Characterization of Cell Death Induced by 2-Methoxyethanol in CD-1 Mouse Embryos on Gestation Day 8

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ABSTRACT

Cell death was analyzed in neurulating mouse embryos after in vivo doses of 2-methoxyethanol (2-ME) that produce anterior neural tube defects. Characterization of 2-ME-induced cell death was performed by evaluating: (1) vital fluorochrome staining in whole embryos applying confocal laser scanning microscopy; (2) characteristics of cell debris in conventional histological sections revealed by light microscopy; and (3) Apoptag in situ immunohistochemical staining for apoptosis using light microscopy. Methods for quantification of cell death identified by these three techniques were explored using computerized image analysis. Physiological cell death in control embryos primarily occurred in the neural crest region during neural fold elevation. Embryos exposed to 2-ME had expanded areas of cell death in the neural crest and also new areas of cell death in medial regions of the anterior neural tube. Both physiological and 2-ME-induced embryonic cell death had morphological, immunohistochemical, and fluorochrome staining characteristics of apoptosis. When fluorescence data from confocal microscopic analysis of vital fluorochrome-stained embryos were analyzed, a dose-dependent increase was found in embryos exposed to 2-ME. Similar results were obtained when cell death was analyzed in either conventional histological sections or sections prepared for immunohistochemical detection of apoptosis. The cell death data obtained in this study correlate with previously observed near-term malformation rates, suggesting that a quantitative relationship exists between 2-ME-induced embryonic cell death and neural tube defects.

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Physiological or programmed cell death is a normal developmental event that occurs consistently at sites where morphogenetic changes, such as limb formation and neural tube closure are taking place (Glücksman, '51; Saunders, '66; Schlüter, '73). Many teratogens, including radiation (Hicks and D'Amato, '66), heat (Edwards et al., '74; Mirkes, '85), nonphysiological oxygen concentrations (Grabowski, '70), and various chemical exposures (Hossain et al., '95; Marin-Padilla, '66; Sadler and Cardel, '76; Schweichel and Merker, '73; Sulik et al., '88; Theodosis and Fraser, '78; Wiley et al., '83) induce excessive cell death in embryos. There are also reports that toxicant-induced inhibition of embryonic cell death is associated with abnormal development (Greene and Pratt, '78; Morriss and New, '79; Scott, '81). Thus, perturbation of physiological cell death in embryos may be an integral step in the pathogenesis of many malformations.

Most investigations of teratogen-altered embryonic cell death have described only qualitative changes in vital dye staining and/or cell morphology in embryos, often without verification that the treatment conditions were relevant for in vivo teratogenesis. To gain a better understanding of the relationship between embryonic cell death and malformations, quantitative analysis of cell death following known teratogenic exposures would be desirable. Data derived from such studies could be used to develop mathematical models of abnormal development similar to models of carcinogenesis (Conolly and Kimbell, '94; Moolgavkar et al., '88) that have potential application in human risk assessment.

Previous work in our laboratory investigated the dosimetry and teratogenicity of the industrial solvent 2-methoxyethanol (2-ME; ethylene glycol monomethyl ether). Administration of 2-ME to pregnant CD-1 mice on gestation day (gd) 8 resulted in exencephaly in near-term fetuses (Terry et al., '94), while administration of the chemical on gd 11 caused paw malformations (Horton et al., '85; Clarke et al., '92). In both cases, increased cell death was observed in the primordia of the organ which eventually becomes malformed (Greene et al., '87; Terry et al., '96), but no quantitative measures of embryonic cell death were reported. On gd 11, 2-ME-induced cell death in the limb bud possessed ultrastructural features consistent with apoptosis, including cytoplasmic and nuclear condensation, cellular fragmentation into membrane-bound bodies and phago-
cytosis of these bodies by neighboring cells (Greene et al., '87). Whether 2-ME-induced cell death in embryos on gd 8 has the same characteristics is unknown. The present study was therefore undertaken to characterize the mode of cell death induced by 2-ME in the anterior neural folds of gd 8 mouse embryos exposed to teratogenic doses of 2-ME and also to explore methods to quantify this cell death.

**MATERIALS AND METHODS**

**Chemicals and supplies**

Acridine orange (AO) was obtained from Fisher Scientific Company (Fairlawn, NJ). Apoptag® In Situ Apoptosis Detection Kits (peroxidase) were obtained from Oncor Incorporated (Gaithersburg, MD). Pentex® crystallized bovine serum albumin (BSA) was obtained from Miles, Inc. (Kankakee, IL). High-performance liquid chromatography (HPLC)-grade 2-methoxyethanol (99.9%) and propidium iodide (PI) were obtained from Sigma Chemical Company (St. Louis, MO).

