Review

Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta)

Shelly Tartakover Matalon a,1, Asher Ornoy b, Michael Lishner a,∗

a Oncogenetic Laboratory, Department of Medicine ‘A’, Sapir Medical Center, Kfar-Saba, Israel
b Laboratory of Teratology, Department of Anatomy and Cell Biology, Hebrew University Hadassah Medical School, Jerusalem, Israel

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1. Introduction

There is a growing number of women who delay pregnancy to advanced age [1]. Since older age is associated with malignant diseases [2], it is expected that the incidence of cancer in pregnancy will rise also. Furthermore, the use of immunosuppressive drugs for autoimmune diseases and following organ transplantation is rising as well [3]. Thus, there is the potential for more frequent exposure of women, embryos and fetuses to cytotoxic and/or immunosuppressive agents during pregnancy. These drugs may affect the placenta directly or may cross the placenta to enter fetal circulation, carrying a risk for fetal maldevelopment and malformations [4,5]. Animal and human data suggest that most antineoplastic drugs may have deleterious effects on the fetus, including increased incidence of prematurity, intrauterine growth retardation (IUGR), and low birth weight. Animal models have also shown a high risk of congenital anomalies, which in human occur mainly when the drugs are administered during the first trimester [6,7].

Many drugs readily cross the placenta to reach pharmacologically significant concentrations in fetal plasma; other drugs cross the placenta less readily and their concentrations are lower in the fetal plasma than maternal plasma [4]. For those latter drugs the placenta may be exposed to higher concentrations of the drugs than the embryo. Thus, any adverse effects of the drugs on development may result from direct effects to the embryo and fetus or from indirect effects through altered placental function [8,9].

2. Cyclophosphamide (CP)

CP is an antineoplastic and immunosuppressive agent. The parent drug (CP) or "pro-drug" is bioactivated to yield phosphoramid mustard and acrolein, which alkylate DNA and proteins [23].
2.1. Pharmacokinetics and metabolism of CP

CP is absorbed well after oral administration. The parent compound is widely distributed throughout the body with a low degree of plasma protein binding (20%). The half-life of CP is between 6 and 9 hours (h) although its metabolites reach maximal levels in the plasma within 2–3 h after an intravenous dose [17]. CP undergoes metabolic transformation to generate active alkylating species mainly in the liver by the mixed-function oxidase system of the smooth endoplasmic reticulum. Cytochrome P450 (CYP) enzymes are responsible for the initial activation reaction of hydroxylating at carbon-4 position of the oxazaphosphorine ring of CP. This reaction produces 4-hydroxycyclo-phosphamide, which exists in equilibrium with aldoephosphamide. Aldophosphamide breaks down by spontaneous beta elimination to release phosphoramidate mustard and acrolein [23]. CYP2A6, CYP2B6, CYP2C8, CYP2C9, and CYP3A4 are cytochrome P450 enzymes that are competent in hydroxylating CP [24]. In addition, mRNAs of certain CYP’s capable of hydroxylating CP are also present in first trimester human placenta including CYP2C and CYP3A4; however, the relative abundance of these CYP mRNAs is low compared to the corresponding levels in liver or lung [24]. CYP3A4 protein is present in first trimester human placenta but no functional activity of that enzyme was detected [25,26].

Autoinduction of CP metabolism is recognized, resulting in increased clearance and shortened half-life of the drug [24]. Induction of cytochrome P-450 (consisting of increases in cellular RNA and protein content and associated catalytic activities) may occur in response to exposure to certain substances. CYP1A is inducible in human placenta by maternal cigarette smoking [27]. CYP1A and CYP2B induction were demonstrated predominantly in the liver, but also in the placenta, of Norway rats (Rattus norvegicus) exposed to certain environmental contaminants [28]. CYP3A4, CYP2C8, and CYP2C9 protein levels increased during exposure of hepatocytes to CP, which thereby enhanced inherent rates of 4-hydroxylation in the cultured cells [29]. In theory, placental exposure to CP may induce catalytic activity of CYP enzymes, which are capable of catalyzing hydroxylation of CP and thereby activate it.

2.2. Mechanism of action of cyclophosphamide

CP metabolites affect tumor cells by mechanisms that may also affect the developing embryo and placenta. These effects are as follows.

