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METHOTREXATE AND FALLOPIAN TUBES IN ECTOPIC PREGNANCY

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The condition of ectopic pregnancy is presented from different points of view, bringing out physiological explanations for its occurrence in primates and the striking absence in other mammals. Ectopic pregnancies have been described in several animal species such as cats, sheep, cows, horses, gerenuks, pigs, hamsters, rats, rabbits, marmosets, baboons, squirrel monkeys, owl monkeys and rhesus macaque. Methotrexate (MTX) has anticancer, antibacterial, antifungal, antiparasitic, immunosuppressive, antimitotic, embryotoxic and teratogenic effects. Methotrexate has species-specific effects. The purpose of this study was to investigate the medical treatment outcome of ectopic pregnancy by local administration of methotrexate and the adverse effects of increasing levels of MTX on the epithelium of the Fallopian tube as viewed by light microscopy. We treated patients with local and systemic regimen of methotrexate. Local applications were performed by direct injection of 0,5 mg/kg or 1 mg/kg methotrexate into the amniotic sac under sonographic control. Fallopian tubes were removed in patients with methotrexate treatment failure. The histological features we observed in the Fallopian tube were similar in all groups. In particular, histological findings showed necrosis and inflammatory infiltrate that was not quantitatively parallel to the increase in MTX dose. Fallopian tubes in human pregnancy could be resistant to the influence of MTX.

Key words: methotrexate, ectopic pregnancy, Fallopian tube, mammals

INTRODUCTION

Methotrexate (MTX) is used mainly as an anticancer drug, and it is an antagonist of folic acid. MTX has been routinely used for the treatment of trophoblastic diseases since 1956, and more recently to deplete the proliferative activity of trophoblast in nonmolar and ectopic pregnancies. The antimitotic effect of MTX is especially evident in trophoblastic tissues (Schiff *et al*., 1992). Methotrexate had a lethal effect on embryos in rats. Further studies on rats showed that MTX can also cause embryonic deformities, the most common being cleft palate and limb abnormalities. As would be expected, the use of MTX after the

thirteenth day of a 21-day gestation (i.e. after the critical period of organogenesis) was associated with a marked reduction in the number of abnormalities. Neural tube defects and poor brain development were seen in rat embryos treated with MTX. The effect of methotrexate on the fetus appears to be species-dependent. It has specific abortifacient effects, as well (Lloyd ME,1999).

Ectopic pregnancies, although uncommon, have been described in several animal species such as cats, sheep, cows, horses, gerenuks, pigs, hamsters, rats and rabbits. Lapin and Yakovleva (1963) state that the incidence of ectopic pregnancy in marmosets, baboons and macaques in the Sukhumi breeding colony was 0.15%. This rate is approximately twenty times lower than that estimated for humans. Only three cases of ectopic pregnancy have been previously described in primates: in a squirrel monkey, in an owl monkey, and as an unexpected finding at necropsy in a rhesus macaque (Schlabritz-Loutsevitch E, Hubbard B, 2004).

Ectopic pregnancy (EP) is a major cause of maternal morbidity and mortality in humans. The treatment of this condition is primarily surgical, but medical management in selected cases is safe, effective and eliminates the morbidity of surgery (Floridon *et al*.,1994; Farquhar, 2005). Methotrexate (MTX) is a folate antagonist that can be used for non-oncologic purposes including the treatment of ectopic pregnancy (Bourget *et al*., 1993). The dose and duration of MTX therapy for EP is much lower than that used in oncology cases, thus reducing side effects and increasing safety. MTX selectively acts on rapidly dividing cells, such as trophoblast cells which comprise the implantation site of early gestation. The two most common methods of administering MTX to patients with EP are intramuscular administration of a single-dose, based on body surface area and calculated as 50 mg/m^2 or local by direct administration dose regimen of 1 mg/kg of MTX. Both methods have a similar side effect profile, resulting in the rare occurrence of nausea, vomiting, stomatitis, elevated liver function tests, anorexia and diarrhoea. The two methods yield success rates similar to those of conservative surgical therapy with similar future fertility. As the efficacy of MTX therapy is between 60% and 95%, women must be followed clinically until there is complete resolution of human chorionic gonadotropin (hCG) titres from the serum (Elito *et al*., 2005).

The first to use methotrexate in cases of ectopic pregnancy with implantation in the Fallopian tube was Tanaka (1982). Besides these first clinical trials on patients with ectopic pregnancies, other studies have also shown that MTX is efficient and safe when administered in various different doses and given by various methods of administration. Later morbidities, such as tubal occlusion (reported incidence 0.9-18.6%) and recurrent ectopic pregnancies (reported incidence 9.1-22.0%), have been attributed to adverse effects of methotrexate on the Fallopian tube (Bayram *et al*., 2005).

