Toxicity of Methoxyacetic Acid in Cultured Human Luteal Cells

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Ethylene glycol monomethyl ether (EGME) and its proximate metabolite, 2-methoxyacetic acid (MAA), increase ovarian luteal cell progesterone production in the female rat in vivo and in cultured rat luteal cells in vitro, respectively. In order to better assess the potential hazard of EGME and MAA to women, these studies were conducted to determine whether the same concentrations of MAA increase progesterone in human luteinized granulosa cells as in rat luteal cells. Human cells were collected from healthy anonymous oocyte donors, washed, plated 25,000 viable cells per well, and treated with 10 IU hCG and 0–5 mM MAA for 6–48 hr. Progesterone in media was significantly elevated after 24 hr incubation at >1 mM MAA. MAA had no effect on ATP levels at 6 or 24 hr. Thus, MAA increased progesterone production in cultured human luteal cells at the same concentration as MAA increased progesterone in rat luteal cells. The implication is that EGME has the potential to alter ovarian luteal function in women. These data should be useful for determining the real health hazards and potential risks of EGME exposure.

Ethylene glycol monomethyl ether (EGME) is a commonly used solvent that has been identified as a reproductive toxicant in humans and in animal models of human disease (reviewed in National Institute for Occupational Safety and Health, 1983, 1991). Recently published epidemiological studies have associated ethylene glycol-based solvent exposure in women with increased risks of spontaneous abortion, menstrual cycle dysfunction, and subfertility (Pastides et al., 1988; Gold et al., 1995; Correa et al., 1996). Specifically, solvent-exposed women had a 4.6-fold increase in risk of infertility (95% confidence interval 1.6–13.3) and a 2.8-fold increase in risk of spontaneous abortion (95% confidence interval 1.4–5.6) (Correa et al., 1996). A dose–response relationship between estimated ethylene glycol exposure and each of these outcomes has also been found.

Concurrent with the publication of these studies, we determined that the luteal cell is a target for EGME and its proximate toxic metabolite, 2-methoxyacetic acid (MAA), in the female rat (Davis et al., 1997). Specifically, in vivo dosing with EGME (300 mg/kg) elevated serum progesterone and suppressed cyclicity, inhibited ovulation, and increased corpora lutea size by inhibiting luteolysis. In vitro, 1–10 mM MAA elevated progesterone after 24 hr of treatment, but had no effect on cAMP at 1, 3, 24, or 48 hr or ATP levels at 24 hr. Based on these data, we concluded that EGME and MAA increase ovarian luteal cell progesterone production independent of LH-stimulated cAMP.

The question remains as to how the reproductive toxicity of EGME and MAA in the rat relates to the potential reproductive toxicity of EGME and MAA in women. To begin to bridge the knowledge gap, this study was designed to determine whether MAA affects progesterone production in cultured human luteinized granulosa cells at concentrations similar to those in the rat and provide data to allow direct comparisons between in vitro effects of MAA in the human luteal cell and the rat luteal cell.

MATERIALS AND METHODS

Chemicals. MAA (CAS No. 625-56-6) was obtained from Aldrich Chemical Co. (Milwaukee, WI) at a purity of 98%. HEPES, DMEM/H-12 with penicillin and streptomycin, phenol red, 10% fetal calf serum, collagenase, dispase, and Percoll were from Gibco (Grand Island, NY). Other reagents were from Sigma (St. Louis, MO) unless specified in the text.

Cell preparation and culture. Human luteinized granulosa cells were collected from healthy anonymous oocyte donors in a standardized in vitro fertilization protocol. Following a normal pelvic ultrasound and a negative serum pregnancy test, the pituitary was desensitized with 1 mg/day subcutaneously leuprolide acetate (Lupron, TAP Pharmaceuticals, Deerfield, IL) beginning in the midluteal phase. After the next menstrual period, the leuprolide was decreased to 0.5 mg/day and follicular recruitment was initiated with human urinary gonadotropins by daily intramuscular injections of Humegon (Organon, West Orange, NJ), Pergonal (Serono, Norwell, MA), or Metrodin (Serono) until the lead follicle reached a mean diameter of 9 mm. Human chorionic gonadotropin (10,000 IU) from Profasi (Serono) was administered im, 37–39 hr before the oocytes and surrounding luteinized granulosa cells were retrieved by transvaginal aspiration under ultrasound guidance. Harvesting granulosa cells from healthy oocyte donors eliminates infertility problems as a potential confounding variable in evaluating the biology of the luteinized granulosa cells.

Discarded luteinized granulosa cells (luteal cells) were collected into Hepes-buffered DMEM/H-12 medium with heparin and treated according to Richardson et al. (1989). Briefly, the aspirates were centrifuged at 200g
FIG. 1. Effects of various concentrations of MAA on human luteinized granulosa cell progesterone production. Human luteal cells were cultured for 24 hr with 10 IU hCG and 0–5 mM MAA. Progesterone was measured in media by RIA and standardized to cell protein. Each value represents the mean ± SE of quadruplicate determinations from three separate experiments.