1. Effect of CP on DNA: The cytotoxicity of alkylating agents may result from high chemical reactivity through covalent linkage of alkyl groups to DNA. Phosphoramidate mustard is the proximate DNA binding metabolite of CP [30,31]. The mustards probably react with purine bases in DNA to form adducts [32].

2. Induction of apoptosis: CP can trigger apoptosis and induce a pronounced cytotoxicity to lymphoid and tumor cells [33,34]. Apoptosis is one of the proposed mechanisms of pathogenesis in mediating direct phosphoramidate mustard teratogenicity [35].

3. Generation of free radicals: CP has the ability to generate free radicals that cause endothelial and epithelial cell damage [36]. One consequence of the intracellular generation of reactive oxygen free radical (ROS) species could be the induction of DNA damage. Moreover, the generation of ROS could lead to lipid peroxidation; however, there is insufficient evidence to implicate lipid peroxidation in the antitumor effects of CP.

Acrolein is a highly electrophilic alpha unsaturated aldehyde. There is little information on its molecular effects. At low doses, acrolein may inhibit cell proliferation and enhance apoptosis. It is possible that acroleins modulate the expression of one or more growth or stress related genes or transcription factors secondary to a reduction in glutathione (GSH), which is rapidly depleted following acrolein treatment. Activation of the transcription factors nuclear factor kappa B (NF-κ B) and activator protein 1 (AP-1) can be inhibited by acrolein [37].

2.3. Transfer of cyclophosphamide through the placenta

There is limited information about the ability of CP to cross the placenta. In one report CP was found in the amniotic fluid at 33 weeks in pregnant women with Hodgkin’s lymphoma who was treated with the drug in combination with prednisone. The level of CP in the amniotic fluid was equivalent to one quarter of the mother’s concentration of the drug [38].

2.4. Teratogenicity of CP

CP teratogenicity was demonstrated in animal models and in human case reports.

2.4.1. Animal models

CP has been found to be teratogenic in all animal species tested. The teratogenic effects that were observed in mice, rats, chicks, rabbits and monkeys consist of CNS anomalies, skeletal defects, and facial anomalies [21,39–41]. Mice exposed to CP in utero were found to display CNS and craniofacial malformations, including hydrocephalus, encephalocoele, exencephaly, and cleft palate [39,42], and limb anomalies including adactyly, ectrodactyly, polydactyly, syndactyly, long bone fusion, and short, curved long bones [39,42–44]. Similar skeletal defects have been found in rats exposed to CP, including adactyly, ectrodactyly, polydactyly, syndactyly and brachydactyly [42,44–47]. Encephalocele, encephalocoele, microphthalmia, microtia and cleft lip or palate were also present in rats exposed to CP in utero [47–49]. In chicks, anophthalmia, microphthalmia,
short limbs and abnormal beaks were found [40]. Brachycephaly, anencephaly, microphthalmia, cleft lip, cleft palate, and skeletal defects including adactyly, oligodactyly, brachydactyly, and humero-ulnar fusion were noted in rabbits exposed to CP in utero [50]. In monkeys, CP (5–10 mg/kg, 2–3 doses) given at the beginning of organogenesis resulted in cleft lip and/or cleft palate, exophthalmos, malformations of skull, bilateral polysyndactyly and fused ribs, absent ulna and ectrodactyly. When CP was administered during mid-organogenesis a flattened nasal bridge, meningoencephalitis, and ectrodactyly resulted. High doses produced abortion (20 mg/kg), while low doses did not result in malformations [51]. The differences between time points and doses reflect the importance of those parameters for inducing teratogenicity.

2.4.2. Human reports

Exposure to CP during the first trimester of pregnancy is significantly associated with early fetal loss [52]. Moreover, growth restriction and congenital anomalies were described among infants of women treated with CP in the first trimester of pregnancy, mostly in combination with other chemotherapeutic agents or irradiation [53]. Congenital malformations that were found include absent big toes in both feet, flattening of nasal bridge, hypoplastic fifth finger and bilateral inguinal hernia in one case [54], absence of all toes and single left coronary artery in a stillborn infant [55], hemangioma and umbilical hernia [56], imperforate anus and rectovaginal fistula [57], dysmorphic facies, cleft palate, multiple eye defects, abnormal shaped and low set ears, absent thumbs, borderline microcephaly, hypotonia and developmental delay at 10 months of age in another child [58]. An additional baby was born with multiple congenital abnormalities. He was diagnosed with papillary thyroid cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59]. Another reported case is a mother with systemic lupus erythematosus exposed to cyclophosphamide in the first trimester. She also took nifedipine, atenolol, clonidine, prednisone, aspirin, and potassium chloride throughout pregnancy. The infant had cancer at 11 years of age and stage III neuroblastoma at 14 years of age. He also had low IQ at 11 years [59].

