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STUDY THE EFFECT OF METRONIDAZOLE DRUG (FLAGYL®) IN THE INDUCING CONGENITAL MALFORMATIONS IN PREGNANT RAT

دراسة تأثير عقار الميترونيدازول (الفلاجيل®) في ظهور التشوهات الجنينية في الجرذ الحامل

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Abstract

A teratogen is an agent or drug or other substance capable of interfering with the development of an embryo and fetus that may lead to congenital malformation. Parasitic illnesses is increasing all over the world, especially in Iraq and developing countries, and Metronidazole (MTZ) drug is the therapeutic agent usually administered to children as well as adults at the reproductive age. In this study, we propose an evaluation of MTZ in order to analyze the potential damage in infants of Rattus norvegicus as an animal model. Adult female pregnant rats were treated with commercial MTZ. Different types of defects were evaluated using prenatal mortality, phenotypic abnormalities as parameters were studied and scored in pup 1st generation of 40 adult mothers. They were divided into four groups: a) untreated pregnant females as a control; b) females treated with MTZ daily for 18 days as therapeutic dose; c) a double therapeutic dose; and d) a triple therapeutic dose. The results of this study showed that the using of Metronidazole (Flagyl®) during pregnancy in pregnant rats was a teratogen causing multiple different anomalies, birth defects and preterm birth to new born infants.

Keywords: Metronidazole; teratogenicity; birth defects; preterm birth.

الخلاصة

العامل الماسخ (المهدد للحمل) هو أي عامل او عقار أو مواد أخرى لها إمكانية التداخل مع تطور جنين الأم الحامل والتأثير بنمو المولود الجديد والذي ربما يتسبب في حدوث التشوه أو العوق . تزداد الاعتلالات الطفيلية حول العالم خاصة في العراق والبلدان النامية والمتطورة لاسيما الإصابة بداء المشعرات Trichomonocidal activity ، والميترونيدازول (الفلاجيل®) هو العقار الأكثر استعمالا لعلاجها وعادة يستخدم للصغار وللكبار في سن الإنجاب . في هذا العمل قمنا بتقييم هذا العقار لمعرفة الضرر الذي يسببه في موت اجنة ومواليد الجرذ النرويجي Rattus norvegicus كحيوان مختبري . تم معالجة اناث الجرذ الحوامل بالميترونيدازول التجاري ثم تقييم موت الأجنة ماقبل الولادة والتشوهات المظهرية ، كمقاييس في الجيل الأول من المواليد لأربعين أنثى بالغة. قسمت حيوانات التجربة إلى أربع مجاميع : اولا) إناث غير معالجة كمجموعة كسيطرة. ثانيا) إناث معالجة بجرعة علاجية من الميترونيدازول يوميا لمدة 18 يوم. ثالثا) ضعف الجرعة العلاجية و رابعا) ثلاثة أضعاف الجرعة العلاجية . أظهرت نتائج البحث الحالي ان استعمال عقار الميترونيدازول (الفلاجيل) للجرذان الحوامل كان عامل ماسخ (مهدد للحمل) مسببا تشوهات مختلفة عديدة ، منها موت الأجنة والعوق الولادي على اختلاف أنواعه والولادات المبكرة.

Introduction

It is estimated that approximately 10–15% of congenital structural anomalies are the result of nongenetic causes, in other words, from the effects of teratogens[1]. A teratogen is an agent, which can cause a birth defect[2]. It is usually something in the environment that the mother may be exposed to during her pregnancy[3]. It could be a prescribed medication, a street drug, alcohol abuse[4], or a disease present in the mother which could increase the chance for the baby to be born with a birth defect[5]. A "teratogen" is any exposure that can cause harm to an unborn or breastfeeding baby[6] or maternal autoimmune disorder[7]. Most literatures[8] reviewed indicated that the danger of structural defects caused by teratogens is greater in embryonic stage[9], also showed that the teratogenic agents can be disastrous to the developing human being from the moment of conception to birth[10]. About 4 to 5 percent of birth defects are caused by exposure to a teratogen[11].

