Cyclosporin A-Induced Embryopathy in Embryo Culture Is Mediated through Inhibition of the Arachidonic Acid Pathway

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Abstract. **Embryos from Swiss Webster mice were grown in culture for 24 hr starting at Day 8.5 of gestation to study the effects of cyclosporin A (CsA) on the developing embryo. The embryos exposed to concentrations of CsA from 0.1 μg/ml to 10.0 μg/ml developed a significant increase in the incidence of malformations from 28.6% to 78.6%, as compared with the 6.8% incidence of malformations in the control embryos. These malformations included defects in the neural tubes, head folds, and facial arches. In addition, inhibition of embryonic growth in CsA-exposed embryos was shown by a lower somite number, crown-rump length, and protein content than those of the control embryos. Supplementation of the culture medium with arachidonic acid or prostaglandin E_2 decreased the incidence of CsA-induced malformations by 50% to 70% and prevented the CsA-induced inhibition of growth. We conclude that CsA causes abnormal embryonic development in mouse embryo culture and that the mechanism of CsA-induced embryopathy involves inhibition of the arachidonic acid pathway.** [P.S.E.B.M. 1993, Vol 202]

The mechanism of teratogenesis of several agents in mice appears to involve inhibition of the arachidonic acid pathway. Malformations that appear to be produced by a deficiency of arachidonic acid include: diphenylhydantoin-induced craniofacial and neural tube defects in embryo culture (1); glucocorticoid- and diphenylhydantoin-induced cleft palate *in vivo* (2); and hyperglycemia-induced teratogenesis *in vivo* and in embryo culture (3).

Cyclosporin A (CsA) has actions that are similar to these agents. Many studies have shown that CsA leads to inhibition of the arachidonic acid pathway and decreased prostaglandin production (4, 5). Furthermore, children treated with CsA chronically and children treated with diphenylhydantoin chronically develop similar abnormal morphologic features, including coarsening of their facial features, gingival hyperplasia, and hypertrichosis (6). These actions of CsA suggest the hypothesis that CsA has the potential to produce congenital malformations by a mechanism similar to that of these other agents, i.e., inhibition of the arachidonic acid pathway.

The objectives of this study were to determine whether CsA causes abnormal embryonic development in mice using an embryo culture technique and, if so, to determine whether the mechanism of CsA-induced embryopathy involves inhibition of the arachidonic acid pathway as demonstrated by prevention of teratogenic effects by exogenous arachidonic acid and prostaglandin E_2.

Materials and Methods

Animals. Swiss Webster mice were obtained from Charles River Laboratory, Wilmington, MA. The mice were maintained under a 14:10-hr light:dark cycle with water and Purina Rat Chow available *ad libitum*. Matings were allowed to occur overnight and confirmed by the presence of a vaginal plug after the mating period. Midnight was designated as the start of Day 0 of gestation.

The Swiss Webster strain of mice was used in this

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study because it is susceptible to diphenylhydantoin- and hyperglycemia-induced embryopathy in embryo culture (1).

Chemicals and Reagents. All chemicals and reagents, except cyclosporin A (CsA), were obtained from Sigma Chemical Co., St. Louis, MO. Sandimmune IV (CsA) was purchased from Sandoz Pharmaceutical Corp., East Hanover, NJ. Sandimmune IV and Cremophor El (vehicle for CsA) were diluted in 0.9% NaCl. Arachidonic acid (AA) and prostaglandin (PG) E_2 were initially dissolved in 100% ethanol and then diluted further in rat serum immediately before use. The maximum final concentration of ethanol in the embryo culture was 0.1%. PGE_2 was used because of its greater stability as compared with other prostaglandins.

Embryo Culture. Pregnant female mice were sacrificed on Day 8.5 of gestation. The uterus was removed and placed in sterile phosphate-buffered saline. The embryos were dissected away from the uterus, decidua, Reichert's membrane, and the parietal yolk sac. The visceral yolk sac and ectoplacental cone were left intact. Prerotational embryos five to eight somites in size were used for culture.

One to three embryos were placed in 15-ml sterile centrifuge tubes containing 1 ml of rat serum, 1 ml of Ham's F12 medium, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 2 m*M* glutamine. The rat serum was obtained from the blood of Sprague-Dawley rats that had been immediately centrifuged. The serum was then stored at −20°C. On the day of culture, the serum was heat inactivated at 57°C for 30 min.

The embryos were separated into nine groups as follows: (i) control with Cremophor El 130 μg/ml, (ii) CsA 0.1 μg/ml, (iii) CsA 0.5 μg/ml, (iv) CsA 1.0 μg/ml, (v) CsA 10.0 μg/ml, (vi) CsA 1.0 μg/ml and AA 20.0 μg/ml, (vii) CsA 10.0 μg/ml and AA 10.0 μg/ml, (viii) CsA 10.0 μg/ml and AA 20.0 μg/ml, and (ix) CsA 10.0 μg/ml and PGE_2 10 ng/ml. The culture tubes containing the embryos were then rotated at 30 rpm for 24 hr in 5% CO_2 at 37°C.

