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Apparent Cyclophosphamide (Cytoxan) Embryopathy: A Distinct Phenotype?

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Cyclophosphamide (CP) is an alkylating agent widely used in treating cancer and autoimmune disease. CP is classified as a pregnancy risk factor D drug and is teratogenic in animals, but population studies have not conclusively demonstrated teratogenicity in humans. Six isolated reports of prenatally exposed infants with various congenital anomalies exist, but to date no specific phenotype has been delineated. The purpose of this report is to document a new case of in utero CP exposure with multiple congenital anomalies and to establish an apparent CP embryopathy phenotype. The mother had systemic lupus erythematosus and cyclophosphamide exposure in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had growth retardation and multiple anomalies including microbrachycephaly, coronal craniosynostosis, hypotelorism, shallow orbits, proptosis, blepharophimosis, small, abnormal ears, unilateral preauricular pit, broad, flat nasal bridge, microstomia, high-arched palate, micrognathia, preaxial upper limb and postaxial lower limb defects consisting of hypoplastic thumbs, and bilateral absence of the 4th and 5th toes. Chromosomes were apparently normal. The reported cases of in utero exposure to cyclosposphamide shared the following manifestations with our patient: growth deficiency, developmental delay, craniosynostosis, blepharophimosis, flat nasal bridge, abnormal ears, and distal limb defects including hypoplastic thumbs and oligodactyly. We conclude that (a) cyclophosphamide is a human teratogen, (b) a distinct phenotype exists, and (c) the safety of CP in pregnancy is in serious question. Am. J. Med. Genet. 86:237–241, 1999.

KEY WORDS: Cytoxan; cyclophosphamide; teratogenesis; malformation

INTRODUCTION

Cyclophosphamide (CP) is an alkylating agent widely used in cancer chemotherapy and in autoimmune disease therapy. Microsomal cytochrome P-450 monooxygenases convert CP to 4-hydroxycyclophosphamide which, in turn, spontaneously breaks down into the active compounds phosphoramide mustard and acrolein [Mirkes, 1985]. CP is clearly teratogenic in animals, producing similar central nervous system and skeletal anomalies in rats, mice, chicks, rabbits, and monkeys [Gilani and Chatzinoff, 1983; Mirkes, 1985]. Animal fetuses exposed to CP have also shown facial anomalies including exophthalmos, cleft lip/palate, abnormal ears, microtia, and midface hypoplasia [Gilani and Chatzinoff, 1983; Mirkes, 1985; Padmanabhan and Singh, 1984]. Despite the clear association of CP with teratogenicity in animals, to our knowledge, only 6 isolated case reports of malformations in human infants with in utero exposure to CP beginning in the 1st trimester exist and a distinct phenotype associated with in utero exposure to CP has not been established [Greenberg and Tanaka, 1964; Kirshon et al., 1988; Murray et al., 1984; Mutchinick et al., 1992; Toledo et al., 1971; Zemlickis et al., 1993] (Table I). There is 1 additional case report of 1st-trimester CP exposure in which the infant had only minor anomalies (umbilical hernia and hemangioma) not thought to be caused by CP [Coates, 1970]. There are no prospective population data available on pregnancy outcome in women taking CP. Past retrospective studies of pregnant patients treated with CP alone or in combination with other drugs or radiation therapy have not clearly docu-

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TABLE I. In Utero Cyclophosphamide Exposure: Clinical Findings*

	Greenberg Tanaka, 1964	Toledo et al., 1971	Murray et al., 1984	Kirshon et al., 1984	Mutchinick et al., 1992	Zemlickis et al., 1993	Current study	Totals
Growth deficiency/SGA	+		+		+	+	+	5/7
Developmental delay				+		+	+	3/7
Craniofacial anomalies								
Craniosynostosis					+		+	2/7
Microcephaly			+	+			+	3/7
Eye								
Hypotelorism							+	1/7
Blepharophimosis				+	+		+	3/7
Microphthalmia				+				1/7
Shallow orbits/proptosis							+	1/7
Ear								
Abnormal ears				+	+		+	3/7
Microtia							+	1/7
Nose								
Flat nasal bridge	+				+		+	3/7
Bulbous tip				+	+		+	3/7
Mouth								
High-arched/cleft palate	+			+	+		+	4/7
Extremities								
Hypoplastic radius					+	+		2/7
Hypoplastic/absent thumbs	+			+	+	+	+	5/7
Hypoplastic 5th finger middle phalanx	+						+	2/7
Absent fingers/toes	+	+			+		+	4/7
Prenatal drug exposure	CP (T1–3)	CP, Rad (T1)	CP, D, Co, Rad (T1)	CP (T1), P (T1–3)	CP (T1)	CP, P (T1–3)	CP (T1), N, C, A (T1–3)	

