (metabolic cooperation).

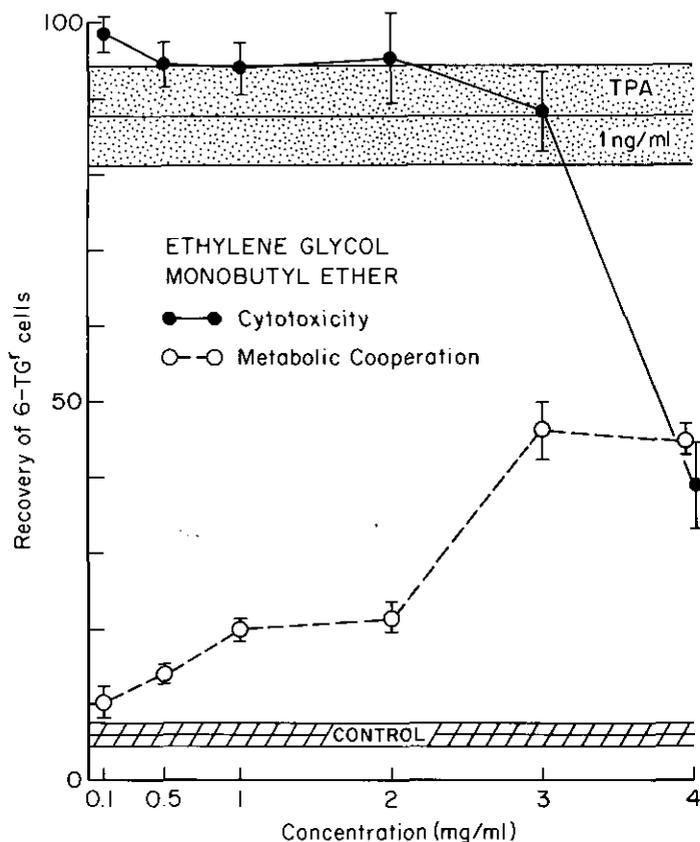


FIGURE 5. Effect of ethylene glycol monobutyl ether on survival of 6-TG⁺ cells cultured alone (cytotoxicity) and in the presence of 6-TG⁺ cells (metabolic cooperation).

increased over background by each of the compounds tested. There were some differences among the glycol ethers in the maximum amount of blocked communication, but these differences were not consistent in the replicate experiment (compare Figs. 6 and 7) and are probably not significant.

Cytotoxicity, as measured by cell survival in plates containing only 6-TG⁺ cells, was related to the structure of the compound. Generally, as the length of the aliphatic chain increased so did the cytotoxicity. EG and EGME were relatively much less cytotoxic than EGEE, EGPE and EGBE. EGEE was very slightly, but consistently more cytotoxic than EGPE, and EGEE and EGPE were both more cytotoxic than EGBE. The potencies of the compounds were also inversely related to the length of the aliphatic chain, with the dose required for maximum blockage increasing as the aliphatic chain shortened.

As can be clearly seen in Figures 6 and 7, the most striking differences among the compounds were in the sizes of the noncytotoxic ranges over which communication was blocked. The effective dose ranges became reduced as the length of the aliphatic chain increased. EG was effective over the broadest range, followed by EGME. The remaining compounds had very narrow effective ranges relative to EG and EGME.

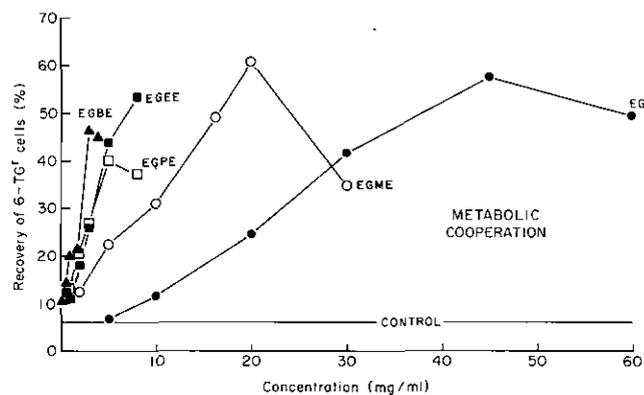


FIGURE 6. Comparison of the abilities of ethylene glycol (EG), ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), ethylene glycol monopropyl ether (EGPE) and ethylene glycol monobutyl ether (EGBE) to inhibit metabolic cooperation, as observed by increased recovery of 6-TG⁺ cells.

