

EFFECT OF ACYCLOVIR ON EMBRYO IMPLANTATION IN MICE



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ABSTRACT

Background

Acyclovir is the antiviral drug used for treatment of herpes simplex and varicella zoster viral infections and its adverse effects are not well studied on the embryo implantation.

Objectives

This study was conducted to determine the effect of Acyclovir on the process of implantation in mice of Swiss Webster Albino type and to detect whether there is any effect of Acyclovir on implantation of embryo in mice.

Materials and Methods

Adult female Swiss Webster albino mice were used in this study, aged 8-10 weeks and of 20-25gms body weight. The study was conducted from October 2009 through January 2010. A group of 24 mature female mice were included in this study and they were divided into two groups: Group 1(experimental group), included 12 mice injected with a single daily dose of 50 mg/kg b.wt. of Acyclovir subcutaneously on day 1-5 of gestation at 10:00 a.m; Group 2 included 12 mice injected D.W subcutaneously and considered as a control group. All animals were sacrificed on day 7 at noon and the uteri of all mice were examined for presence of implantation sites.

Results

In this study, out of 12 pregnant females, only two embryos were implanted from only one mouse (8.33%), the remaining 11 (91.67%) showed no sign of implantation at day7 postcoitus. The mean implant in the experimental group was 0.16 ± 0.75 , whereas in the control group a total of 84 embryos from 12 pregnant females were implanted and the mean implant was 7 ± 1.41 . Statistically, the result was highly significant since the p-value was < 0.01 .

Conclusion

Exposure to Acyclovir at early days of pregnancy resulted in inhibition of embryo implantation.

Keywords: *Acyclovir, Implantation, Embryo, Mice.*

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INTRODUCTION

In mammals, the meeting of the oocyte and sperm, and subsequent fertilization take place in the ampulla of the oviduct. During the following days, the embryo travels down the oviduct to the uterus, and prepares for implantation⁽¹⁾.

The receptive state of the uterus is defined as the limited time when the uterine milieu is favorable to blastocyst acceptance and implantation. In mouse, implantation of embryos occurs on day 4 (day 1 = detected vaginal plug). The window for successful implantation could be defined as a limited time span when the activated stage of the blastocyst is superimposed on the receptive state of the uterus⁽²⁾.

Acyclovir or Aciclovir, chemical name acycloguanosine, is a guanosine antiviral drug, marketed under trade names such as Acyclovir or Zovirax. It is one of the most commonly used antiviral drugs primarily used for the treatment of herpes simplex viral infection, herpes zoster and varicella zoster infections⁽³⁾. A derivative of Acyclovir, called Valacyclovir can be used as an alternative to treat the same types of conditions; a common name of it is Valtrex. Since Valacyclovir is changed to Acyclovir in woman's body, the effect on the pregnancy and breast feeding is thought to be the same^(4,5).

Acyclovir is poorly water soluble, the maximum water solubility at 37 °C is 2.5 mg/ml and has poor oral bioavailability (15-30%), and hence intravenous administration is necessary if high concentrations are required⁽⁶⁾.

Acyclovir is widely distributed into the body tissue and fluids and when it is taken by pregnant women it crosses the placenta and reaches the fetus^(7, 8) and because the implantation and gastrulation process are sensitive periods so it may cause teratogenic effects. However, there are only a few experimental studies specifically looking at Acyclovir and pregnancy in animal⁽⁹⁻¹¹⁾. The lack of prenatal toxicity of Acyclovir in some studies had been explained by the relatively high specificity of the drug to inhibit DNA metabolism in predominantly virus- infected cells⁽¹²⁾.

MATERIALS AND METHODS

Adult female Swiss Webster albino mice were obtained from the animal facilities of the

Department of Biology, Faculty of Science and Education at University of Sulaimani. The mice average age was 8-10 weeks and their average body weight ranged between 20-25gms. They were housed in plastic cages under same environmental conditions of light (14 hrs. Light: 10 hrs dark), temperature (20-23°C) and almost normal humidity. The animals had free access to standard laboratory diet, prepared in animal house consisting of wheat, barley, sunflower, corn and protein (local supplier), and water ad libitum^(13, 14). This study was done from October 2009 through January 2010.