**Animals and dosing**

Crl:CD-1 (ICR) BR (CD-1) mice purchased from Charles River Laboratories (Raleigh, NC) were housed in a mass air displacement room (Biolene, Hazelton Systems, Vienna, VA) maintained at 22 ± 1.5°C, 50 ± 10% humidity, and a 12-hr light–dark cycle. The mice were fed National Institute of Health (NIH) 07 open formula pelleted food (Zeigler Brothers, Gardner, PA) and filtered tap water ad libitum. Nulliparous females, 8–10 weeks old, were paired 1:1 with males during the final 2 hr of the dark cycle (from 0700 to 0900 hr), and mating was confirmed by the presence of a vaginal plug. Eight o’clock (0800 hr) on the day of mating was considered gd 0, 0 hr (gd 0:0).

Between 0815 and 0845 hr on gd 8, a single oral dose of 0 (water), 250, or 325 mg 2-ME/kg body weight was administered to the pregnant dams. Then, 4 hr later, the dams were killed by CO2 asphyxiation and 8–10 embryos from each litter were explanted in Tyrode’s buffer containing 2 mg BSA/ml (Tyrode’s/BSA). The embryos were staged by somite number and either stained with vital fluorochromes or fixed in preparation for analysis of cell death as described below. The embryos selected for analysis were of similar developmental stage, both between treatment groups and between methods.

**Supervital fluorochrome staining for cell death**

AO was used to identify apoptotic cell death. This metachromatic dye has been found to label cells undergoing apoptosis in Drosophila embryos (Abrams et al., '93). The advantages and limitations of AO staining have been described in detail elsewhere (Darzykowski and Kapuscinski, '90; Kasten, '67). The staining procedure was adapted from Gao et al. ('94). The AO stock solution (10 mg/ml in 0.1 M HCl) was diluted to 5 μg/ml in Tyrode’s solution containing 30% heat-inactivated rat serum. Embryos were incubated in this solution for 5 min at 37°C. The embryos were rinsed 3 × 2 min in Tyrode’s/BSA at room temperature and whole-mounted ventral side up in Tyrode’s/BSA on welled microscope slides with coverslips. The stained and mounted embryos were held in the dark at room temperature before imaging with the confocal microscope. The amount of AO staining was found to increase slowly over time subsequent to staining. To minimize this potentially confounding variable, all embryos were imaged in a random sequence within 3 hr of staining.

PI was used as a fluorescent label for cells that had lost their membrane integrity. A working PI solution of 10 μg/ml was prepared in Tyrode’s solution containing 30% heat-inactivated rat serum. The embryos were incubated in the PI solution for 20 min at 37°C and then rinsed and mounted as described above.

**Confocal laser-scanning microscopy of fluorochrome-stained embryos**

The fluorochrome-stained embryos were imaged using a Zeiss LSM-10 confocal laser-scanning microscope (CLSM) equipped with a 10× objective lens with a numerical aperture (NA) of 0.3. The 514-nm line of the argon laser was used to excite either fluorophore, and the pinhole size was set at 50 μm. Fluorescent emission was collected through a 575- to 645-nm bandpass filter. These filter settings were used rather than the usual FITC filters because they produced images better suited for image analysis (there was a greater difference in intensity between background fluorescence and the bright fluorescence associated with cell death). Approximately 10 serial optical sections were collected in z-plane steps of 15 μm from both the dorsal and ventral side of each embryo (coronal orientation). Images of a stage micrometer were also acquired for calibration of the image analysis program. The depth of scanning (laser penetration) in the tissue was found to be approximately 60 μm, similar to the depth of the neuroepithelium at this developmental stage. Thus, it was assumed that the measurements taken represent neuroepithelial cells, but not the underlying mesenchyme. It was also assumed that small differences in the orientation of the embryos affected neither the data acquisition nor analysis.

The overall pattern of cell death in fluorochrome-stained embryos was assessed by overlaying serial optical sections to obtain a composite image. Because of the lack of background fluorescence in PI-stained embryos, images of PI were overlaid on brightfield images of the same embryo to localize areas of cell death. The composite images were saved as digital files and photographed with a digital slide maker (Cl-5000 Digital Palette, Polaroid, Cambridge, MA).

