

Methotrexate Induced Gross Malformations in Chick Embryos

P.E. Natekar

Department of Anatomy, Goa Medical College, Bambolim 403 202, Goa, India

Telephone: (Office) 0832-2458701-09 Ext.2250, (Residence) 0832-2458787

(M) 09326150001 E-mail: drpenatekar@hotmail.com

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ABSTRACT Methotrexate is a drug that is used to treat rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, acute lymphoblastic leukemia, ectopic pregnancy and other conditions. Since methotrexate is well known for its teratogenic effects in humans, the present study is conducted to observe its effect on chick embryos. A single injection of 0.012 mg of methotrexate was injected into the yolk sac of chick embryos on 5th day of incubation. The eggs were sacrificed on 19th day of incubation to see for any gross malformations. The spectrum of gross malformations recorded were stunted growth, scanty feathers, beak deformities, short wings and ectopia viscerale. These findings support the malformations caused by the teratogenic drug methotrexate.

INTRODUCTION

Methotrexate is a drug that is used to treat rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, acute lymphoblastic leukemia, ectopic pregnancy and other conditions. According to Gubner et al. (1951) methotrexate was used for the first time for the patients with connective tissue diseases like rheumatoid arthritis. Studies by Faber et al. (1948) reveal that methotrexate is used for temporary remissions in leukemia and also in remissions in choriocarcinoma in women. A study by Wilson (1973) reveals that methotrexate which was taken during pregnancy resulted in fetal death, which led its use as a human abortifacient. Studies by Feichteinger et al. (1989) and Panksy et al. (1989) suggested that methotrexate has been also used in unruptured ectopic pregnancy, and tubal pregnancy respectively so as to cause miscarriages in patients. According to Pymar et al. (2000) methotrexate primarily affects the cytotrophoblast and inhibits the implantation process and hence is used in inducing miscarriages in patients with ectopic pregnancy. Studies by Hausknecht et al. (1995) reveals that a single high dose of methotrexate given before 8 weeks of gestation causes abortion in over 95% of cases

Since methotrexate is well known for its teratogenic effects in humans, therefore the present study is conducted to its effect in developing chick embryos by administering methotrexate in them.

AIMS AND OBJECTIVES

Animal studies are important as they have

enlightened us by knowing the mechanism of teratogenesis as similar patterns of anomalies in humans should also be suspected. No such studies of teratogenesis are conducted in humans. The anomalies recorded in humans are either because the patients had taken methotrexate earlier or anomalies evaluated were due to outcome of the pregnancy and also due to the long term effect of drugs. It was also noted that some of the anomalies in other experimental animals were also different. In the present paper, study is conducted to observe the effect of drug methotrexate on chick embryos.

MATERIAL AND METHODS

Fertilized eggs of white leghorn chicken obtained from the Government poultry farm were incubated at 37 degrees to 38 degrees centigrade. Eggs were turned manually twice a day throughout the period of incubation. 0.012 mg of freshly prepared solution of methotrexate in 0.012ml of normal saline was injected by simple techniques by Singh and Sinha (1973) on the 5th day of incubation. According to Singh and Gupta (1972) this day has been found to be most productive in malformations in chick embryos. About 550 fertilized eggs were taken for this study and were divided into three groups a) Methotrexate treated b) Saline treated and c) Control group. 400 eggs were injected with 0.012mg of methotrexate and 100 eggs were injected with 0.012ml of sterile normal saline under similar conditions. 50 eggs were kept without any injection. All the eggs from these three groups

were sacrificed on 19th day of incubation and were examined for any gross malformations. The malformations were recorded after comparing with the control group.

RESULTS

The fertility of each egg was checked by candling once a day and was allowed to grow. Each embryo was examined for any malformations and mortality of rate in each group was noted. None of the embryos died in the control group and saline treated group while 28% died in methotrexate treated group. No deformities were seen in the control group and saline treated group whereas the malformations observed in methotrexate treated group are given in the table 1.

DISCUSSION

Most of the women take prescribed or non prescribed drugs or use social drugs such as tobacco and alcohol at sometime during pregnancy. Women of childbearing age with chronic medical condition are often concerned about hazards from drug exposure during pregnancy and lactation. A study by Schardein (1985) revealed that 45% of women may use at least one drug on prescription and may use more drugs bought over the counter. Studies by Wilson (1973) revealed that 20% of infant mortality is due to major birth defects. Of those defects about 25% are of genetic origin (genetically inherited diseases, new mutations and chromosomal abnormalities) and 65% are of unknown etiology (multifactorial, polygenic, spontaneous errors of development and synergistic interactions of teratogens) and only 2- 3% defects are thought to arise in association with drug treatment.

Teratology is the study of environmentally

Table 1: Percentage and deformities seen in control, saline and methotrexate group.

S. Deformities No.	Control group (%)	Saline treated (%)	Methotrexate treated (%)
1.Stunted growth	Nil	Nil	89
2.Beak deformity (short beak)	Nil	Nil	36
3.Limb deformities	Nil	Nil	49
4.Scanty feathers	Nil	Nil	84
5. Short wings	Nil	Nil	65
6.Ectopia Viscerale	Nil	Nil	41
7.Immediate death	Nil	Nil	28

induced congenital anomalies. A teratogen is an agent, which by acting on the developing embryo or fetus can cause a structural anomaly. However malformations induced by drugs are important because they are potentially preventable. Teratogens act with specificity, in that they produce specific abnormalities at specific times during gestation.