2.5. Molecular and structural effects of CP on placentae and embryos

2.5.1. Animal models

Few studies have shown the effect of CP on cellular processes and related factors in the placenta. Following injection of CP at the ninth day of gestation (histoembryonic phase of nutrition) in rabbits, activated CP could be measured in the maternal blood but not in the yolk sac fluid; however, CP exposure inhibited the absorptive activity of the endoderm of the visceral layer of the yolk sac placenta, which may lead to quantitative and/or qualitative nutritional changes of the developing embryo [63]. Thus, no direct effect of CP on the embryo could be demonstrated. Teratogen-induced apoptosis was observed in the yolk sac of 9-day-old mouse embryos following CP exposure; however, cells of the yolk sac were substantially more resistant to teratogen-induced activation of the mitochondrial apoptotic pathway compared to other embryonic tissues [64]. In another study, mRNA transcripts of CSF-1 decreased in the uteroplacental units of mice exposed to CP. CSF-1 plays an important role in female reproduction and normal embryo and placental development [12,65]. Treatment of mice with CP induced pregnancy loss, which was accompanied by an increase in TNF-α mRNA in the uteroplacental unit. TNF-α has been implicated in mediating post-implantation embryo loss or the embryonic maldevelopment induced with developmentally toxicants or maternal metabolic imbalances [15,66]. Torchinsky and co-workers [67,68] proposed that TNF-α may function to prevent the birth of offspring with structural anomalies. In a similar study, treatment of mice with CP was accompanied with substantially lower TGF-β2 levels in the uteroplacental unit of CP-treated mice [69]. Interestingly, stimulation of the maternal immune system in pregnant mice changed GM-CSF, TNF-α and TGF-β3 levels, and reduced embryonic response to CP in mice [69–71]. Changes in the decidua were also described in rats following CP treatment [72]. Maternal administration of CP induced chromosomal aberrations and decreased cell number and DNA synthesis in preimplantation mouse embryos [73]. Modulation of gene expression in neonatal rat testis was observed following CP exposure to fetuses during testicular differentiation [74]. The occurrence of malformations following phosphoramid mustard exposure in rats was also accompanied by apoptosis [35]. Similarly, exposure of murine embryos in vitro to 4-hydroperoxycyclophosphamide (4-OHCPA), an activated analog of CP, induced limb and head malformations and apoptosis [75,76]. Increased level of p53 protein and G1 arrest was observed in the malformed fetal head area [76]. In another study CP caused neural damage to the fetal brain [77]. TNF-α and TGF-β were involved in the progression of that damage [77]. Induction of pregnancy loss with CP resulted in the appearance of some apoptotic cells in the uterus, and involvement of p53 and Bcl-2 in its regulation [78].
2.5.2. Human studies

CP inhibited aerobic CO₂ production and decreased anaerobic glycolysis in vitro in human placenta from early pregnancies [79]. Moreover, CP increases the frequency of sister chromatid exchange in direct preparations of human chorionic villi in the absence of supplementary enzymatic activation systems [80]. Sister chromatid exchanges are considered to be sensitive indicators of genetic effects after exposure to mutagenic agents. This suggests that chorionic villi are very sensitive to the mutagenic effect of CP.

In summary CP is an alkylating antineoplastic compound that may induce DNA damage and enhance apoptotic processes that are frequent in first trimester placenta and embryos. Moreover, CP affects the level of several factors that have important role in placental and embryonic development. These effects may lead to placental and embryonic structural and functional damage. The compound may cross the placenta. CP is classified Class D in FDA pregnant categories (evidence of possible risk to the human fetus). This drug has a consistent pattern of defects, which involves facial clefts, and limb reduction defects in animal models. A pattern of malformation exists also in human embryo exposed in utero to CP. Common insults include cranio-facial abnormalities: microcephaly, craniosynostosis, blepharophimosis, flat nasal bridge, abnormal ears, high arched or cleft palate, and distal limb defects including preaxial upper limb anomalies and absent digits. Thus, the malformations present in animals are remarkably concordant with those seen in some of the patients. If an immunosuppressive drug is needed during the first trimester of pregnancy (and even throughout pregnancy) a better indication is AZP instead of CP. CP is suspected to be teratogenic in the first trimester of pregnancy. Since there is not enough human data concerning the effect of CP on human pregnancy, if possible it should not be used during that period of pregnancy [81]. If, however, CP is the drug of choice for the particular illness, than, based on animal studies, it may be advisable to recommend pregnancy interruption. The human studies are incomplete but they do not seem to support a very high teratogenicity. Therefore, if CP is to be given to the woman under such circumstances, she should be counseled regarding the potential hazards to the embryo and fetus and make her decision as to the continuation of pregnancy.