**Quantification of apoptosis from CLSM data**

The digitized images of AO fluorescence were downloaded onto an Image-1 image analysis system (Universal Imaging Corporation, West Chester, PA). A calibra-
tion was performed from the stage micrometer images, using the calibration function in the Image-1 software. The analysis was limited to the neural tube region anterior of the first somite, as abnormal closure in this region is associated with exencephaly (Campbell et al., '87). The area of brightly stained fluorescent bodies in each optical section was quantified by threshold measurement of greyscale pixels. Appropriate threshold values were determined by zooming the images until individual pixels could be seen and then by manually evaluating various threshold settings for their ability to include all the bright pixels. This threshold was 80–120 greyscale values for all embryos. Any pixel containing a greyscale value higher than the selected threshold was included in the area measurement, while any pixel with a lower greyscale value was excluded. The area of bright fluorescence associated with apoptosis was then normalized to the total area imaged (in-focus tissue in the optical section), which was measured using a lower greyscale threshold (range, 10–20). The data are expressed as the percentage area affected (area of cell death as percentage of total tissue area). Thresholds were set for individual embryos, rather than uniformly, because stain intensity was variable. This technique gave more consistent data within dose groups than setting uniform thresholds.

Preparation of histological sections

After removal of the extraembryonic membranes, embryos were fixed in Bouin’s solution for 2–4 hr at room temperature for hematoxylin and eosin (H&E) staining or in 10% neutral buffered formalin for 8 hr for Apoptag® immunohistochemistry. The fixed embryos were rinsed and stored in 70% ethanol at 4°C for several weeks.

To facilitate handling during paraffin embedding, the embryos were embedded in agarose (1.5% solution in water at 45°C). Once cooled, agarose casts were placed in tissue cassettes and dehydrated through a graded series of ethanols, followed by xylene. The samples were then infiltrated and embedded in paraffin. Serial coronal sections were cut at 5 μm on a rotary microtome.

The slides were stained according to a conventional H&E protocol for adult tissues using Harris hematoxylin, 1% acid alcohol destain, and 1% alcoholic Eosin Y counterstain. The slides mounted in Permount® (Fisher Scientific, Fairlawn, NJ).

Quantification of cell death from H&E-stained sections

Percentage area measurements. To compare the data obtained from CLSM to those obtained from conventional histological sections, the percent area of cell death was determined in serial histological sections. Consistent with the optical sections obtained from the confocal scope, every third section of each embryo was stained and analyzed, and analysis was restricted to the neuroepithelium anterior to the first somite. A 20× objective lens (NA = 0.65) was used to measure the total neuroepithelial cell area, while a 60× lens (NA = 1.4) was used to measure the area of prospective apoptotic bodies within the neuroepithelium. The 20× lens was used because it could rapidly measure large areas of neuroepithelium, without the potential error associated with measuring multiple adjoining fields (measuring the same area twice or missing some area). This potential error was minimal when measuring apoptotic bodies with the 60× objective because the bodies were usually well separated and distinct. Digital images were acquired from the microscope through a Dage-MTI CCD 72 black-and-white video camera integrated with the Image-1 image analysis system. Images of a stage micrometer were used to calibrate each objective lens using the calibration function in Image-1. Preliminary experiments were carried out to ensure comparability of the data obtained from the two lenses. When measurements of the same area were made with the two lenses (this required carefully measuring multiple fields with the 60× lens), they agreed within 10%.

The total area of the neuroepithelium (cytoplasm and nuclei) in each tissue section was determined using the measure objects function in Image-1. This function allows the operator to use a mouse to circumscribe a region of interest in an on-screen image and automatically calculates the enclosed area based on a calibration file. Apoptotic bodies were identified manually within the neuroepithelium on the basis of morphological criteria. In well-prepared H&E sections, they can be observed under high magnification as circular, intensely stained bodies usually surrounded by an unstained halo (e.g., see Fig. 4). Measurement of apoptotic bodies was performed using the measure ellipse function in Image-1, which automatically measures the area within a circumscribed ellipse or circle. Bodies containing nuclear material (basophilic stain) were included in the analysis, while those containing only eosinophilic staining were excluded. The sum of the area of all bodies identified in all sections from an embryo was divided by the total neuroepithelial cell area measured in that embryo and multiplied by 100 to obtain the proportion area affected. The number of apoptotic bodies/mm² and the average size of the bodies were also calculated.