At the end of the culture period, all embryos were assessed for viability. Embryos having an active heart beat and yolk sac circulation were considered viable. Only viable embryos were examined for malformations and included in this analysis.

The viable embryos were examined for defects in neural tubes, facial arches, and head folds. The somite number was counted and crown-rump length was measured. The crown-rump length could be measured only in those embryos that had rotated. Thus, the most severely affected embryos that did not rotate were not included in this analysis. Morphologic development was scored based on a system suggested by Sadler and Warner (7). This system scores the embryos based on their degree of yolk sac vascular development, rotation, neural tube closure, facial arch development, limb development, and heart rate. Each of these features is assigned a score from 0 to 5 based on their developmental stage. The overall morphologic score for an individual embryo is the total of its scores for each of the above features.

Protein Assay. Each embryo was dissolved overnight in 100 μl of 1 *N* NaOH. Protein determination was performed using the Lowry method (8).

Statistics. Categorical variables were compared using Fisher's exact test. Continuous variables were compared using a two-tailed Student's *t* test. Correlations were analyzed using linear regression and calculating a Pearson's correlation coefficient.

Results

Teratogenic Effects of CsA. As the concentration of CsA was increased from 0.1 μg/ml to 10.0 μg/ml in the culture media, the incidence of embryonic malformations increased from 28.6% to 78.6% (Table I). Even at the lowest concentration of CsA (0.1 μg/ml) the total incidence of malformations and the incidence of neural tube defects were significantly higher than those of the control group. A dose-dependent relationship was demonstrated between the concentration of CsA and the incidences of neural tube defects ($P < 0.05$, $r = 0.90$) and facial arch defects ($P < 0.05$, $r = 0.97$).

Examples of embryos exposed to CsA and control embryos are shown in Figures 1 through 3. The neural tube defects consisted of open neural tubes in the cranial and cervical regions. Abnormalities of facial arches consisted of either asymmetric arches, missing arches, or arches with an excessive downward angulation. Abnormal head folds were either misshapen or asymmetric. A cranial neural tube defect was not considered a head fold defect unless the head fold was also grossly asymmetric or misshapen. Rotation was considered abnormal if the head of the embryo was not turned at least 90° in relation to the body.

Growth and development of the embryo also was decreased by CsA (Table II). The somite number was not different among the groups at the start of the culture period. After the culture period, somite number, crown-rump length, total protein content, and morphologic scores were significantly less in the CsA-exposed embryos than in the control group.

There were no differences in the incidences of nonviable embryos among the nine groups of embryos. No group had more than one nonviable embryo.

Effect of AA and PGE_2 Supplementation. Both AA and PGE_2 supplementation decreased the incidence of CsA-induced embryopathy (Table III and Figs. 4 and 5). Supplementation of the embryos exposed to 1.0 μg/ml of CsA with 20.0 μg/ml of AA resulted in greater than 60% decreases in the total incidence of malformations and in the incidences of each specific defect,

Table I. Effect of CsA on the Incidence of Embryonic Malformations

Group	Total (*n*)	Abnormal *n* (%)	Specific defects			
			Neural tube *n* (%)	Head fold *n* (%)	Facial arch *n* (%)	Rotation *n* (%)
Control	44	3 (6.8)	1 (2.3)	0 (0)	2 (4.5)	2 (4.5)
CsA						
0.1 μg/ml	21	6 (28.6)[a]	4 (19)[a]	2 (9.5)	1 (4.8)	3 (14.3)
0.5 μg/ml	20	5 (25)	5 (25)[b]	3 (15)[a]	1 (5)	2 (10)
1.0 μg/ml	25	14 (56)[c]	11 (44)[c]	7 (28)[c]	3 (12)	10 (40)[c]
10.0 μg/ml	28	22 (78.6)[c]	22 (78.6)[c]	10 (35.7)[c]	8 (28.6)[b]	10 (35.7)[c]

[a] $P < 0.05$ vs control.
[b] $P < 0.01$ vs control.
[c] $P < 0.001$ vs control.