Once the egg is fertilized (conception), and connected to the uterus, a common blood supply exists between the mother and the embryo. In other words, if something is in the mother's blood, it can now cross over to the developing fetus[12]. Teratogens are thought to have the ability to affect the fetus up to 10 to 14 days after conception. Birth defects are known to occur in 3-6% of all newborns[13]. However, it is extremely difficult to make accurate estimates of exposure risk due to the large number of pharmaceutical, industrial and agricultural chemicals which increases the risk of exposure to multiple agents and their potential synergistic effects[14,15].

During the development of a baby, there are certain organs forming at certain times, if a teratogen has the potential to interfere with the closure of the neural tube, for example, the exposure to the teratogen must occur in the first 3.5 to 4.5 weeks of the human pregnancy, since this is when the neural tube is closing. There are some organ systems that are sensitive to teratogens throughout the entire pregnancy, such as the central nervous system[16]. One teratogen, for example, that affects the central nervous system is alcohol[17], it was at any time during the pregnancy, has the potential to cause birth defects and health problems in the baby, since the central nervous system is sensitive to teratogens the entire nine months of gestation, about 25% of all recognized pregnancies end in miscarriage. The risk of a miscarriage drops to 10% in the eighth week[18].

Exposure to teratogens can result in a wide range of structural abnormalities such as cleft lip, cleft palate, dysmelia (limbs defect), anencephaly, ventricular septal defect[19]. Specific birth defects are not characteristic of any single agents. Hardy *et al.* [20] monitored that results in preterm birth or low birth weight. Other recent studies have indicated that development of bacterial vaginosis during pregnancy is linked to an increased risk of preterm or low-birth-weight infants[21].

Metronidazole (MTZ) Flagyl®, a nitroimidazole that has been on the world market since 1961, is used to treat trichomoniasis, bacterial vaginosis, amebiasis, giardiasis, and numerous anaerobic bacterial infections. MTZ is the therapeutic agent usually administered to children as well as adults at the reproductive age to treat parasitic illnesses , which is increasing all over the world, especially in Iraq and other developing countries[5]. Human studies undergoing therapeutic abortion, Heisterberg[22] showed that MTZ crossed the placental membrane; placental concentrations were approximately 50-65% of those in maternal plasma. Amon and Amon[23] found that, on occasion, the fetal MTZ tissue concentrations exceeded those found in maternal plasma.

The present study was conducted to assess whether the use of MTZ during pregnancy is associated with a higher risk of congenital malformations as main goal.

Materials and methods

Forty virgin female rats and 16 male, one male for each 3 females in one cage, belongs to *Rattus norvegicus,* aged 6-10 weeks old were used in this study which carried out in the Alrazzaza and West Alfurat Researches Unit, Karbala University, Iraq. The pregnant rats were maintained under light program of Light – Dark (LD) 12:12 and fed *ad libitum*. The females were randomly divided into four groups,control T1 gp were administered distilled water only,T2 gp were given 9mg\200gm body weight as therapeutic dose of Metronidazole (MTZ), T3 gp were given 18 mg\200 gm body weight as double therapeutic dose of MTZ; and T4 gp were given 27mg\200 gm body weight as triple therapeutic dose of MTZ. The calculation of dose was measured according to formula of conversion adopted by Pagat and Barnas[24]. The animals were mated and the day on which spermatozoa were found in the vaginal smear and the presence of vaginal plug was designated day zero of pregnancy as outlined in table (1).

Table 1: The experimental design:

Duration of treatment	groups			
	Control (Distilled Water) (T1)	Therap. Dose of MTZ 9mg (T2)	Double Therap. Dose of MTZ 18mg (T3)	Triple Therap. Dose of Metro. 27mg (T4)
18 days	No of rats :10	No of rats : 10	No of rats : 10	No of rats: 10

The drug used was commercial Flagyl® tablets, each containing 500 mg of the active ingredient Metronidazole (MTZ) (From Sanofi Aventis Co. France). The calculated doses (therapeutic, double and triple therapeutic dose) of suspension solution in distilled water of MTZ drugs were administered as an oral dosage one time daily for eighteen days. Distilled water was administered via the same route to the control animals group. All pregnant rats were observed carefully during the period of pregnancy which is about 21 days (± 1day), to detect if there is abortion cases or any abnormal clinical singes and checked for any morphological abnormalities or defects on the new born pups due to the using of Flagyl. After delivery a records of the weight, crown rump lengths and the pups were also observed for gross malformations.