Before receiving Acyclovir, the sexually mature females were daily examined by investigating their vaginal smears for 14 days to establish their normal pattern of cyclical activity. Then all females with proestrus or oestrus stages, stages in normal oestrus cycle in mice were caged overnight with males of the same strain and of proven fertility (three females with single male per box)⁽¹⁵⁾. Observation of vaginal plug on the next morning at 08:00 a.m. indicated a successful mating, and the number of days post coitum (p.c) was counted as day one of gestation, is the day of vaginal plug detection. All of 24 mature female mice were used in this study and they were divided into two groups: Group 1 was the experimental one. It consisted of 12 mice that were injected with a single daily dose of 50mg/kg b.wt. of Acyclovir subcutaneously on day 1-5 of gestation at 10:00 a.m. and Group 2, was the control group. It included 12 mice and injected with distilled water subcutaneously of same volume and time of the injection as that of the drug supplied to group 1.

All animals were sacrificed on day 7 at noon and the uteri of all mice were examined for presence of implantation sites. Implantation sites prior to gestational swellings were observed at day 6 of gestation by the Technique of Orisini⁽¹⁶⁾, figure 1. Thus horns of the uterus were removed, cleared from fat and pinned out through aluminium foil to a supporting card. The horns were then subjected to the following procedures:

1-Fixed in a mixture of 30 ml of 95% alcohol, 10 ml formalin, 10 ml glacial acetic acid, and 50 ml water overnight.

2-Pins were removed in order to prevent metallic staining of the adjacent tissues.

3-Uterine horn was bleached and dehydrated through two changes of ascending alcohol concentrations (50%, 70%, 80%, 95%, and 100%)

each for 40 minutes (all of them containing a few drops of hydrogen peroxide solution). When this procedure was completed, implantation sites were visible, figure 1.

4- After dehydration, clearing was done by passing the horn into 100% alcohol and benzol (50: 50) for 30 minutes.

5- For final clearing and storage, horns were transferred to benzyl benzoate for 30 minutes.

The Statistical Package for Social Science (SPSS) Chicago, IL, USA version 17 was used for data entry and analysis, chi-square test (X^2) was used. P-value less than 0.01 was considered statistically significant.

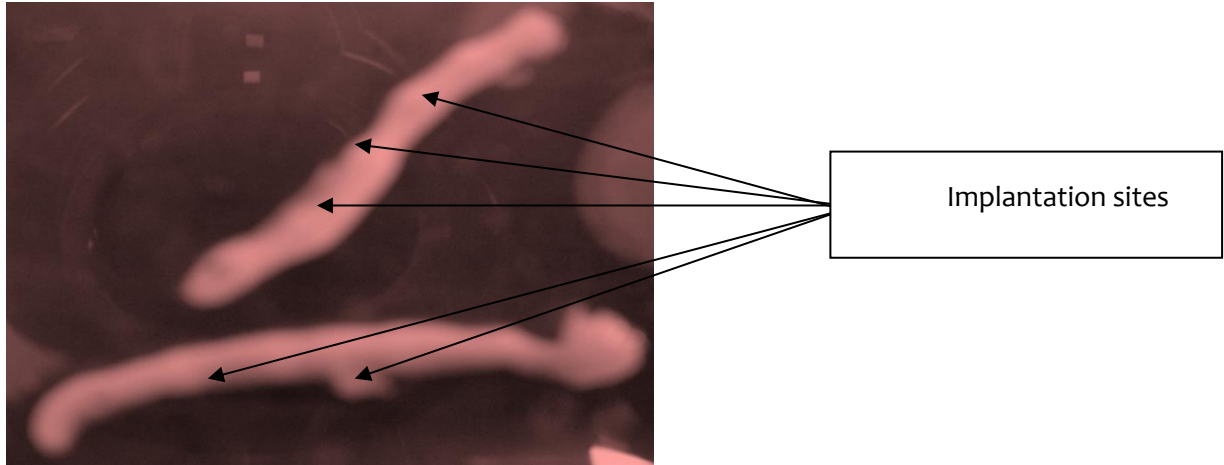


Figure 1. Early implantation sites.

RESULTS

In this study, out of 12 pregnant females in the experimental group, only two embryos were implanted from the same mouse (8.33%), the remaining 11 (91.67%) mice showed no sign for implantation at day 7 p.c.

The mean implant in the experimental group was 0.16 ± 0.75 , whereas in the control group a total of 84 embryos from 12 pregnant females were implanted and the mean implant was 7 ± 1.41 , table 1. The result was highly significant since the p-value of the test was < 0.01 .