3. Azathioprine (AZP) and 6-mercaptopurine (6-MP)

AZP is metabolized non-enzymatically to 6-mercaptopurine (6-MP) [82], an analogue of hypoxanthine.

3.1. Pharmacokonetics and metabolism of AZP and 6-MP

Absorption of an oral dose of 6-MP in humans is incomplete and variable, averaging approximately 50% of the administered dose. Intravenous administration of 6-MP resulted in a plasma half-disappearance time of 21 min in children and 47 min in adults. 6-MP is metabolized rapidly to active intracellular derivatives. The drug enters the anabolic and catabolic pathways of purines, and the active intracellular metabolites have appreciably longer half-lives than the parent drug [24]. 6-MP competes with hypoxanthine and guanine for the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) that catalyzes the first step of 6-MP metabolism, yielding thionosine monophosphate (TIMP). TIMP, the principal nucleotide metabolite of 6-MP, is subjected to anabolic reactions and forms thionosine nucleotides (TGNS) that incorporates into the DNA, or is catalyzed by the enzyme thiositurine S-methyltransferase (TPMT) to form mTIMP. TIMP and mTIMP are inhibitors of phosphoribosyl pyrophosphate (PRPP) amidotransferase, an enzyme that is important in de novo purine synthesis [82]. TPMT catalyzes the S-methylation of 6-MP and other thio-urine drugs [83]. Both HGPRT and TPMT can be found in the human placenta and 6-MP may be degraded at that site [84,85].

Purine metabolism plays an important role in embryonic development [86-88]. In the degradative pathways of purine metabolism adenosine is deaminated by adenosine deaminase (ADA) to inosine. Inosine can be further broken down to hypoxanthine [89]. ADA is enriched at the maternal-fetal interface of mice [86], and exists in human placenta [90]. During early post-implantation stages ADA is highly expressed in both maternally derived decidual cells and in trophoblast cells. High levels of expression of ADA at the maternal fetal interface suggests an important role in nucleoside metabolism during post-implantation development. Genetic studies suggest that placental ADA plays an important role in protecting the fetus from endogenous purine nucleoside (adenosine and 2′-deoxyadenosine) intoxication during development [86]. Both adenosine and 2′-deoxyadenosine are potent bioactive nucleosides. 2′-Deoxyadenosine is a cytotoxic metabolite that may cause embryolethality and fetal liver damage associated with ADA deficiency in mice and cell death in embryonic chicken sympathetic ganglia and brain [87,88]. It has been suggested that the accumulation of 2′-deoxyadenosine leads to p53-dependent apoptosis in the early embryo and subsequent loss of viability [87].

3.2. Mechanism of action of azathioprine (AZP)

AZP is metabolized non-enzymatically to 6-MP [82]. The precise mode of action of 6-MP remains unclear, however, the cytotoxicity of AZP/6-MP has been attributed to at least three mechanisms as follows.

1. AZP/6-MP are catalyzed to inhibitors of enzymes that are important in de novo purine synthesis.
3. AZP and 6-MP promote a rapid apoptotic cell death [14]. Moreover, 6-MP induces a fall in NOS activity, and a decrease in the Bcl-2/Bax ratio in B lymphocytes, which favors apoptotic processes in those cells [14].

3.2. Transfer of AZP and 6-MP across the placenta

AZP and 6-MP cross the human placenta. The placental concentration of AZP is 64–93% of the maternal blood level, while AZP and 6-MP fetal blood concentration represent 1–5 and 1–2% of their respective maternal blood levels [92]. Hence the placenta strongly impedes the diffusion of AZP and 6-MP into the fetus.