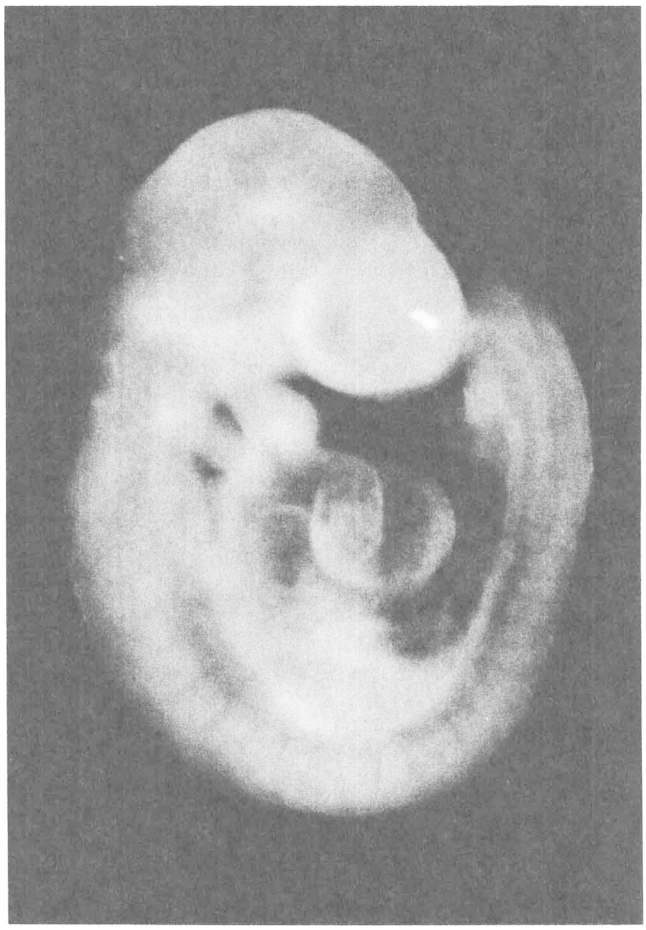

Figure 1. Normal control embryo (magnification ×30).

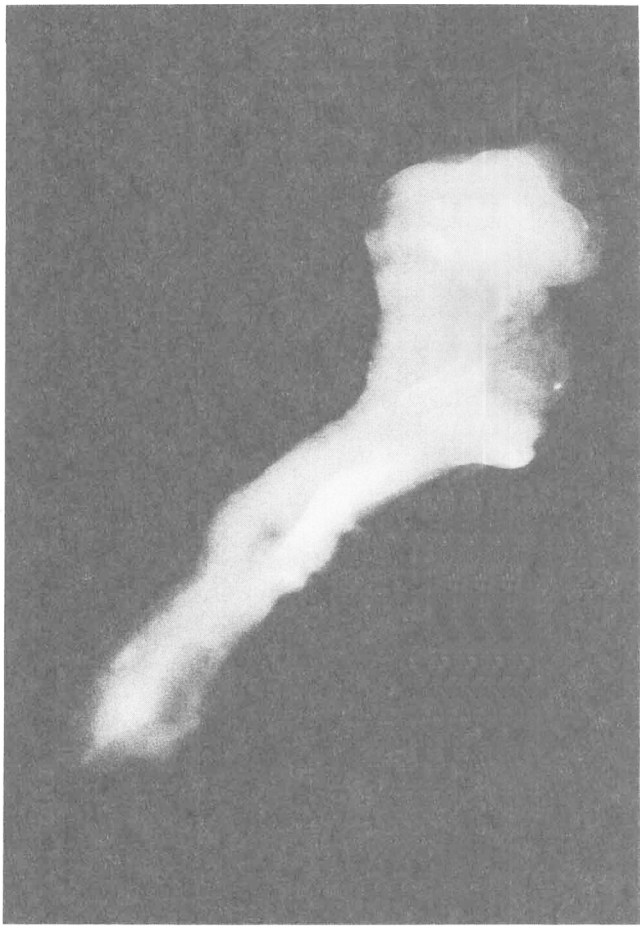

Figure 2. Embryo exposed to 10 μg/ml of CsA. The neural tube is open. The head fold is misshapen and the facial arches are displaced. Rotation has not occurred (magnification ×30).

except facial arch defects. The lack of significant change in the incidence of facial arch defects is probably because of the small number of these defects in those embryos exposed to CsA at 1.0 μg/ml.

In embryos exposed to 10.0 μg/ml of CsA, supplementation with 10.0 μg/ml of AA significantly decreased only the incidence of neural tube defects. However, when the concentration of AA was increased to 20.0 μg/ml, there were decreases of 50% in the total incidence of malformations and in the incidences of each specific defect. A dose-response relationship was demonstrated between the amount of AA supplementation and the total incidence of malformations ($P < 0.05$, $r = -0.99$) and the incidence of head fold defects ($P < 0.05$, $r = -0.99$).