Apoptotic index. For comparison of our data with quantitative data on apoptosis in other experimental systems, an apoptotic body index (number of apoptotic bodies/number of nuclei × 100) was determined for each embryo analyzed by the H&E method described above. To do this, the average number of neuroepithelial cell nuclei per square millimeter of cellular area was counted in at least six of the slides from each embryo. The average number of apoptotic bodies/mm² obtained above was then divided by the average number of nuclei/mm² and multiplied by 100, to obtain the apoptotic index.
Immunohistochemical labeling for apoptosis

DNA fragmentation was detected on formalin-fixed embryo sections using the Apoptag® peroxidase kit. Every third section of each embryo was stained and quantified as described below. The manufacturer's instructions were adapted to embryonic sections as follows. The sections were digested with 15 µg Proteinase K/ml (Boehringer Mannheim) at 37°C for 10 min before the tailing reaction. An aminoethyl carbazole (AEC) chromogen substrate (Zymed Laboratories, San Francisco, CA) was used instead of the diamino benzidine substrate supplied. Light counterstain using hematoxylin (25 min at room temperature) replaced the methyl green recommended. Following counterstaining, the sections were sealed with a small amount of Crystal Mount (Biomedia Corp., Foster City, CA). After drying, the slides were mounted in Permount® and coverslipped.

Quantification of cell death in Apoptag®-stained sections

Digital color images were acquired from a Nikon Optiphot microscope through a Sony CCD/RGB video camera integrated with the Image-1 image analysis system. Color image analysis was used to quantify the area of peroxidase product (red-brown chromogen) in every third section of each embryo. Thresholds were manually selected for each slide to match the hue, saturation and intensity of color of the peroxidase product, with the H&E-stained background set to zero. The area of chromogen was then measured using the measure objects function in the Image-1 software. This area was normalized to the total cellular area of neuroepithelium determined as described above for H&E sections. It was assumed that small variations in section thickness did not affect the results.

Statistical analysis

Analyses were performed using JMP software (SAS Institute, Cary, NC). Percentage data were arcsine-square root transformed prior to analysis. The data were tested for homogeneity of variance and then analyzed using one-way analysis of variance (ANOVA). Where multiple comparisons were required, a significant F-test was followed by Dunnett's test. For clarity of presentation, the data are shown untransformed. Litter means were obtained by averaging the following number of individual embryos/litter: AO data (4); Apoptag® data (2–3); H&E data (1–2).

RESULTS

In utero exposure to teratogenic doses of 2-ME had no observable effect on the developmental stage of embryos collected on gd 8.04 as measured by somite number. Control and treated groups all had 6 ± 2 somites (mean ± SD).
In contrast to AO, intense PI fluorescence was not observed in the neural crest region but occurred primarily in remnants of the visceral yolk sac and amnion, which had been mechanically damaged during dissection to expose the embryo proper. Although PI would not be predicted to penetrate tissue as readily as AO, because of its hydrophilicity, our previous work in limb buds showed that PI could indeed access cells well below the surface ectoderm (Stedman and Welsch, '93). This, together with the fact that much of the cell death observed with AO staining was near the surface of the embryo in the neural crest, suggests that the differences in staining observed with the two dyes were not attributable to an inability of PI to access regions of cell death.

**Localization and characterization of 2-ME-induced cell death**

In embryos from dams treated with 2-ME, AO fluorescence along the neural crest was enhanced and appeared in more anterior locations than that observed in similarly staged control embryos (Fig. 2). In addition, AO fluorescence was observed in medial regions of the anterior neural folds, where it was not seen in control embryos. Embryos exposed to 2-ME and stained with PI appeared very similar to control embryos, with minimal fluorescence in the neural fold region but intense fluorescence where remnants of the amnion and visceral yolk sac remained (not shown).

Light microscopic observations of H&E-stained histological sections confirmed that the increase in AO fluorescence in embryos exposed to 2-ME was due to increased cell debris that had morphological characteristics of apoptotic bodies (Fig. 3). The cell debris was primarily comprised of circular structures that stained intensely with eosin, possessed chromatin, and were surrounded by a clear halo. Most of these bodies resided in the cytoplasm of neuroepithelial cells, presumably having undergone phagocytosis. Cell debris was also visible in areas of early migratory neural crest, but the
cephalic mesenchyme was generally much less affected than the neuroepithelium. H&E-stained sections corresponding to the midbrain and rostral hindbrain appeared to possess the greatest increase in cell debris in treated embryos, as compared with controls. Many of the bodies identified in H&E-stained sections labeled positive with Apoptag® immunohistochemistry (Fig. 4).