3.3. Teratogenicity of AZP

AZP and 6-MP teratogenicity was demonstrated in animals and in humans.

3.3.1. Animal models

AZP and 6-MP had deleterious effects on different stages of embryonic and fetal development. Following daily injection of high doses (20 mg/kg) AZP to rats from the first day of gestation adverse effects were evident by the blastocyst stage [93]. Increased frequencies of cleft palate, open eye, and skeletal anomalies as well as significant decrease in thymic size were observed in offspring of mice injected intraperitoneally (i.p.) during organogenesis with 4–13 times the human therapeutic dose of AZP [94, 95]. Fetal hydrops, anemia and hematopoietic depression were also observed in those mice [95]. In contrast, no anomalies were observed in the offspring of mice treated with AZP doses that were within the human dose range, although increased frequencies of fetal loss and growth retardation were observed [94–96]. Increased frequencies of limb malformations, ocular anomalies, and cleft palate occurred among the offspring of pregnant rabbits injected i.p. with AZP at doses equivalent to two to six times those used in humans [96]. No malformations occurred in the offspring of rats injected i.p. with similar AZP doses [96]. However, an increased percentage of fetal deaths and fetoplacental growth restriction was induced in rats following daily i.p. injection of AZP doses equivalent to human doses [97]. Increased incidence of cleft palate, skeletal, and urogenital anomalies, diaphragmatic hernia, and other malformations was observed among fetuses of rats given i.p. very high doses that are equivalent to 37–150 times the human doses [98–101]. In another study, increased frequencies of fetal death and CNS and limb anomalies were observed among offspring of rats, rabbits and mice treated during organogenesis with doses equivalent to 1–125 times the human dose [102, 103]. No malformations were induced in the offspring of rats treated orally during organogenesis [104]. In an in vitro study, AZP affected the brain, the caudal trunk, the heart and forelimb regions, and the vesicular structures in 9.5- to 11.5-day-old rat embryos that were cultured (during organogenesis) in the presence of low doses of AZP [105].

3.3.2. Human reports

Exposure of pregnant women to AZP during pregnancy may affect the embryo. The major side effects reported after antenatal exposure to AZP were spontaneous abortions, prematurity (40–52%), IUGR (19–40%) and LBW [32, 106, 107]. The frequency of prematurity and fetal growth retardation appears to be increased in pregnancies of renal transplant recipients treated with AZP, especially if the woman has reduced renal function, previous rejection, or requires high dose immunosuppressive therapy [108–112]. Fetal wastage has been reported after 6-MP exposure in all trimesters [113]. Moreover, hematological and immunological disturbances, chromosomal changes and malformations were observed following exposure of first trimester embryos to the drug.

3.3.2.1. Malformations. The infants of women treated with AZP during pregnancy show only a slight increase in the frequency of congenital malformations that varied from 0 to 11.8% [114]. Malformations included microcephaly, hydrocephalus, anencephaly, unusual facial features, hypoplasia, malformed hand, polydactyly, cleft palate, congenital heart disease [114]. Out of about 80 cases of women exposed in the first trimester to 6-MP during pregnancy, only the following malformed infants were reported: a baby with cleft palate, stunt growth, corneal opacity microphthalmia and poorly developed external genitalia, that died within 10 days (The fetus was exposed to 6-MP and Busulfan) [115]; and a stillborn baby (abruptio placentis) with polydactyly that was born following exposure to 6-MP and CP [116]. No congenital anomalies were observed in two small series of six and nine children born to women treated during pregnancy with cancer chemotherapeutic regimens that included 6-MP. Four and five of these women, respectively, were treated during the first trimester [61, 117].

Two additional children with anomalies associated with antenatal exposure to AZP have been reported: one had pulmonic stenosis [118] and the other (who was not exposed during the first trimester) had an atrial septal defect [119]. Congenital anomalies were observed in 4 of 103 and in 2 of 48 infants born to women who received renal transplants prior to becoming pregnant [108, 120]. Approximately 90% of these women were treated with AZP during pregnancy. There was no specific pattern as all of the reported anomalies varied among the newborns. In another series of 24 liveborn children of kidney transplant recipients, no congenital anomalies were observed [109]. The frequency of congenital anomalies among 27 clinical series of infants of renal transplant recipients who were treated with AZP throughout pregnancy, that were presented in Poláška et al.’s analysis, were 0–11.8%. The number of infants included in each series was from 6 to 110 [114].