In addition, supplementation with PGE_2 at 10.0 ng/ml in those embryos exposed to 10.0 μg/ml of CsA

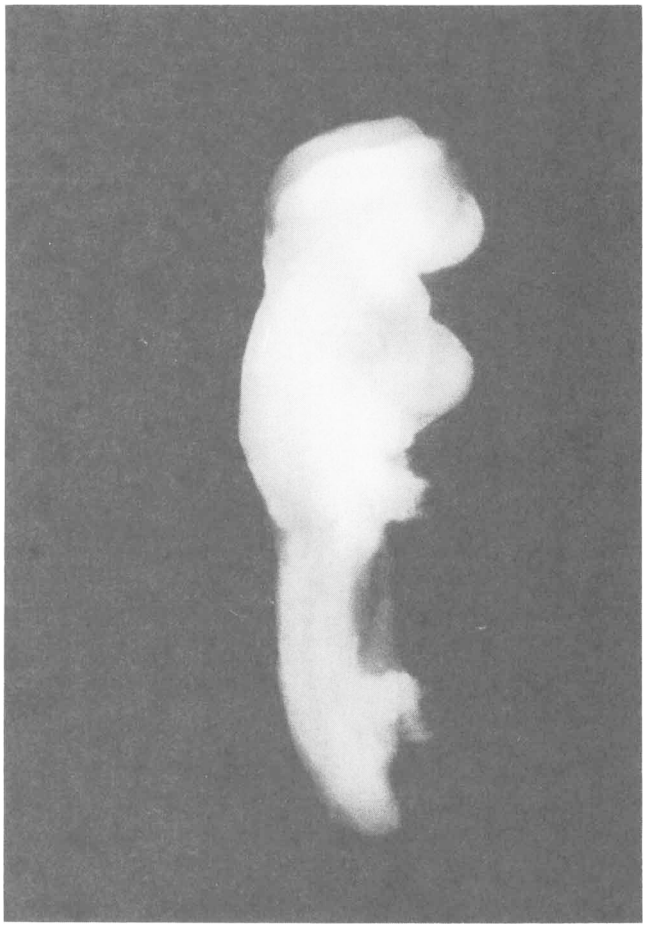

Figure 3. Embryo exposed to 1.0 μg/ml of CsA. The neural tube is open and rotation is not complete.

resulted in a greater than 60% decrease in the total incidence of malformations and greater than 70% decreases in the incidences of each specific defect. The decreases in all categories were significant.

An additional 15 embryos were cultured in media containing only PGE_2 at 10 ng/ml. There were no defects in neural tubes, facial arches, or head folds in this group of embryos.

The delay in growth and development secondary to CsA also was prevented by AA or PGE_2 supplementation (Table IV). As compared with the group exposed only to 1.0 μg/ml of CsA, the group exposed to both 1.0 μg/ml of CsA and 20.0 μg/ml of AA had significantly greater protein content and morphologic score. The crown-rump length and the somite number were not different. When embryos exposed to 10.0 μg/ml of CsA were supplemented with 10.0 μg/ml of AA, there were significant increases in crown-rump length and morphologic score. When supplementation with AA was increased to 20 μg/ml, all categories of growth and development were significantly increased. Supplementation with PGE_2 at 10.0 ng/ml also resulted in significant increases in all categories of growth and development.

Discussion

We found that CsA caused abnormal development in Swiss Webster murine embryos in culture. As the concentration of CsA increased from 0.1 μg/ml to 10.0 μg/ml in the culture medium, the incidence of congenital malformations and the degree of growth delay increased. The concentrations of CsA used in this study overlap the therapeutic trough plasma concentrations in humans of 0.05–0.25 μg/ml.

This is the first report to show embryonic malformations secondary to CsA. Previous reports in rats *in vivo* by Mason *et al.* (9) and in ICR mice *in vivo* by Fein *et al.* (10) have shown a fetotoxic effect as shown by increased resorptions and decreased litter size. These reports also show a decrease in fetal weight secondary to CsA administration; however, fetal malformations were not found. Currently, other animal models demonstrating a consistent pattern of CsA-induced embryopathy do not exist.

The lack of CsA-induced fetal malformations in previous studies may be related to absent or limited placental transfer of CsA in rodents. Bäckman *et al.* (11) did not observe passage of radioactive CsA from

Table II. Effect of CsA on Embryonic Growth and Development[a]

Group	Somite (*n*)	Crown-rump length[b] (mm)	Protein (μg/embryo)	Score
Control	24.2 ± 1.9	2.25 ± 0.2	86.5 ± 14.2	21.3 ± 1.6
CsA				
0.1 μg/ml	22.4 ± 2.3[c]	2.08 ± 0.2[d]	70.1 ± 12.7[e]	20.5 ± 2.5
0.5 μg/ml	23.6 ± 2.1	2.11 ± 0.2[d]	64.5 ± 8.3[e]	19.9 ± 2.7[c]
1.0 μg/ml	21.4 ± 2.6[e]	2.05 ± 0.2[c]	53.5 ± 7.8[e]	17.2 ± 4.2[e]
10.0 μg/ml	21.0 ± 2.7[e]	2.00 ± 0.2[e]	59.4 ± 26.3[e]	16.1 ± 3.8[e]

[a] All values are mean ± SD.
[b] Crown-rump length measured in completely rotated embryos only.
[c] $P < 0.01$ vs control.
[d] $P < 0.05$ vs. control.
[e] $P < 0.001$ vs. control.