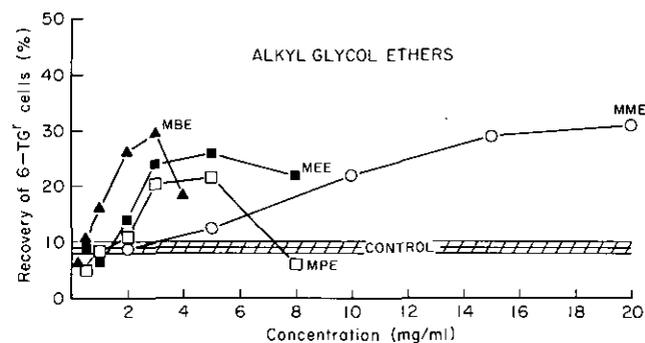


FIGURE 7. Summary of replicate experiment in which ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), ethylene glycol monopropyl ether (EGPE) and ethylene glycol monobutyl ether (EGBE) were tested for their abilities to inhibit metabolic cooperation, as observed by increased recovery of 6-TG⁺ cells.

Discussion

These studies demonstrated that EG, EGME, EGEE, EGPE and EGBE were effective blockers of metabolic cooperation between V79 cells in culture. EG and EGME were distinct from the other compounds tested in several respects. They were less toxic and less potent than EGEE, EGPE and EGBE. In addition, EG and EGME effectively blocked communication over relatively large noncytotoxic dose ranges. The range of effective doses and the cytotoxicity of the compounds corresponded to the length of the aliphatic chain; the longer the aliphatic chain, the more cytotoxic and potent the compound. The maximum degree of blockage, however, did not appear to be related to the structure of the compounds.

The assay employed in this study has been widely applied to the study of tumor promoters, most of which have been shown to be effective blockers of metabolic cooperation. Trosko et al. (13) proposed a hypothesis that teratogenesis and tumor promotion may share

inhibition of intercellular communication as a common mechanism of these two phenomena. Other teratogens recently found to be positive in this assay include phenobarbital, phenylhydantoin, chlorpromazine, Mirex and Kepone (Trosko et al., unpublished observations).

Although the role of gap junction communication in embryogenesis and the potentially harmful role of inhibition of such communication are yet to be defined, it is likely that gap junctions do have an important function in morphogenesis. Our data support the hypothesis that EG and ethylene glycol monoalkyl ethers may act as teratogens by blocking junction-mediated intercellular communication. Further support for this idea is lent by teratology studies which found that exposure to EGME and EGEE significantly lengthened gestation in rats and mice (22,23). Since labor is dependent upon the formation of gap junctions between the myometrial cells (4), increased length of parturition might be a sign of gap junction interference. Other organ systems that rely on gap junctions such as the heart, intestines, and male reproductive system have also been adversely affected by some of these compounds (23). When considered together, these findings support the hypothesis that inhibition of gap junction communication may be a mechanism of action *in vivo*.

Blockage of intercellular communication may be most significant as a teratogenic mechanism for EG and EGME because they are effective over such broad noncytotoxic ranges. Although the other compounds in the series also blocked communication, they were more cytotoxic and they interrupted communication in relatively narrow windows of concentration. Consequently, interrupted intercellular communication may be mixed with cytotoxicity in the embryo and the mother, and thus be less specific as a mechanism of teratogenesis for EGME, EGPE and EGBE.

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