Kallen reported about one infant with a cardiovascular defect, one with pes equinovarus, and one with undescended testicle among 33 births of women who were treated for rheumatic disease with AZP during pregnancy [111]. No
congenital anomalies occurred among infants born to women who had previously received liver or cardiac transplantation and were treated with AZP during pregnancy [121–123] or among 46 infants of women treated with AZP during the first trimester of pregnancy for inflammatory bowel disease or severe systemic lupus erythematosus [124,125]. One case of hydrocephalus was observed among 33 infants that were born to patients with bowel disease and who took 6-MP at the time of conception or throughout the entire pregnancy [126]. Increased risk for malformations was observed in a small Danish study of 11 pregnant women [127].

3.3.2.2. Hematological and immunological disturbances.
In infants whose mothers received AZP/6-MP (in combination with other drugs) during pregnancy, neonatal leukopenia, thrombocytopenia [112,113,128], microangiopathic hemolytic anemia and thymic/bone marrow hypoplasia were reported [129]. Disturbances in the immune system were also found, such as decreased serum IgG, IgM and/or IgA levels [113] and the presence of autoantibodies [106].

3.3.2.3. Chromosomal changes.
Prenatal exposure to AZP/6-MP during the second trimester was also associated with chromosomal abnormalities [106,113,130]. These chromosomal aberrations included chromatid gaps, breaks, deletions and extra fragments, translocations, and bridging fusions. The clinical significance of these findings is unclear but it can represent a risk for future cancer or for genetic damage in the next generation.

3.4. Structural effects of AZP on the placenta
Studies in rats showed that AZP inhibits placental differentiation, induces a degeneration of the trophoblastic cells, and causes a smaller placenta with a reduced cell number [106]. Moreover, placentas of rats that received 6-MP (55 mg/kg) on day 11 of gestation analyzed at term were smaller in diameter and had lower weight compared to control rats. Histologically, the placenta from 6-MP-treated rats showed a highly disproportionate reduction in the labyrinthine layer with larger, less subdivided maternal sinuses than in controls, reduction of fetal vasculature, and a diminished morphology [131].

In summary, AZP and its metabolite 6-MP are purine analogues and can be teratogenic in animals, however, available human data suggest that the risk of congenital anomalies as a result of 6-MP treatment in early pregnancy is low. In rodents (rats, mice) 6-MP mainly induced CNS (central nervous system), eye, jaw and limb defects; however, no recurrent pattern of congenital anomalies in infants exposed to 6-MP emerges from the human studies. The mode of exposure, doses and inherent differences between animals and humans preclude direct conclusions from animal data to humans. Humans take AZP or 6-MP orally, whereas most animal studies employ intraperitoneal injections. The bioavailability of these drugs is substantially reduced when they are administered orally [114]. Embryos during the animal experiments may actually be exposed to higher levels of the drugs than the human embryo. In spite of the wide spread use of the drug, there is low teratogenicity in few reports and no consistent pattern of defects. Thus, AZP/6-MP may be used in pregnancy if the benefit to the mother justifies the possible risk to the fetus [81].

4. Doxorubicin (DOX)
DOX is an anthracycline, antineoplastic antibiotic. It acts by forming stable complexes with DNA and by interfering with the synthesis of nucleic acids.

4.1. Pharmacokinetics and metabolism of the drug
DOX displays linear pharmacokinetics after intravenous administration. It is widely distributed in the plasma and tissues, and a plasma protein binding ranging from 50 to 85%. The drug is extensively metabolized in the liver by aldo-keto reductase, to yield the dihydrodiol derivative doxorubicinol, which retains antitumour activity [132]. Aldo-keto reductase activity is present also in the placenta [133].

4.2. Mechanism of action of the drug
Anthracycline derivatives are frequently used in the treatment of numerous human malignancies. A number of different mechanisms have been proposed for the cytostatic and cytotoxic actions of these agents [134,135] as follows.