The purpose of this study was to investigate the medical treatment outcome of ectopic pregnancy by local administration of methotrexate and adverse effects of increasing levels of MTX on the tissue of the Fallopian tube as viewed by light microscopy.

MATERIAL AND METHODS

This study was carried out on 120 patients which were treated with methotrexate. We treated patients by local and systemic regimen of methotrexate. Local applications were performed by direct injection of methotrexate into the amniotic sac under sonographic control in 78 cases. Parenteral intramuscular methotrexate treatment was performed in 42 patients. Fallopian tubes were removed in patients with methotrexate treatment failure. Tube specimen was fixed in 10% formalin for a period of 24 h for examination under the light microscope. Sections 5 ìm thick were cut from paraffin blocks and stained with H&E. These sections were examined with an Olympus BH-2 microscope, and the detection areas for inspection were marked for further slicing. The marked sections were cut into thin slices with the same ultramicrotome, and these thin slices were stained with uranyl acetate and with lead citrate. We performed double blind control study and Fisher exart probability test (Pf).

RESULTS

The medical treatment of ectopic pregnancy was effective in 102 patients (85%). In 18 cases we performed surgery because of methotrexate treatment failure. Local route of methotrexate treatment we applied in 78 patients. In 43 cases we administered 0.5 mg/kg and treatment was successful in 35 patients. In 35 woman we administered 1 mg/kg and it was successful in 29 cases. There was not a significant difference between the two regimens (Table 1).

Table 1. Outcome of medical treatment

Dosage	N	Outcome			
		cured		surgery	
		n	%	n	%
0.5 mg/kg	43	35	54.7	8	57.1
1 mg/kg	35	29	45.3	6	42.9
Total	78	64	100.0	14	100.0
$X^2 = 0.028$; DF = 1; $p > 0.05$					

Table 2. Serum level β-hCG (IJ/L) before and after methotrexate therapy

		$\bar{X}1$	SD	SE
Before therapy		5448	8571	2710
After therapy	1-3 days	4345	7391	4267
	4-6	3883	6942	2314
	7-9	3252	8330	1666
	10-12	2593	6751	2387
	13-15	1621	5096	1315
	16-18	708	1297	580
	19	215	449	183

The efficacy of methotrexate therapy was followed clinically and sonographically until there was compete resolution of human chorionic gonadotropin (hCG) titres from the serum. Successful medical treatment were correlated with decreasing serum hCG levels (Table 2 and Table 3).

We performed histological analyses of surgically removed Fallopian tubes in 18 cases. In group (E1) with locally injected MTX (0.5 mg/kg) there were 8 cases. In the second group (E2) with locally injected MTX (1 mg/kg) there were 6 cases. Parenteral intramuscular metotrexate (50 mg/m^2) treatment failed in 4 cases (E3). Specimens were taken from Fallopian tubes and examined under light microscope. In all groups, the surface epithelial cells were normal in all samples. The lamina propria was infiltrated by numerous inflammatory cells, with a prevalence of polymorphonuclear leucocytes. We explored the occurrence of inflammatory reactions and necrosis in all groups, as a double blind control analyses. There was no significant statistical difference between the three groups ($p>0.05$) (Table 3, Figure 1, Figure 2, Figure 3).

Tabela 3. Histological finding in Fallopian tubes after methotrexate treatment

Group	n	Histological finding	
		Inflammatory infiltrate	Necrosis
E_1	8	5	4
E_2	6	3	3
E_3	4	2	1
$E_1 : E_2$		Pf = 0.529 ; p>0.05	Pf = 0.704 ; p>0.05
$E_1 : E_3$		Pf = 0.575 ; p>0.05	Pf = 0.424 ; p>0.05
$E_2 : E_3$		Pf = 0.738 ; p>0.05	Pf = 0.452 ; p>0.05
E_1 =Local injection of 0.5 mg/kg methotrexate E_2 =Local injection of 1mg/kg methotrexate E_3 =Parenteral intramuscular admnistration of 50 mg/m^2			