*Frequencies of specific congenital anomalies associated with in utero cyclophosphamide exposure reported in the literature are shown above. Other rarely reported anomalies include a single coronary artery [Toledo et al., 1971], inguinal hernia [Greenberg and Tanaka, 1964], and imperforate anus [Murray et al., 1984]. In addition to the anomalies tabulated above, 1 patient also had an abnormal inferior vena cava, esophageal atresia, crossed-renal ectopia, and cryptorchidism and developed papillary thyroid cancer and a neuroblastoma in later childhood [Zemlickis et al., 1993]. SGA, small for gestational age; T, trimester; A, atenolol; C, clonidine; Co, cobalt therapy; CP, cyclophosphamide; D, doxorubicin; N, nifedipine; P, prednisone; Rad, radiation therapy.

mented teratogenicity in humans [Nicholson, 1968; Sanders et al., 1996]. In general, treatment with antineoplastic agents, including CP, has been considered relatively safe if treatment is given in the 2nd or 3rd trimesters [Nicholson, 1968; Pizzuto et al., 1980; Sanders et al., 1996; Sweet and Kinzie, 1976]. It has been difficult to determine the specific potential for CP to cause birth defects because CP was frequently used in combination with other drugs or radiation therapy in cases of abnormal pregnancy outcome [Greenberg and Tanaka, 1964; Kirshon et al., 1988; Murray et al., 1984; Toledo et al., 1971; Zemlickis et al., 1993]. The purpose of the present article is to report a patient exposed to CP in utero with multiple anomalies, similar to those reported in animal studies, and to establish a distinct phenotype associated with in utero CP exposure by comparison of our patient to previously published cases. Thus, we question the safety of using CP during pregnancy.

CLINICAL REPORT

The patient was born at 37 weeks of gestation to a 23-year-old G2P1 Hispanic mother by Caesarean section for toxemia and decreased fetal heart rate variability. The mother was unaware of her pregnancy until 26 weeks of gestation. An ultrasound evaluation at 31 weeks of gestation detected possible craniosynostosis and hypotelorism and amniocentesis documented a normal karyotype (450 band resolution). The mother had been undergoing treatment for lupus nephritis and hypertension during pregnancy. She received a total of 4 intravenous doses of Cytoxan® (20 mg/kg/dose). Based on ultrasound data from an examination in the 26th week of gestation, 3 doses of CP were most likely given before conception and an additional dose was calculated to have been administered during the 6th week postconception on Day 37. The mother also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. There was no history of consanguinity or birth defects in the family. The birth weight was 1705 g (<5th centile), length 46 cm (5–10th centile), and head circumference (OFC) 30.2 cm (<5th centile). Apgar scores were 5 and 7 at 1 and 5 min, respectively. She was intubated because of respiratory distress, but was extubated the next day and weaned to room air soon thereafter. Multiple anomalies were noted at birth including microbrachycephaly, coronal craniosynostosis, blepharophimosis, shallow orbits, proptosis, hypotelorism (interpupillary distance at the 3rd centile), broad, flat nasal bridge, bulbous nasal tip, overfolded small ears, left preauricular pit, microstomia, high-arched palate, micrognathia, hypoplastic thumbs, 5th finger clinodactyly, and absent 4th and 5th toes bilaterally (Fig. 1 A–D, Table I). She remained hospitalized for 1 week and was then discharged home.