1. Interference with macromolecular biosynthesis. These drugs inhibit DNA synthesis, due to DNA interaction and/or inhibition of DNA polymerase activity.
2. Chelation of iron and generation of reactive oxygen species (ROS) resulting in DNA damage and lipid peroxidation. However, there is insufficient evidence to implicate lipid peroxidation in the antitumor effects of the anthracyclines.
3. DOX adducts to single and double stranded DNA. This may lead to DNA cross-linking.
4. Induction of DNA damage through interference with topoisomerase II. Topoisomerase enzymes interconvert different topological isomers of DNA [89]. Its interac-
tion with the DNA topoisomerase II complex is likely to be a primary triggering event for growth arrest.
5. Growth arrest in G2 phase of the cell cycle.
6. DOX induces apoptosis in different cells, including: murine thymocyte, p388 leukemic cells and human leukemic cells.

4.3. Transfer of doxorubicin through the placenta

It has not been determined how well DOX crosses the placenta. The transplacental passage of DOX, was studied by in vitro perfusion of term human placenta [136]. DOX, has not been found even when the highest concentrations of the drug were used. Moreover, DOX and its metabolites were not detected in the amniotic fluid, collected through amniocentesis in a 31-year-old woman with 28 weeks pregnancy and breast cancer treated weekly with DOX chemotherapy [137]. In contrast, two pregnant women with lymphoproliferative disorders were treated with DOX-containing regimens. Both patients delivered shortly after administration of DOX. One child was healthy and the other was stillborn. Anthracycline was found in the maternal and fetal parts of the placenta of the first woman, but was undetectable in the cord plasma. Also, DOX was undetectable in fetal liver, lung, kidney and heart [138]. Moreover, DOX was found in the fetal liver, kidney and lung, but not in the amniotic fluid of a woman with Hodgkin’s lymphoma, who aborted at the 17th week of gestation [139]. Those findings suggest that DOX may cross the placenta.

4.4. Teratogenicity of doxorubicin

4.4.1. Animal model

DOX is teratogenic in laboratory animals, as demonstrated by a number of in vivo and in vitro experiments [53]. It produced increased malformation rates when tested in rats [140,141]. Oguro et al. [142] gave up to 1 mg per day of DOX i.p. to pregnant rats and intravenously to pregnant mice during organogenesis and observed no teratogenicity. Moreover, DOX and its metabolites were not detected in the amniotic fluid, collected through amniocentesis in a 31-year-old woman with 28 weeks pregnancy and breast cancer treated weekly with DOX chemotherapy [137]. In contrast, two pregnant women with lymphoproliferative disorders were treated with DOX-containing regimens. Both patients delivered shortly after administration of DOX. One child was healthy and the other was stillborn. Anthracycline was found in the maternal and fetal parts of the placenta of the first woman, but was undetectable in the cord plasma. Also, DOX was undetectable in fetal liver, lung, kidney and heart [138]. Moreover, DOX was found in the fetal liver, kidney and lung, but not in the amniotic fluid of a woman with Hodgkin’s lymphoma, who aborted at the 17th week of gestation [139]. Those findings suggest that DOX may cross the placenta.

4.4.2. Human reports

The effect of DOX on apoptotic events during pregnancy was described in two studies: Esophageal atresia and tracheoesophageal fistula were induced by injection of DOX to pregnant rats. Apoptotic nuclei were found in the region of the upper esophageal pouch. In DOX-treated embryos, the number of apoptotic nuclei was significantly lower in day 12 and significantly higher in day 13 than in the control embryos. It was suggested that DOX affected apoptotic events that are required for normal tracheoesophageal embryogenesis [150].

4.6. In summary DOX is an anthracycline, antineoplastic antibiotic. The passage of DOX via the placenta remains controversial. Evidence exists that DOX affects embryonic apoptosis and is embryotoxic in animal models. The drug may induce teratogenic effects in humans and thus is classified as class D. Since there is not enough human data concerning the effect of DOX on human pregnancy, if possible it should not be used during the first trimester of pregnancy [81]. The drug can probably be administered when indicated in the second and third trimesters.