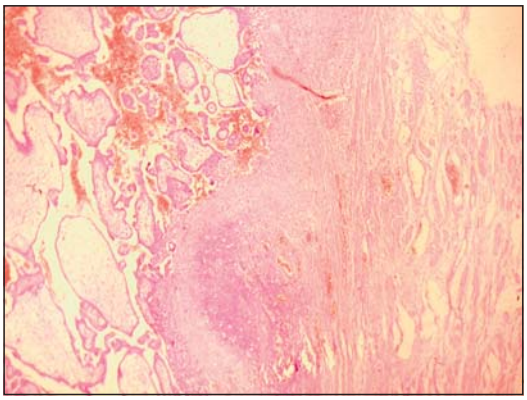

Figure 1. Fallopian tube after local injection of 0.5 mg/kg MTX

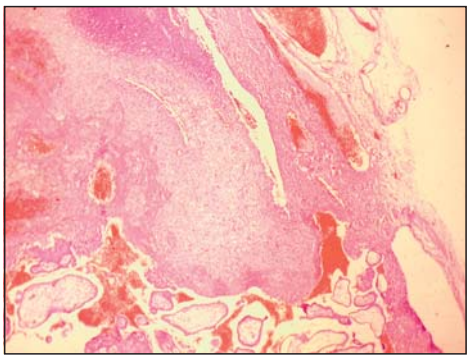

Figure 2. Fallopian tube after local injection of 1 mg/kg MTX

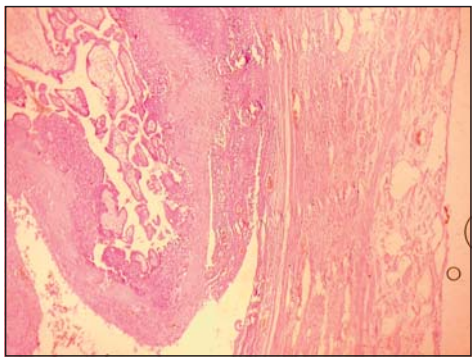

Figure 3. Fallopian tube after parenteral intramuscular administration of 50 mg/m^2 MTX

DISCUSSION

Methotrexate has antimitotic and embryotoxic effects. Embryonic growth delay induced by methotrexate was first described in chickens. Later work in this species suggested that the limb abnormalities seen were caused by transient inhibition of cell division, rather than by cell death. Further studies suggested species-specific effects, with embryotoxicity in cats, and embryotoxicity and teratogenicity in rats, mice and rabbits. Twenty-five monkeys were given MTX at different dosages and durations in pregnancy. Only one showed evidence of embryotoxicity, with evidence of abnormal ossification of long bones and thoracic vertebrae (Lloyd ME, 1999).

The condition of tubal ectopic pregnancy is presented from diverse points of view, bringing out physiological explanations for its occurrence in primates and striking absence in other mammals. Part of the flexibility underlying ectopic pregnancy in humans originates from the absence of a uterine luteolytic mechanism, enabling early embryonic development in the Fallopian tube without compromising function of the corpus luteum. Attention is devoted to a potential overlap between the composition of tubal and uterine fluids, and to specific mixing between the two fluid compartments, expressed in an ability of the human oocyte or zygote to tolerate transplantation to the uterus (Hunter, 2002). Putting to one side the occasional bizarre or exceptional claim, tubal ectopic pregnancies would appear to be restricted to primates. A model for tubal pregnancy has been established by the arrest of embryos in the Fallopian tubes using appropriately positioned ligatures. Such artificially-retained embryos may progress to the blastocyst stage in the Fallopian tube, but development thereafter is severely compromised and degeneration soon follows (Jacobson *et al*., 1987; Kooi *et al*. 1992; Lecuru *et al*., 1992; Popp *et al*., 1993; Tepper *et al*., 1992).

Artificial insemination of does with semen from males selected for high growth rate has resulted in an increase in the reproductive rate. New rabbit meat production systems have caused the appearance of an increased incidence of

ectopic pregnancy. During a necropsy study of 550 adult fertile female New Zealand white rabbits (Oryctolagus cuniculus) from two rabbit farms in Spain, twenty-eight ectopic pregnancies were diagnosed. Ectopic pregnancy would not be such an unusual finding in rabbits if we were to make regular necropsies on animals. The most frequent symptom in these animals was failure in mating. Therefore ectopic pregnancy should be considered in the diagnostic approach when assessing rabbit doe pathology. Many unknown factors exist and further investigations on the matter are necessary. It is important to explore the effect of arteficial insemination (use of menotrophin, GnRH) in rabbit farms utilizing artificial insemination and natural mating protocols (Segura *et al*., 2004).

The incidence of ectopic pregnancy has increased from 0.4 to 2% within the last two decades in humans. Major risk factors in ectopic pregnancy include previous tubal surgery, previous ectopic pregnancy, previous salpingitis, assisted reproduction and previous pelvic infection. In women who have undergone previous tubal surgery the potential risk of developing ectopic pregnancy is 20 times that in other women. Because of the high risk attached to previous tubal surgery, for the last two decades MTX has been used as a medicamentous alternative to surgery in nonruptured ectopic pregnancies (Farquhar, 2005; Murray, 2005). MTX in our study is administered at various dosage and route of administration: locally 0.5 mg/kg or 1 mg/kg, and systemically in single dose of 1 mg/kg or 50 mg/m^2 (i.m.).