Table III. Effect of AA and PGE Supplementation on the Incidence of CsA-Induced Malformations

Group	Total (*n*)	Abnormal *n* (%)	Specific defects			
			Neural tube *n* (%)	Head fold *n* (%)	Facial arch *n* (%)	Rotation *n* (%)
Control	44	3 (6.8)	1 (2.3)	0 (0)	2 (4.5)	2 (4.5)
CsA 1.0 μg/ml	25	14 (56)[a]	11 (44)[a]	7 (28)[a]	3 (12)	10 (40)[a]
CsA 1.0 μg/ml + AA 20.0 μg/ml	20	4 (20)[b]	3 (15)[b]	1 (5)[b]	2 (10)	1 (5)[c]
CsA 10.0 μg/ml	28	22 (78.6)[a]	22 (78.6)[a]	10 (35.7)[a]	8 (28.6)[d]	10 (35.7)[a]
CsA 10.0 μg/ml + AA 10.0 μg/ml	19	11 (58)[a]	7 (36.8)[a,c]	5 (26.3)[d]	2 (10.5)	6 (31.6)[d]
CsA 10.0 μg/ml + AA 20.0 μg/ml	21	7 (33)[c,d]	6 (28.6)[d,e]	3 (14.3)[f]	0 (0)[c]	0 (0)[c]
CsA 10.0 μg/ml + PGE_2 0.01 μg/ml	29	9 (31)[e,f]	7 (24.1)[d,e]	2 (6.9)[c]	2 (6.9)[b]	2 (6.9)[c]

[a] $P < 0.001$ vs control.
[b] $P < 0.05$ vs corresponding CsA group.
[c] $P < 0.01$ vs corresponding CsA group.
[d] $P < 0.01$ vs control.
[e] $P < 0.001$ vs corresponding CsA group.
[f] $P < 0.05$ vs control.

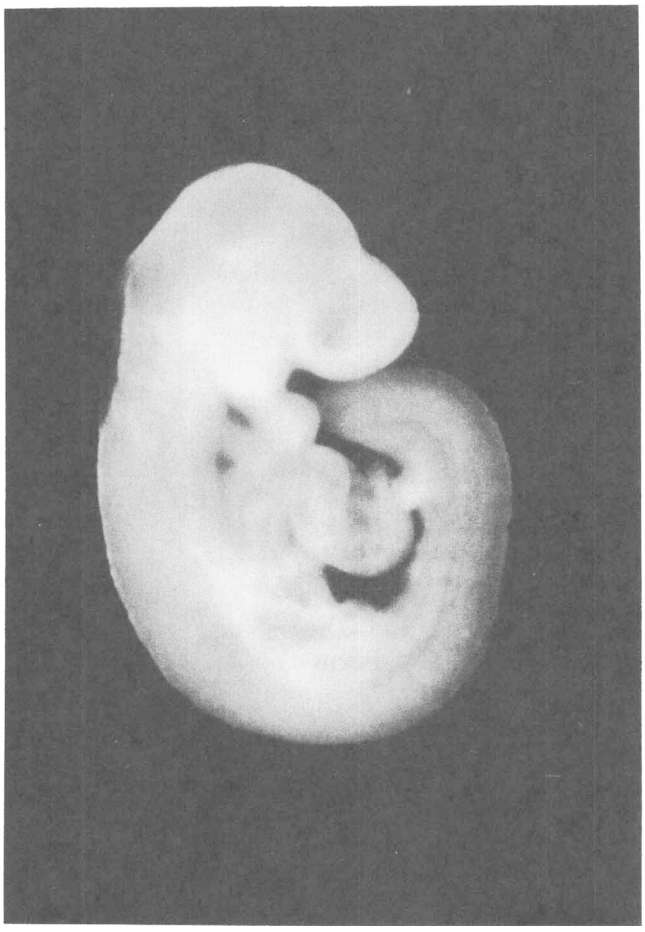

Figure 4. Normal-appearing embryo after exposure to 10 μg/ml of CsA with 20 μg/ml of AA (magnification ×30).