Results

The study was conducted on potential teratogenic effects during pregnancy. We report on a case of a multiple congenital anomalies in a newborn infant whose mother was on MTZ (Flagyl) treatment throughout pregnancy. No considered abnormalities could be detected in the pups of the control group. Variation in the duration of gestation period were observed. It was 22.7 days for T1gp, 21.0 d. for T2, 21.0 d for T3 and 23.0 d for T4 gp, and the total Infants (pups) were decreased T1=70 ; T2= 66 ; T3= 68 and T4= 60 pups.

Table 2 showed that there were 2 defects in control group T1 represented 1.4 % from the 70 pups. While there was 1 defect in T2 gp represented 0.6 % from the 66 pups. There were 2 defects in T3 gp represented 1.3 % from the 68 pups and there were 9 defects in T4 gp represented 5.4 % from the 60 pups.

Table 2: The total number of mothers, infants and the defects number and percentages treated with MTZ.

Total number(n)of Mothers treated with MTZ	Total Infants (pups)	Defects	% of infants with congenital anomalies
Control T1: n-10	70	2	2.85 %
Treated dose T2: n-10	66	1	1.51 %
Double dose T3 : n-10	68	2	2.94 %
Triple dose T4 : n-10	60	9	15 %

From table 3, a multiple congenital anomalies in a newborn infant whose mother was on MTZ treatment through the pregnancy, the types and numbers of each defect was 2 pups (2.85 %) with low-birth weight in T1 gp; 1 defect in T2 gp (1.51 %); 2 in T3 gp (2.94 %) and 2 defects in T4 gp (15 %). While only in T4 gp there was 2 defects with harelip ,3 defects with short tail (fig. 1), 1 defect with absence of one upper incisor teeth (fig. 2) and 1 defect with hydrocephalus. There were no other types of defects in all groups. The total number of defects were 2 in T1gp, 1 in T2, 2 in T3 and 9 defects in T4 gp.

Table 3 : Congenital anomalies reported when MTZ was used during pregnancy in the treated groups of rats.

Type of anomalies	groups			
	Control (Distilled Water) (T1)	Therap. Dose of Metro. 9mg (T2)	Double Therap. Dose of Metro. 18mg (T3)	Triple Therap. Dose of Metro. 27mg (T4)
Short tail	_	_	_	3
Low-birth weight infant	2	1	2	2
Harelip	_	_	_	2
Partial anodontia	_	_	_	1
Hydrocephalus	_	_	_	1
Preterm delivery	_	_	_	_
Micrognathia	_	_	_	_
accessory digit	_	_	_	_
Allopurinol	_	_	_	_
unilateral renal agenesis	_	_	_	_
microphthalmia,	_	_	_	_
optic nerve hypoplasia,	_	_	_	_
club foot , stillborn	_	_	_	_
microcephaly, anencephaly	_	_	_	_
Total	2	1	2	9

The results revealed that the averages of tail length of triple dose gp T4 was decreased (6.3cm) comparing with control group T1(9.6 cm), and the pup weight was decreased also from 6.2 gms for control T1 gp to 5.0 gms for T4 gp , and the pregnancy period was increased corresponding to control T1gp from 22.7 days up to 23.0 d. for T4gp as indicated in table 4.

Table 4 : Averages for some infants defects at weaning age.

Some averages at weaning	Control gp (Distilled Water) (T1)	Therap. Dose of Metro. 9 mg (T2)	Double Therap. Dose of Metro. 18 mg (T3)	Triple Therap. Dose of Metro. 27 mg (T4)
Tail length (cm)	9.7	9.1	8.6	6.3
Body length (cm)	10.1	9.9	10.5	9.4
Pup weight (gm)	6.2	6.5	6.0	5.0
Mum weight (gm)	310	330	330	350
Pregnancy period, days	22.7	21.0	21.0	23.0