Byram *et al*. (2005) in the study which was carried out on 24 female rats (Albino Wistar type, 250–300 g) treated with different doses of MTX. The authors concluded that the structural derangements resulting from administration of MTX in doses in excess of 1 mg/kg could cause a reduction in the surface epithelium's ability to make rhythmic lashing movements and could impair the patency of the Fallopian tubes. All these disturbances could be involved to some degree in the genesis of infertility and recurrent ectopic pregnancy. Therefore, the dosage of MTX should be limited to use of the lowest effective dose to avoid these adverse effects.

The histological features observed in the Fallopian tube were similar in all groups. In particular, we observed only an inflammatory infiltrate that was not quantitatively parallel to the increased dose. Cilial loss was prominent and the basal membrane was irregular in all groups. Epithelial cell nuclei were of irregular arrangement. Inflammatory infiltrate can be result of previous tube infection, thus making it impossible to differentiate between this kind of reaction and the effect of methotrexate. Also, inflammatory reaction can be the consequence of vascular changes in tubar ectopic pregnancy. In the histological examination we used a double blind study to analyse tubal pathological changes. These findings did not indicate that the derangements, resulting from methotrexate especially with dosages of 1 mg/kg, can cause necrosis and inflammatory reactions of the tubes and impair the patency of the Fallopian tubes. Probably, a direct intrasaccular injection of methotrexate prevents tissue damage of the Fallopian tubes. MTX has anticancer, antibacterial, antifungal, antiparasitic and also immunosuppressive effects (Chan, 2006; Schurman, 2006). Fallopian tubes in human pregnancy could be resistant to the influence of methotrexate.

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METOTREKSAT I FALOPIJEVE TUBE KOD EKTOPIČNE TRUDNOĆE

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SADRŽAJ

Metotreksat je citostatik izbora u lečenju gestacijskih trofoblastnih bolesti. On ima antimitotski, citotoksički, embriotoksički, imunosupresivni, antiinflamatornni i antiinfektivni efekat što ukazuju rezultati istraživanja *in vitro* i *in vivo*. Izgleda da su njegova dejstva specifična za vrstu i humanu populaciju. Metotreksat se koristi u lečenju različitih malignih bolesti a od nedavno se koristi i u lečenju psorijaze, reumatoidnog artritisa i ektopične trudnoće. Izgleda da je ektopična trudnoća posebnost humane vrste, mada je anatomski, genitalni trakt žena sličan onom u viših primata. Ektopična trudnoća se registruje još kod ženki pacova, kunića, zamoraca, mačaka, jelena, goveda, svinja i majmuna, ali je njena učestalost daleko niža nego u humanoj populaciji. Pelvisna inflamatorna bolest koja je specifična za humanu vrstu možda bi mogla biti jedan od uzroka ove značajne razlike. Smatra se i da humani trofoblast ima daleko veću invazivnost od životinjskog trofoblasta. Primena novih tehnoloških postupaka i arteficijelne inseminacije dovela je do višestrukog povećanja incidence ektopične trudnoće kod ženki kunića. Eksperimenti u *in vitro* uslovima i na animalnom modelu (ženke kunića i pacova) pokazali su da lokalna aplikacija metotreksata dovodi do oštećenja epitela i strome jajovoda i smanjuje fertilnost ovih životinja. Subperitonealne injekcije metotreksata dovode do tipičnih histoloških lezija i povećavaju adherentnost cilija. Zato se smatra da kod lečenja ektopične trudnoće lokalnom aplikacijom metotreksata treba primeniti minimalnu efikasnu dozu. U ovoj studiji koja je obuhvatala 120 bolesnica sa ektopičnom trudnoćom metotreksat je davan lokalno u dozi od 0,5 mg/kg ili 1 mg/kg i sistemski intramuskulno u dozi od 50 mg/m^2. Kod pacijentkinja kod kojih ovaj tretman nije uspeo primenjivano je hirurško lečenje i jajovodi su odstranjivani. Histološkom analizom ovih tuba ispitivano je lokalno dejstvo metotreksata. Efekat leka je procenjivan na osnovu prisustva nekroze i inflamatorne reakcije. Konstatovano je da nije bilo statistički značajne razlike između ispitivanih grupa i da lokalna aplikacija metotreksata nije oštetila jajovod. Lokalna aplikacija MTX vrši se direktno u gestacijski mešak koji se tada devitalizuje što verovatno sprečava direktan kontakt sa tkivom jajovoda i njegovo oštećenje. Gravidna humana tuba je otporna na direktno delovanje metotreksata.