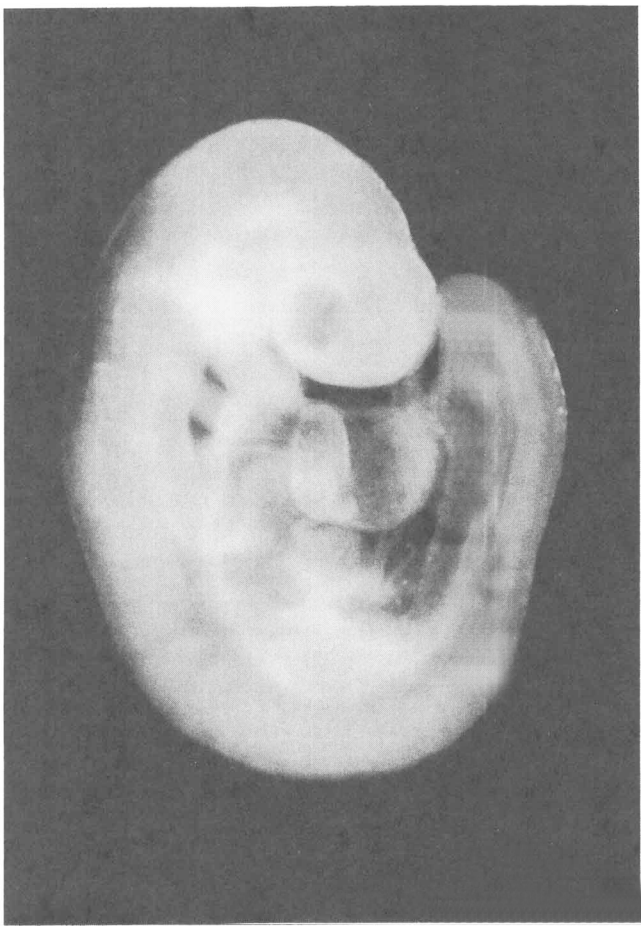

Figure 5. Normal-appearing embryo after exposure to 10 μg/ml of CsA with 10 ng/ml of PGE_2 (magnification ×30).

Table IV. Effects of AA and PGE Supplementation on CsA-Induced Delayed Growth and Development[a]

Group	Somite (*n*)	Crown-rump length (mm)	Protein (μg/embryo)	Score
Control	24.2 ± 1.9	2.25 ± 0.2	86.5 ± 14.2	21.3 ± 1.6
CsA 1.0 μg/ml	21.4 ± 2.6[b]	2.05 ± 0.2[c]	53.5 ± 7.8[b]	17.2 ± 4.2[b]
CsA 1.0 μg/ml + AA 20.0 μg/ml	22.5 ± 2.1[c]	2.10 ± 0.2[d]	73.2 ± 6.5[b,e]	20.6 ± 2.8[f]
CsA 10.0 μg/ml	21.0 ± 2.7	2.00 ± 0.2[b]	59.4 ± 26.3[b]	16.1 ± 3.8[b]
CsA 10.0 μg/ml + AA 10.0 μg/ml	22.1 ± 2.6[b]	2.17 ± 0.2[a]	61.7 ± 15.8[b]	18.6 ± 3.4[b,g]
CsA 10.0 μg/ml + AA 20.0 μg/ml	24.1 ± 2.1[f]	2.31 ± 0.4[f]	92.6 ± 21.1[e]	20.3 ± 1.8[d,e]
CsA 10.0 μg/ml + PGE_2 0.01 μg/ml	22.7 ± 2.6[c,g]	2.19 ± 0.3[g]	79.9 ± 14.4[f]	19.7 ± 3.1[c,e]

[a] All values are mean ± SD.
[b] $P < 0.001$ vs control.
[c] $P < 0.01$ vs control.
[d] $P < 0.05$ vs control.
[e] $P < 0.001$ vs corresponding CsA group.
[f] $P < 0.01$ vs corresponding CsA group.
[g] $P < 0.05$ vs corresponding CsA group.

pregnant mice to their fetuses after a single injection. Classen and Shevach (12) showed that in pregnant mice that had received a continuous infusion of CsA for 4 days, the fetal tissue levels were as little as 6% of maternal levels. Therefore, the fetotoxic effect of CsA may be mediated by alteration in maternal physiology rather than a result of a direct effect on the fetus.

Despite the previous lack of evidence of CsA-induced teratogenesis in rodents, the murine embryo culture model that was used in this study was chosen for a variety of reasons. First, the direct effects of CsA on the embryo could be evaluated without interference from maternal and placental variables. Second, the concentration of CsA in the culture medium and the gestational age of the embryos could be precisely controlled. Finally, the mechanism of CsA-induced embryopathy could be easily investigated by the addition of arachidonic acid or PGE_2 to the culture medium. Because this system had been used previously to demonstrate that inhibition of the arachidonic acid pathway leads to teratogenic effects (1, 3), it seems appropriate to use the same method to study the effects of CsA, which also inhibits the arachidonic acid pathway.

In human pregnancy, experience with CsA is limited, and specific teratogenic effects of CsA have not been documented consistently. In a review of 51 pregnancies with fetal CsA exposure by Cockburn *et al.* (13) three infants had congenital malformations, but these malformations were thought to be coincidental events because of the lack of a common pattern. One of these infants had anencephaly, a neural tube defect. The other two malformations were absence of the corpus callosum and congenital cataracts. In addition, Pickrell *et al.* (14) did not find any malformations in his review of 16 cases of fetal CsA exposure. However, the incidences of low birth weight in these studies were 44% and 56%, respectively. On the other hand, a case report by Pujals (15) describes an infant with hypoplasia of the right leg after maternal CsA administration during pregnancy. The small number of human pregnancies involving fetal CsA exposure may be inadequate to detect a low frequency rate of congenital malformation.