At age 2½ weeks, she was noted to have respiratory distress and was admitted to the U.C.S.F. Neonatal Intensive Care Unit. She remained hospitalized for 8 weeks. A bronchoscopy evaluation detected tracheomalacia and upper airway obstruction and she had a tra-

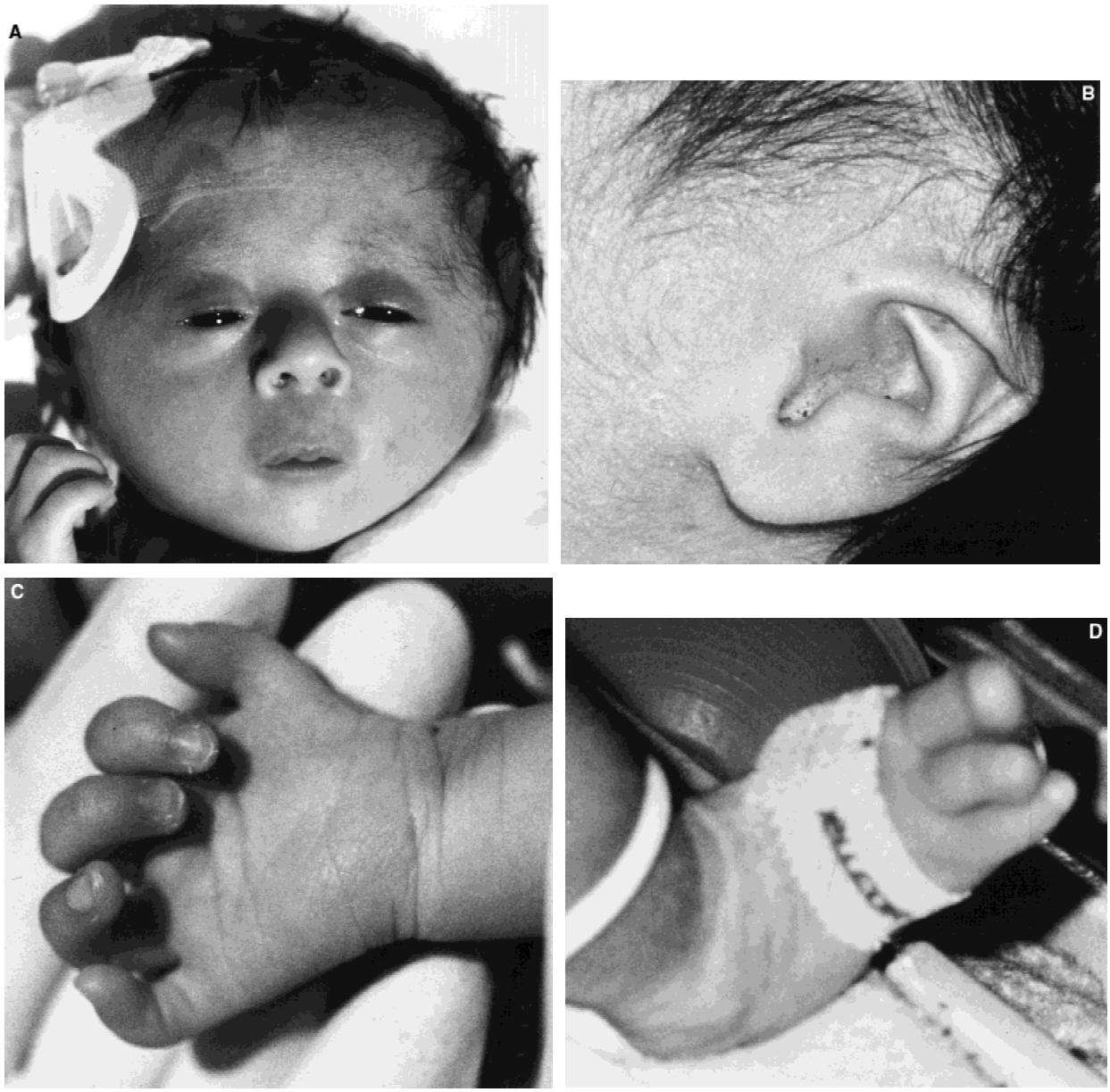

Fig. 1. The patient at age 2½ weeks. **A:** Note hypotelorism, shallow orbits, blepharophimosis, flat nasal bridge with prominent tip, long philtrum, and microstomia; **B:** small ear with overfolded superior helix, prominent lobe, and preauricular pit; **C:** proximally placed and hypoplastic thumb; **D:** and postaxial oligodactyly.