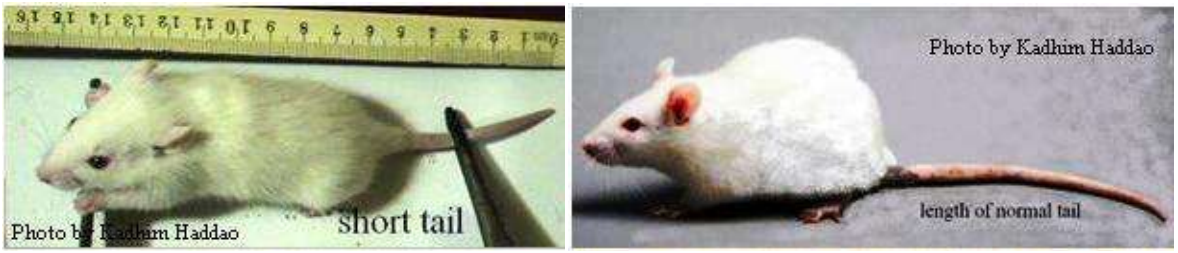

Figure 1 : Short tail anomalies.

Figure 2 : Partial anodontia.

Discussion

The unborn child, although seemed to be protected, comfortable in the womb environment is not completely immuned against to external and internal influences surrounding the mother that can cause serious and debilitating effects at different developmental stages of the individual. Most literature reviewed indicated that the danger of structural defects caused by teratogens is greater in embryonic stage. Previous study has showed that the teratogenic agents can be disastrous to the developing human being from the moment of conception till birth and/or throughout life, birth defects only result when a given dose has been exceeded[2], and after that, the likely effects increase in line with the dose, this idea is true as showed in tables 2, 3 and 4 in which the triple doses of MTZ have caused multiple defects. The variation in the gestation period observed in the present study was in accordance with the results presented by Barnett[25]. From table 4 the newborns pup's average weight of T4 gp was 5 gms. It was less than the control gp. T1,this was confirmed by Calhoun[26]. The normal length of rat can be up to 25 cm, with the tail a further 25 cm, the same length as the body[26] in our results the tail length was 6.3 cm for T4 gp this shortening was due to the MTZ treatment.

MTZ crosses the placental barrier, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, and because Metronidazole is a carcinogen in rodents[27], this drug should be used during

pregnancy only if clearly needed [28]. Use of Metronidazole (Flagyl) for trichomoniasis during pregnancy should be restricted to those in whom alternative treatment has been inadequate because it crosses the placental barrier entering the fetal circulation rapidly and its effects on the human fetal organogenesis are not known[29].

However, numerous reports have been published in which the effects of MTZ taken to treat an infection during pregnancy were examined [18]. Physicians often hesitate to use it during pregnancy, particularly in the first trimester [30,31].The use of Metronidazole in pregnancy remains controversial because the drug is known to be mutagenic in bacteria and carcinogenic in rodents. Multiple studies have failed to show any sign that either topical or oral use is hazardous in early pregnancy, but it is, of course, difficult to prove a negative. It will always be particularly difficult to prove that use during early pregnancy does not increase the risk of the child developing cancer in later life but there is no evidence that the drug is a direct carcinogen in adults[29].

Hillier et al.[29] reported that in a study of the association between antibacterial Vaginitis and preterm delivery of low-birth-weight infants in which more than 10,000 pregnant women were enrolled, women with bacterial vaginitis during the second trimester of pregnancy were 40% more likely to deliver a premature, low-birth weight infant.

Watts[32] has shown that Trichomonas, a motile organism, can attach to various bacteria. They suggest that trichomonads may possibly carry pathogens through the female genital tract into the uterus and tubes and may be an important factor or cofactor in causing salpingitis that results in tubal infertility which known to be factors in neonatal infections, premature labor, and chorioamnionitis. Other recent studies have indicated that development of BV during pregnancy is linked to an increased risk of preterm or low-birth-weight infants[33].

Hauth et al.[34] studied 600 pregnant patients at risk for preterm delivery, randomly assigning them to receive one of two treatments during the second trimester: metronidazole, 250 mg, three times daily for seven days plus erythromycin base, 333 mg, three times daily for 14 days. Treatment was found to decrease preterm deliveries as our results revealed for T2 and T3 gp (table 4).

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