Unlike in rodents, CsA crosses the human placenta easily. A report by Flechner *et al.* (16) showed that maternal blood and cord blood levels of CsA were equal. Furthermore, the concentration of CsA in the amniotic fluid and placenta was even higher than in maternal plasma. In another study, Venkatarmanan *et al.* (17) showed that cord blood levels of CsA were approximately 50% of maternal blood levels, but some active metabolites of CsA were present at equal concentrations in maternal and cord blood. Therefore, a potential risk for teratogenesis secondary to CsA may exist in humans.

We also found that the embryopathic effects of CsA in mice are decreased by supplementation with either AA or PGE_2, which suggests that the mechanism of CsA-induced embryopathy involves inhibition of the arachidonic acid pathway. A relative deficiency of AA or prostaglandins has been shown to lead to malformations secondary to maternal diabetes (3) and the teratogens, diphenylhydantoin and glucocorticoids (1, 2). Glucocorticoid- and diphenylhydantoin-induced teratogenesis appears to involve receptor-mediated production of lipocortins that inhibit phospholipase A_2 and subsequent AA release from membrane phospholipids (18). Maternal diabetes, on the other hand, appears to inhibit phosphatidylinositol turnover through a defi-

ciency of *myo*-inositol uptake with subsequent inhibition of protein kinase C and phospholipase A_2 (19, 20). Thus, it appears that inhibition of the arachidonic acid pathway may be a common mechanism through which multiple agents cause teratogenesis at different sites.

This study is consistent with studies in other systems that have shown that CsA causes inhibition of prostaglandin production. In cultured human umbilical vein endothelial cells, CsA at 10.0 μg/ml leads to an 80% decrease in PGI_2 release (5), and in aorta smooth muscle cells, CsA leads to decreased PGE_1 release (4). Using skin allographs, Fan and Lewis (21) showed that CsA caused decreased release of arachidonic acid. In the kidney, CsA causes a decrease in the glomerular production of PGE_2 and 6-keto-$PGF_{1\alpha}$ (22), and Metz-Kurschel *et al.* (23) showed that rioprostil (BAY 06893; Bayer AG), a PGE_1 analog, prevented CsA-induced nephrotoxicity in rats.

Some studies have concluded that CsA enhances prostaglandin production. However, many of these studies have examined urinary excretion of AA metabolites in response to CsA. Urinary prostaglandin excretion may not be a good indicator of prostaglandin synthesis because the concentration of urinary prostaglandins is dependent on urinary flow and pH. In addition, urinary prostaglandin excretion may reflect only the effect of CsA on the renal medulla and not the effect on the renal cortex (24).

The results of our study support the hypothesis that CsA inhibits the AA cascade above the level of cyclooxygenase since AA itself greatly reduced CsA-induced teratogenesis. This is consistent with other reports. Fan and Lewis (25) showed that CsA had a direct inhibitory effect on phospholipase A_2 at concentrations from 1.0 μg/ml to 30.0 μg/ml. In cell culture, however, CsA concentrations from 0.3 μg/ml to 10.0 μg/ml caused a 29–61% decrease in prostaglandin production. The inhibitory effect of CsA on prostaglandin synthesis by rat macrophages was prevented by supplementation with exogenous arachidonic acid (25). A study by Kurtz *et al.* (4) also showed prevention of the decrease in PGE_2 release secondary to CsA in aortic smooth muscle cells with supplementation of arachidonic acid. Walker *et al.* (26) were able to show that CsA inhibited phosphorylation by protein kinase C in renal tubular epithelial cells that subsequently inhibited 12-*O*-tetradecanoylphorbol 13-acetate-induced release of PGE_2 (26).

The fact that AA and PGE_2 did not completely prevent the embryopathic effects of CsA may be explained in several ways. First, the concentrations of PGE_2 and AA may not have been optimal in our study. Second, not all biochemical aspects affected by the arachidonic acid pathway may have been corrected, such as possible decreases in leukotriene synthesis. Third, CsA may have effects on other pathways that also may be involved in teratogenesis.

We have found that CsA causes abnormal embryonic development in mouse embryo culture at concentrations considered within the therapeutic range in humans. The embryopathic effects of CsA were greatly reduced by exogenous AA and PGE_2. Although animal studies sometimes correlate with effects seen in humans, this is not always the case. Nevertheless, close monitoring of the outcome of CsA use in pregnancy seems warranted. In addition to CsA's extensive use after transplantation, its potential uses in the treatment of other diseases, such as psoriasis, aplastic anemia, diabetes, and connective tissue diseases, are now being investigated. With more widespread use, more women of reproductive age will be treated with CsA. Thus, a greater incidence of fetal exposure to CsA and the possibility of a greater risk of CsA-induced teratogenesis may exist.