**Image analysis of 2-ME-induced cell death**

Litters from dams given 2-ME displayed dose-dependent increases in AO fluorescence measured by image analysis of the data collected with CLSM (Fig. 5). For the high-dose group, quantitatively similar results were obtained when either Apoptag®- or H&E-stained serial histological sections were analyzed for cell debris in the neuroepithelium (Table 1). Analysis of H&E-stained sections also provided data concerning the number and size of individual apoptotic bodies (Table 2). These data showed that while 2-ME exposure increased the number of bodies per unit area, the average surface area of individual bodies in H&E-stained sections was unchanged.

Comparing the apoptotic index data presented here with previously published information (Bursch et al., '84; Ledda-Columbano et al., '91; Wheeldon et al., '95; Kolaja et al., '96) indicates that the embryonic neuroepithelium normally has much higher levels of apoptosis than normal adult liver. However, increasingly malignant tumors apparently resemble embryos by having high rates of both cell proliferation and cell death (Schulte-Hermann et al., '95).

**DISCUSSION**

One goal of the studies presented here was to characterize cell death induced by 2-ME in the anterior neural
folds of gd 8 mouse embryos. By several criteria, the cell debris in 2-ME-exposed embryos was indistinguishable from that observed in control embryos associated with physiological cell death. In histological sections, the cell debris fit the well-characterized description of apoptotic bodies published by Kerr and Harmon ('91). The differential labeling of dead cells with AO, but not with PI, was also consistent with the idea that apoptotic cells retain their membrane integrity (Del Bino et al., '91). In cultured thymocytes, however, late-stage apoptotic cells have been shown to become permeable to PI (Elstein and Zucker, '94). Our assay system may be less sensitive to low levels of PI fluorescence than the flow cytometric assay used in the latter study, or rapid phagocytosis of apoptotic bodies by neighboring neuroepithelial cells may make these bodies inaccessible to PI in intact embryos. The important finding is that in both control embryos and those from 2-ME-treated dams, there was a strikingly different pattern of staining with AO compared with PI. Thus, severe membrane damage was not a feature of 2-ME-induced cell death in viable embryos. Another characteristic of apoptotic cell death observed in both control and 2-ME-exposed embryos was DNA fragmentation detected in situ by Apoptag® immunohistochemistry (Gavrieli et al., '92). Positive Apoptag® staining is not conclusive evidence for apoptotic cell death, but the intensity of the label observed, together with the morphology, is highly suggestive of apoptosis (Gold et al., ’94). Furthermore, the average surface area of apoptotic bodies identified in H&E-stained sections was unchanged in embryos exposed to 2-ME. Together these data support the notion that 2-ME-induced cell death occurs via the same intrinsic program as physiological cell death, which is believed to proceed through an apoptotic mechanism (Kerr and Harmon, ’91; Mori et al., 1995). This conclusion is not surprising considering that 2-ME-induced cell death in embryonic limb buds, adult thymus and adult testis all have similar morphological features (Creasy and Fos ter, ’84; Greene et al., ’87; Balakumaran et al., ’95).

Fig. 4. In situ immunohistochemical detection of DNA fragmentation. A: Apoptag®-labeled coronal section from the prospective forebrain region of a gd 8 control embryo. The red-brown chromogen indicates free 3'-OH-DNA ends produced during apoptosis. Normal physiological cell death is occurring in the neuroepithelium (NE) and the foregut diverticulum (FD). Scale bar = 50 µm. B: High-power view of the foregut diverticulum seen in A. Most of the apoptotic bodies are Apoptag® positive, but a few do not stain. C: Neural crest region from the midbrain of a control embryo. No Apoptag®-positive bodies are visible. D: Similar section from an embryo of a dam exposed to 325 mg 2-ME/kg for 4 hr. Many peroxidase-positive bodies reside in the midbrain neuroepithelium (NE); some are also evident in the early migratory neural crest (MNC). Scale bar = 12.5 µm (B–D).
optical sections/embryo. Bars represent the means percentage of the area of background stain in approximately 20 serial area of bright stain, associated with apoptotic cells, was measured as a optical sections collected with confocal laser-scanning microscopy. The analysis was used to measure acridine orange fluorescence in digitized staining in the anterior neural folds of gd 8 mouse embryos. Image Fig. 5. Image analysis of apoptosis identified by acridine orange of DNA synthesis have revealed morphological features of embryonic cell death induced by known inhibitors proliferative rates (Manson, '86). Ultrastructural stud-
ies of embryonic cell death induced by known inhibitors of DNA synthesis have revealed morphological features consistent with apoptosis (Schweichel and Merker, '73; Sadler and Cardell, '76), lending further support for this model.