Methotrexate is an antimetabolite drug which means it is capable of blocking the metabolism of cells. It acts by inhibiting the metabolism of folic acid. Methotrexate is cell cycle S-phase selective, and has greater negative effects on rapidly dividing cells (such as malignant and myeloid cells), which are replicating their DNA and thus inhibits the growth and proliferation of these cells. Methotrexate drug is used in treatment of cancers like carcinoma of breast and acute lymphoblastic leukemia, autoimmune diseases including psoriasis, psoriatic arthritis, rheumatoid arthritis, Crohns disease and in combination with misoprotol to terminate early pregnancies, particularly ectopic pregnancies. A study by Klareskog et al. (2004) reveals that methotrexate is used with infliximab or etanercept in patients of rheumatic arthritis.

In human, methotrexate is known to cause teratogenic effects for which various studies have been carried out. Studies by Powell (1971) and Buckley et al. (1997) reported malformations in humans as follows: CNS abnormalities which included spina bifida, hydrocephaly, anencephaly, meningomyelocoele and mental retardation. Skeletal abnormalities like cleft palate, high arched palate, micrognathia, ocular hypertelorism, external ear anomalies, abnormal cranial ossification, and abnormalities in first branchial arch derivative, syndactyly of fingers, absent digits, large fontanelles. Cardiac abnormalities like dextrocardia were also recorded.

Granzov et al. (2003) reported incomplete cleft palate associated with asymmetric deformities of the toes on both feet in case of an infant whose mother was exposed to methotrexate during pregnancy. A study by Boogerd (1990), reported early blindness and coma in meningeal carcinomas. According to Blackburn (1989) methotrexate caused impotence, when it was used in rheumatic arthritis. Ishii (1989) reported oral mucositis. Studies by Jeurissen (1989) reported nodulosis and vasculitis.

The teratogenic effects of methotrexate have also been studied in animals. According to

Karnofsky et al. (1949) methotrexate delays the embryonic growth in chickens. Studies by Brewton et al. (1990) in chicks suggested that the limb abnormalities seen were caused by transient inhibition of cell division, rather than by cell death. Further studies by Skalko et al. (1974), Khera et al. (1976) and Jordan et al. (1977) suggested species specific effects with embryotoxicity with cats and embryotoxicity and teratogenicity in rats, mice and rabbits. According to Jordan et al. (1977) revealed that administration of methotrexate in rabbits caused hydrocephalus, microphthalmia, cleft lip and palate, dysplastic vertebrae and distal limb dysplasias. Studies by Wilson et al. (1979) showed that in rats the malformations were largely confined to caudal vertebrae following exposure to methotrexate. Further studies by De Sesso et al. (1992) revealed that when rabbits were injected with methotrexate, the commonest abnormalities were that of limb and digit abnormalities, micrognathia, cleft palate and hydrocephalus. Most of the above studies were carried out in rats and rabbits.

As far as chick embryos are concerned many studies have been carried out by administering other anticancerous drugs. Studies by Singh et al. (1971) Singh et al. (1972), Singh et al. (1973), Singh et al. (1974), Singh et al. (1976) noticed spectrum of malformations induced by cyclophosphamide in chick embryos. Studies by Kar et al. (1974) and Malik et al. (1976) also noticed malformations induced in chick embryos by cyclophosphamide.

In our present study chick embryos were selected since not much study was being carried out by administering methotrexate. Methotrexate was injected on 5th day of incubation and the egg being in a closed shell system was constantly exposed to it. No malformations were observed in control and saline treated group. The spectrum of malformations induced in chick embryos due to methotrexate included stunted growth, visceroptosis, scanty feathers, limb deformities, short wings and beak deformities. According to Shepard (1979) teratogenic specificity also applies to species for example aspirin and corticosteroids have been found to be teratogenic in mice and rats but appear to be safe in humans. Thalidomide on the other hand, was not shown to be teratogenic in rats, a tragic fact that resulted in significant human morbidity.

CONCLUSION

All new drug applications filed with the United States Food and Drug Administration (FDA) include data from animal developmental and reproductive toxicologic studies. Although major new teratogenic drugs in humans have been predicted from animal studies, there are problems in extrapolating animal data to humans. Animals have a different gestational clock to humans. There is marked interspecies variability in susceptibility to teratogens and no experimental animal is metabolically and physiologically identical to humans. Animal studies are important because, in some instances, they have shed light on mechanisms of teratogenicity and because when such an agent causes similar patterns of anomalies in several species, human teratogenesis should also be suspected. For obvious reasons no studies of teratogenicity are conducted during embryogenesis of humans. The studies, are therefore, either retrospective in nature (case reports, case series and case control studies), or prospective cohort studies, where a specific maternal exposure in question is ascertained during pregnancy and the pregnancy outcome is evaluated and compared to a control group.

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