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1. Kay ED, Goldman AS, Daniel JC. Common biochemical pathway of dysmorphogenesis in murine embryos: Use of the glucocorticoid pathway by phenytoin. Teratogenesis Carcinog Mutagen **10**:31–39, 1990.
2. Goldman AS. Biochemical mechanism of glucocorticoid- and phenytoin-induced cleft palate. Curr Top Dev Biol **19**:217–239, 1984.
3. Goldman AS, Baker L, Piddington R, Marx B, Herold R, Eglar J. Hyperglycemia-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. Proc Natl Acad Sci USA **82**:8227–8231, 1985.
4. Kurtz A, Pfeilschifter J, Kuhn K, Koch KM. Cyclosporin A inhibits PGE_2 release from vascular smooth muscle cells. Biochem Biophys Res Commun **147**:542–549, 1987.
5. Voss BL, Hamilton KK, Samara EN, McKee PA. Cyclosporine suppression of endothelial prostacyclin generation. Transplantation **45**:793–796, 1988.
6. Reznik VM, Jones KL, Durham BL, Mendoza SA. Changes in facial appearance during cyclosporin treatment. Lancet **1**:1405–1407, 1987.
7. Sadler TW, Warner CW. Use of whole embryo culture for evaluating toxicity and teratogenicity. Pharmacol Rev **36**:145S–150S, 1984.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem **193**:265–275, 1951.
9. Mason RJ, Thomson AW, Whiting PH, Gray ES, Brown PAJ, Catto GRD, Simpson JG. Cyclosporine-induced fetotoxicity in the rat. Transplantation **39**:9–12, 1985.
10. Fein A, Vechoropoulos M, Nebel L. Cyclosporin-induced embryotoxicity in mice. Biol Neonate **56**:165–173, 1989.
11. Bäckman L, Brandt I, Dallner G, Ringden O. Tissue distribution of [^{3}H]cyclosporine A in mice. Transplant Proc **20**:684–691, 1988.
12. Classen JB, Shevach EM. Evidence that cyclosporine treatment during pregnancy predisposes offspring to develop autoantibodies. Transplantation **51**:1052–1057, 1991.

13. Cockburn I, Krupp P, Monka C. Present experience of sandimmun in pregnancy. Transplant Proc **21**:3730–3732, 1989.
14. Pickrell MD, Sawers R, Michael J. Pregnancy after renal transplantation: Severe intrauterine growth retardation during treatment with cyclosporin A. Br Med J **296**:825, 1988.
15. Pujals JM, Figueras G, Puig JM, Lloveras J, Aubia J, Masramon J. Osseous malformation in baby born to woman on cyclosporin. Lancet **1**:667, 1989.
16. Flechner SM, Katz AR, Rogers AJ, Van Buren C, Kahan BD. The presence of cyclosporine in body tissues and fluids during pregnancy. Am J Kidney Dis **5**:60–63, 1985.
17. Venkatarmanan R, Koneru B, Wang CP, Burkart GJ, Caritis SN, Starzl TE. Cyclosporine and its metabolites in mother and baby. Transplantation **46**:468–469, 1988.
18. Gupta C, Katsumata M, Goldman AS, Herold R, Piddington R. Phospholipase A_2 inhibitory proteins mediate glucocorticoid teratogenicity in vitro. Proc Natl Acad Sci USA **81**:1140–1143, 1984.
19. Greene DA, Lattimer S, Ulbrecht J, Carroll P. Glucose-induced alterations in nerve metabolism: Current perspective on the pathogenesis of diabetic neuropathy and future directions for research therapy. Diabetes Care **8**:290–299, 1985.
20. Goldman AS. Pathophysiology of congenital malformations. In: Polin RA, Fox WW, Eds. Fetal and Neonatal Physiology. Philadelphia: WB Saunders, Vol **1**: pp36–46, 1992.
21. Fan TPD, Lewis GP. Effect of cyclosporin A and inhibitors of arachidonic acid metabolism on blood flow and cyclooxygenase products in rat skin allografts. Br J Pharmacol **81**:361–371, 1984.
22. Bunke M, Wilder L, McLeish K. Effect of cyclosporine on glomerular prostaglandin production. Transplant Proc **20**(suppl 3):646–649, 1988.
23. Metz-Kurschel U, Wagner K, Mueller MK. The synthetic prostaglandin E_1 analogue rioprostil reduces the nephrotoxic potential of cyclosporine A. Transplant Proc **22**:1713–1716, 1990.
24. Bennett WM, Elzinger L, Kelley V. Pathophysiology of cyclosporine nephrotoxicity: Role of eicosanoids. Transplant Proc **20**(suppl 3):628–633, 1988.
25. Fan TPD, Lewis GP. Mechanism of cyclosporin A-induced inhibition of prostacyclin synthesis by macrophages. Prostaglandins **30**:735–747, 1985.
26. Walker RJ, Lazzaro VA, Duggin GG, Harvath JS, Tiller DJ. Cyclosporin A inhibits protein kinase C activity: A contributing mechanism in the development of nephrotoxicity. Biochem Biophys Res Commun **160**:409–415, 1989.