5. Conclusions: molecular mechanisms related to CP, AZP and DOX

CP, 6-MP and DOX affect pregnancy outcome in human and animals [21,114,140,149]. The drugs may damage DNA structure, inhibit proliferation or induce apoptosis of different cells that are important during placental and embryonic development in the first trimester of pregnancy. Normal and damaged embryos express several apoptosis-related genes.
during mammalian preimplantation and embryonic development. The ratio of pro-apoptotic and anti-apoptotic genes is probably important in regulating development in that stage [151]. Apoptosis also contributes to the appropriate formation of various organs and tissues during embryogenesis [67] and is normal feature of trophoblast cells throughout gestation [10]. Studies suggested that abnormal pregnancies such as first trimester abortions and ectopic pregnancies are associated with enhanced placental apoptosis [10]. Thus, defective apoptosis during that period may cause developmental abnormalities [11]. 6-MP, CP and DOX facilitate or induce apoptosis and thus may damage embryonic development and placental function. 6-MP and CP may also modulate activity and levels of regulators that are involved in the placental and the embryonic development. These include Bcl2/Bax ratio, CSF-1 and TGF-β1, which are affected by 6-MP and CP, respectively, and have a role in embryonic/placental development [12,63,69,91]. Bcl-2 is expressed in the placenta at low levels during the entire human gestational period. On the other hand, Bax is low during the first trimester but increases towards the end of gestation. In accordance with the change of ratio of these two molecules, an increase of apoptotic cells is observed in the third trimester. These data suggest that the different expression of the above mentioned genes is at least in part responsible for the delicate balance between cell proliferation and apoptosis in the human placenta during pregnancy [152]. Another important factor that is influenced by CP is CSF-1. CSF-1 level increases during pregnancy in mice and humans [153]. CSF-1 and its receptor exists in the human placenta and the uteroplacental unit of mice [12,153], and have a role in human trophoblastic growth and invasion [153]. The effect of that cytokine on the embryo was studied in a mouse model, which showed that CSF-1 increases the rate of development of the preimplantation embryo [154]. Moreover, CSF-1 is neurotrophic in embryonic neuronal cultures and its absence in mice results in severe electrophysiological abnormalities in the cortex [154]. CP also affects TGF-β levels. TGF-β genes expression was found in human villous (especially in the extravillous cytotrophoblasts) and decidual tissues [155,156], and in maternal decidual and uterine epithelial tissues of mice [157]. TGF-β inhibits trophoblastic cell proliferation and migration [158]. Maternal serum TGF-β2 levels significantly increase in cases of severe human preeclampsia and eclampsia, and in mice with pregnancy loss in comparison to controls [69,156]. By affecting Bcl2/Bax ratio, CSF-1 and TGF-β2 levels one may influence placenta and embryo development. Granulocyte macrophage-colony stimulating factor (GM-CSF), TNF-α and TGF-β levels are influenced also by the condition of the maternal immune system [69–71]. Stimulation of the maternal immune system in pregnant mice, changes those cytokines levels and reduces embryonic response to teratogenic insults, such as that of CP [69–71]. Those cytokines were suggested as mediators in rescuing from the teratogenic insult [69,71,153]. The immunoregulation through those factors reflects their significant effect on pregnancy and the importance of the drugs ability to change their levels. These data reflect the wide range of mechanisms that may be responsible for the effect of CP on pregnancy outcome. AZP may damage DNA structure, inhibit proliferation and enhance apoptosis. However, there is no evidence to suggest that AZP may adversely affect pregnancy outcome indirectly via cytokine levels or gene regulation [67,68]. In some cases the placenta is exposed to higher amounts of the drugs than the embryo and may thus be significantly damaged. This may suggests that placental damage is one of the mechanisms that are responsible for the embryonic damage.

6. Summary

CP, AZP and DOX are three commonly used antineoplastic and immunosuppressive drugs. Due to increasing incidence of pregnancies in older women, their use both as immunosuppressive and antineoplastic during pregnancy is rising. Many experimental animals data support the notion that these drugs are embryotoxic and teratogenic. However, human data is limited, and sometimes their use during pregnancy is mandatory. Considering pregnancy interruption prior to the necessary use of some of these drugs seems to be a debatable option but it should be offered to the pregnant women especially if CP treatment is required. One should therefore counsel these women according to the currently existing data. Those data suggests that CP is considered to be a threat for the first trimester of pregnancy and should not be used during that period unless it is the drug of choice for a particular illness. Very little information exists about the use of DOX during pregnancy. Thus, if possible, it should be avoided during the first trimester of pregnancy unless it is the drug of choice for a particular illness. The available human data, suggest that the risk for congenital anomalies as a result of 6-MP treatment early in pregnancy is not high. Thus, AZP/6-MP may be used in pregnancy if the benefit clearly justifies the possible risk to the fetus. In order to further study the direct effects of the drugs on the human placenta and its regulators, human placental cultures and in vitro cultures of rodent embryos surrounded by their yolk sacs may be informative.

References