The cell death observed in embryos from dams exposed to 2-ME primarily affected cells in the neural crest region, and the greatest changes in both AO fluorescence and cell debris in histological sections were found in the prospective midbrain and rostral hindbrain. These are regions in which normal physiological cell death seems to occur at a later developmental stage (Ambroso, unpublished observations). Further studies are required to determine whether the neuroepithelial cells affected by 2-ME are actually cranial neural crest precursors, and whether they are the same cells that are programmed to die in control embryos at later developmental stages.

A second goal of the present work was to explore methods to quantify embryonic cell death, in order to eventually obtain data suitable for mathematical modeling of abnormal development. All three methods used here to analyze apoptosis detected an increase in embryos exposed to 2-ME, but the analysis of H&E-stained sections appeared to be the most sensitive end point (showed a larger difference between control and treated groups). In fact, the sensitivity of the H&E method would further increase if bodies without visible chromatin were included in the analysis (these comprised ~20% of the total when counted), as has been reported in studies of apoptosis in rat liver (Bursch et al., '84). The H&E method may be more sensitive because identification of individual apoptotic bodies was performed manually under high magnification, and relied on morphological features as well as staining characteristics. The H&E method, however, was time consuming and required familiarity with embryonic histology. The other two methods used more selective staining which allowed the analysis to be semi-automated and therefore faster, but both these techniques were limited by stain variability and were less able to accurately measure individual bodies because of the lower magnification. Because of these limitations, further method development is required before data from these two methods should be used for modeling purposes. For robust analysis of embryonic cell death, CLSM of fluorochrome-stained embryos and light microscopy of conventional H&E-stained sections appear to complement each other well.

**TABLE 2. Effect of 2-methoxyethanol (2-ME) on the number and surface area of apoptotic bodies observed in the neuroepithelium of mouse embryos on gestation day 8**

<table>
<thead>
<tr>
<th>2-ME (mg/kg)</th>
<th>Litters (n)</th>
<th>No. of apoptotic bodies/mm²</th>
<th>Mean surface area (µm²/body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>3</td>
<td>151 ± 119</td>
<td>9.8 ± 0.3</td>
</tr>
<tr>
<td>325</td>
<td>4</td>
<td>994 ± 127**</td>
<td>9.8 ± 0.2</td>
</tr>
</tbody>
</table>

*Apoptotic bodies were identified and measured in H&E-stained histological sections as described under Materials and Methods. Data are litter means ± SEM. **P < 0.05.

**TABLE 1. Quantitative analysis of apoptosis identified by Apoptag™ or H&E staining in the neuroepithelium of mouse embryos on gestation day 8**

<table>
<thead>
<tr>
<th>2-ME (mg/kg)</th>
<th>%Area of apoptosis (Apoptag™)</th>
<th>%Area of apoptosis (H&amp;E)</th>
<th>Apoptotic index** (H&amp;E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>0.1 ± 0.1</td>
<td>0.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>325</td>
<td>1.0 ± 0.1***</td>
<td>5.3 ± 1.4***</td>
<td></td>
</tr>
</tbody>
</table>

H&E, hematoxylin and eosin.

*Image analysis data obtained as described under Materials and Methods. Data are litter means from n = 2-3 litters for Apoptag™ and means ± SEM from n = 3-4 litters for H&E.

**H&E, hematoxylin and eosin.

*Image analysis data obtained as described under Materials and Methods. Data are litter means from n = 2-3 litters for Apoptag™ and means ± SEM from n = 3-4 litters for H&E. **(No. of apoptotic bodies/no. of nuclei) × 100; calculated to compare with previously published adult data (see under Discussion). ***P < 0.05.

The molecular mechanism by which 2-ME might trigger apoptosis in embryos remains unknown, although it has been hypothesized that metabolites of 2-ME disrupt nucleic acid biosynthesis (Mebus and Welsch, '89; Stedman and Welsch, '89). Because neuroepithelial cells are dividing extremely rapidly, a decrease in nucleic acid availability could block mitosis and lead to cell death. This mechanistic model fits the pattern of other cytotoxic teratogens that selectively induce cell death in embryonic regions exhibiting high proliferative rates (Manson, '86). Ultrastructural studies of embryonic cell death induced by known inhibitors of DNA synthesis have revealed morphological features...
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LITERATURE CITED