RESULTS

MAA significantly increased progesterone production in human luteal cells at ≥1 mM concentrations after 24 hr of treatment over that produced by maximally effective concentrations of hCG (Fig. 1). Temporal evaluation of the progesterone hypersecretion demonstrated that 5 mM MAA significantly increased progesterone after 24 hr, but not by 6 hr, and did not increase progesterone production above maximal stimulation with hCG at 48 hr (Fig. 2). MAA (5 mM) had no effect on cellular ATP levels at 6 hr (8 ± 2 vs 11 ± 2 μmol/mg protein) or 24 hr (24 ± 9 vs 23 ± 5 μmol/mg protein). To determine whether progesterone hypersecretion in the luteal cell was a specific effect of MAA, cells were treated with phenoxyacetic acid (PAA), a structurally related glycol ether that has no associated reproductive toxicities (Breslin et al., 1991). PAA (1–10 mM) had no effect on basal or hCG-stimulated progesterone levels at any time point (data not shown).

DISCUSSION

This study shows that MAA, the proximate toxic metabolite of EGME, significantly increases progesterone production in a concentration- and time-dependent manner in cultured human luteinized granulosa cells. Moreover, MAA stimulates progesterone production at the same concentrations in the human cells as in cultured rat luteal cells (Davis et al., 1997). The in vitro effects of MAA on rat luteal cells are comparable to the in vivo effects of EGME in adult female rats at equivalent concentrations (Foster et al., 1987;
cells in order to separate physiologic differences and account for a proportion of the reported reproductive dysfunctions in occupationally exposed women.

These data should be useful for predicting the exposure levels at which EGME pose a health risk to women. In both the human and rat luteal cell, MAA had no effect on progesterone or ATP levels below 1 mM concentrations. This concentration correlates to the expected peak blood level after a single oral dose of 100 mg/kg EGME in vivo in the rat (Foster et al., 1987). Similarly, in vivo, EGME had no effect at concentrations less than 100 mg/kg in either the female (Davis et al., 1997) or the male rat (Chapin et al., 1985). Given the correlation between rodent and human in vitro no-effect levels and rodent in vitro and in vivo no-effect levels, we would predict a priori a similar no-effect level in women. Since there are differences, however, between the toxicokinetics of EGME in rodents and in humans (Groeseneken et al., 1989) and different routes of exposure in our rodent studies and in women, it will be necessary to establish the pharmacokinetic data of EGME in women in order to accurately predict exposure risks. Previous studies have addressed experimental and occupational exposures in men to determine such parameters (Groeseneken et al., 1986a,b; Veulemans et al., 1993) which may be feasible to determine in women as well.

While the concentration-dependent stimulation of progesterone production by MAA is the same in both human luteal cells and rat luteal cells, there are some apparent differences between the effects of MAA on rat luteal cells and human luteal cells in vitro. For example, in the human cells, the temporal pattern of effect suggests that MAA enhances hCG-stimulated progesterone production, but does not increase progesterone beyond a maximally stimulated state. In cultured rat luteal cells, MAA appears to maintain progesterone production and prevent luteolysis. However, the apparent differences in the MAA-induced stimulation of progesterone is most likely due to inherent differences in the cell culture methodologies. That is, the human cells are highly stimulated luteinized granulosa cells since they are acquired immediately after a superstimulated cycle during IVF procedures and, consequently, cells had been cultured without hCG for 24 hr to reinstate hCG responsiveness when MAA was added. In contrast, the rat luteal cells are fully differentiated and acquired 3–4 days after immature rats are superovulated; cells are exposed to MAA immediately upon culture; and cells produce detectable levels of progesterone without hCG stimulation. While many experiments could be designed to exactly match the conditions of human and rat cells in vitro in order to separate physiologic differences from methodological differences, the key experiments will be to determine the signaling pathways and molecular mechanisms by which MAA alters progesterone production in the human or the rat.

The stimulation of progesterone by MAA or EGME in vivo would be expected to alter the cycle. In the rat, the inappropriate secretion of progesterone results in an anovulatory, pseudopregnant state. The rat, however, has an estrous cycle without a luteal phase (Everett, 1961) which is distinct from the human menstrual cycle with a luteal phase. Consequently, it is more difficult to extrapolate a specific outcome from the in vivo effect in the rat to a potential in vivo effect in women. The outcome of exposure in women could also be complicated by factors such as when in the cycle exposure occurs. That is, the in vitro studies showed that MAA had no effect on a maximally stimulated luteal cell. Similarly, EGME may have no effect during peak luteal phase production of progesterone, but may have effects early or late in the luteal phase. Such differential effects could explain the association of both long and short cycles with EGME exposure (Gold et al., 1995). Further studies in women as well as in animal models are necessary to establish the real risks that EGME pose to the reproductive health of women.

In summary, this study establishes that MAA alters progesterone production in the human ovarian luteinized granulosa cell. MAA affects the same cell in a similar manner and at the same concentrations in both human and rat in vitro studies. Since EGME affects ovarian function in the rat in vivo, the implication is that EGME has the same potential to alter ovarian function in women. These results are an important step in bridging the gap between identification of hazards in animal models of human disease and assessing the risk of exposure to women’s health.

REFERENCES

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