cheostomy placed. A complete skeletal survey detected brachycephaly, probable coronal craniosynostosis, anterolisthesis of the S1 vertebral body, hypoplasia of the middle 5th finger phalanges, and absent 4th and 5th metatarsals and 4th and 5th toes. A head CT examination at age 3½ weeks showed sagittal, bilateral coronal, and bilateral lambdoid craniosynostosis, an open metopic suture, and prominent ventricles. A head MRI examination at age 6½ weeks showed mild ventriculomegaly, partial absence of the septum pellucidum, an abnormal simple gyral pattern, abnormal base of the skull with Chiari I malformation and "dysplastic" C1 vertebral body, and anomalous transverse sinuses. An ophthalmology evaluation at age 8 weeks detected mildly hypoplastic optic nerves, exotropia, and possible cortical visual impairment. Audiologic testing detected a unilateral mild to moderate conductive loss. Peripheral blood chromosomes were apparently normal (46,XX). At age 2 months, she underwent repair of the coronal synostosis. At age 8½ months she had orbital advancement surgery and placement of a ventriculoperitoneal shunt. Her most recent evaluation at age 17½ months found her to be below the 3rd centile in all growth parameters. Her development is delayed, with gross motor skills at approximately a 10- to 11-month level.

DISCUSSION

Craniosynostosis or cranial dysostosis and limb malformations may be seen in many Mendelian conditions,

including Baller–Gerold syndrome and Yunis–Varon syndrome, as well as in chromosome anomalies. However, the specific pattern of clinical findings present in our patient does not appear to be diagnostic of a known malformation syndrome or chromosome defect. We were unable to find a similar constellation of features reported after a search of dysmorphology and cytogenetic databases. Still, although chromosome analysis was apparently normal, a microdeletion or other cytogenetically undetectable chromosome rearrangement cannot absolutely be excluded as a potential cause of our patient's phenotype. Because our patient had some findings seen in fetal aminopterin syndrome, including craniosynostosis and limb anomalies, and had been exposed to multiple drugs in utero, we next considered a teratogen as a possible explanation for her features. When we compared our patient's findings to those of others exposed to CP in utero, it was apparent that a pattern of malformation may exist. Common abnormalities include microcephaly, craniosynostosis, blepharophimosis, flat nasal bridge, abnormal ears, high-arched or cleft palate, and distal limb defects including preaxial upper limb anomalies (thumb hypoplasia/aplasia) and absent digits (Table I).

CP is an antineoplastic and immunosupressant agent clearly shown to be teratogenic in different animals at doses similar to therapeutic doses used in humans, but its potential to cause birth defects in humans has not been proven [Gibson and Becker, 1968; Mirkes, 1985; Padmanabhan and Singh, 1984; Sanders et al., 1996]. Although CP has been used to treat maternal illness in the 3rd trimester without apparent harm to the fetus [Nguyen Tan Lung et al., 1995], some reports have emphasized caution with respect to CP use, especially during the 1st trimester, and the need for more comprehensive studies [Bermas and Hill, 1995; Ramsey-Goldman et al., 1993; Sanders et al., 1996]. Animal studies have shown that CP needs to be bioactivated by the P-450 system in order to be teratogenic [Mirkes, 1985]. The teratogenic effects are mediated by metabolites of CP, namely phosphoramide mustard and acrolein, which are thought to act by causing cross-linking or strand breakage of DNA, and hence interfere with DNA synthesis [Mirkes, 1985].