Table 1. Effect of Acyclovir (50mg/kg b.wt.) on the mean implants per pregnant mice at day 7 of gestation

Groups of mice	Number of pregnant mice	No. of non pregnant mice	Mean implant/ pregnant mouse
Control			
N=12	12 (100%)	0 (0%)	7 ± 1.41
Treatment			
N=12	1 (8.33%)	11(91.67%)	0.16 ± 0.75
Test	Chi-square=20.31, d.f=1		
	P-Value $< (0.01)$ (HS)		

DISCUSSION

Blastocyst implantation and successful establishment of pregnancy require substantial interactions between the embryo and the maternal environment. In the late luteal phase, physiological changes occur in the endometrium to allow blastocyst implantation. The "window of implantation" represents the period of maximum uterine receptivity for implantation. At the 8-cell to 16-cell stages, the embryo enters the uterine environment, developing into a blastocyst, in which the first events of cellular differentiation are observed. At the blastocyst stage, the embryo hatches from the surrounding zona pellucida and subsequently implants in the uterus⁽¹⁷⁻²⁰⁾. It is better for Acyclovir as any drug to be avoided during pregnancy especially the first trimester but, it should be administered in life-threatening conditions like herpes simplex encephalitis and hepatitis and in these cases the intravenous route is preferred than oral one because it is more effective⁽³⁾.

Concerning the effect of Acyclovir on implantation process, the result of our study showed that injecting the pregnant mice with a single daily dose (50mg/kg b.wt.) of Acyclovir subcutaneously caused a significant effect on the process of implantation, s.c.t administration was preferred than oral route because it was easier in mice. Almost all embryos were not implanted probably due to an alteration in the histological structure as well as the hormonal secretion making the uterus unsuitable to receive the blastocysts. This result was corresponded to the findings of a study showed that Acyclovir caused reduction in the implantation rate and resorption of fetuses⁽²¹⁾.

Both peri- and post-natal study was done on rats treated with Acyclovir at 50 mg/kg/day s.c., and there was a statistically significant decrease in group mean numbers of corpora lutea, total implantation sites, and live foetuses⁽²¹⁾. However this approach remains a promising one and merits further investigation, because in another study the results showed that subcutaneous injection of Acyclovir at dose of 25 mg/kg b.wt. twice daily did not impair implantation in rats⁽²²⁾, also Acyclovir did not impair fertility and implantation in mice administered at dose (450 mg/kg/day) orally⁽⁹⁾, those results were in disagreement with our results. On the other hand Acyclovir administered at higher doses (50 mg/kg/day, s.c.)

in rats and rabbits (11 to 22 and 16 to 31 times human levels, respectively) the implantation efficacy, but not the litter size, was decreased⁽⁹⁾, which confirmed our result.

Glaxo (2007) reported that higher doses (100 mg/kg b.wt) of Acyclovir decreased implantation efficacy in rats and rabbits, while in other study, it was found that Acyclovir cream (5%) applied twice a day around the mouth of pregnant mouse at days 3, 4, 5, and 6 of gestation revealed a significant decrease in the number of implanted embryos, significant increase in abortion and significant decrease in thickness of the endometrium⁽²³⁾. All these results confirmed our results. Taken together, those observations indicated that the higher dose group had greater resorped fetuses than that in the low doses group. Therefore it is clear that the dose of teratogens plays a great role in induction the adverse effect on the pregnancy. However, from the above studies one can conclude that clear dose-response relationships were observed on the blastocyst-endometrium interaction. The effect of Acyclovir differs among the mammalian species⁽²⁴⁾, depending on the pharmacokinetics mechanism, the route of administration (the intravenous administration causes more toxic effects than oral), the rate of distribution and sensitivity of different tissues to the drug⁽²⁵⁾. In addition, the absence of prenatal toxicity of Acyclovir in some studies had been explained by the relatively high specificity of the drug to inhibit DNA metabolism in predominantly virus- infected cells⁽¹²⁾ but as it is found in current study it affected the normal cells too. So the adverse effect of Acyclovir on the implantation in our study can be explained on the bases that, the period of maximal cytotoxicity seen in embryos (between ovulation and blastocyst formation) closely coincides with a period of mitochondrial morphologic evolution in the mouse embryo. It is also possible that drug exposure may result in sub cellular toxicities affecting embryonic development in a more indirect manner^(26, 27).

In conclusion exposure to Acyclovir at early days of pregnancy results in inhibition of embryo implantation and the drug should be prescribed during pregnancy only when the potential benefits justify the possible risks to the fetus.

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