Remarkably similar malformations have been noted in different species exposed to CP in utero. The teratogenic effects seen in animals including mice, rats, chicks, rabbits, and monkeys consist of CNS anomalies, skeletal defects, and facial anomalies [Gibson and Becker, 1968; Gilani and Chatzinoff, 1983; Mirkes, 1985; Padmanabhan and Singh, 1984]. Mice exposed to CP in utero were found to have CNS and craniofacial malformations including hydrocephalus, encephalocele, exencephaly, and cleft palate [Francis et al., 1990; Gibson and Becker, 1968] and limb anomalies including adactyly, ectrodactyly, polydactyly, syndactyly, long bone fusion, and short, curved long bones [Francis et al., 1990; Gebhardt, 1970; Gibson and Becker, 1968; Manson and Smith, 1977]. Most reports on the teratogenicity of CP in mice did not provide specific details of the skeletal abnormalities, although preaxial ectrodactyly has been noted [Manson and Smith, 1977; Manson et al., 1982]. Similar skeletal defects have been found in rats exposed to CP, including adactyly, ectrodactyly, polydactyly, syndactyly, and brachydactyly [Chaube et al., 1967, Hales et al., 1992; Jeyaseelan and Singh, 1984]. A detailed account of the limb malformations in rats generally was not given, but preaxial polydactyly [Chaube et al., 1967] and postaxial oligodactyly [Jeyaseelan and Singh, 1984] have been reported. Exencephaly [Chaube et al., 1967; Singh, 1971], encephalocele [Chaube et al., 1967], microphthalmia [Ashby et al., 1976], microtia [Padmanabhan and Singh, 1984], and cleft lip or palate [Chaube et al., 1967] were also present in rats exposed to CP in utero. In chicks, microphthalmia, anophthalmia, abnormal beaks, and short or curved limbs (either unilateral or bilateral) were found [Gilani and Chatzinoff, 1983]. Anencephaly, brachycephaly, microphthalmia, cleft lip ± cleft palate, cleft palate, and skeletal defects including adactyly, oligodactyly, brachydactyly, and humeroulnar fusion were noted in rabbits exposed to CP in utero [Fritz and Hess, 1971]. Finally, two different patterns of malformation were noted in *Rhesus* monkeys depending on the timing of CP administration. CP (5–10 mg/kg/dose, 2–3 doses) given at the beginning of organogenesis resulted in cleft lip ± cleft palate (in 6/8 animals), exophthalmos (3/8), malformed skull (1/8), bilateral polysyndactyly (1/8), and fused ribs, absent ulna, and ectrosyndactyly (1/8). When CP was administered during the midpoint of organogenesis, (7–10 mg/kg/dose, 3 doses) a flattened nasal bridge (5/8), meningoencephalocele (4/8), and ectrosyndactyly (2/8) were present. Treatment with 20 mg/kg CP (3 doses) resulted in abortion or in utero death in 4/4 animals, while treatment with 2.5 mg/kg CP (6 doses) did not result in any abnormalities in 3/3 animals [McClure et al., 1979]. Thus, the malformations present in animals, including monkeys, are remarkably concordant with those seen in our patient. Furthermore, the studies in *Rhesus* monkeys highlight the importance of both the dose of CP administered and the gestational timing of exposure in causing teratogenic effects.

CP has been shown to cross the human placenta, as documented by an amniotic fluid concentration approximately 25% of the plasma level at 29 weeks of gestation in a patient undergoing therapy for Hodgkins lymphoma [D'Incalci et al., 1982]. CP potentially could be bioactivated by the fetal microsomal P-450 system into mutagenic and cytotoxic metabolites [D'Incalci et al., 1982]. Based on an ultrasound examination during the 26th week of gestation, the time of in utero exposure to CP was estimated at 37 days postconception in our patient.Therefore, to our best estimate, our patient was most likely exposed to CP during the 6th-week postconception, a critical period in morphogenesis, especially with respect to limb bud differentiation [Moore, 1982]. Furthermore, the birth defects reported in animal studies of CP exposure are similar to the defects reported in humans. The data from animal studies thus supports this pattern of anomalies as being specific to in utero CP exposure. It is important to note that, when we exclude reports of patients being treated with other drugs, 2 patients with findings very similar to those of our patient were exposed to CP alone [Greenberg and Tanaka, 1964; Mutchinick et al., 1992]

(Table I). Although our patient was exposed to other drugs during pregnancy (primarily antihypertensive agents), these agents have not been shown to produce defects similar to those found in our patient [Briggs et al., 1994]. Therefore, we propose that CP causes a distinct embryopathy.

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