EFFECT OF PROGESTERONE AND PREGNANCY ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE 2 IN VIVO

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Summary: This project involved an investigation into the effect of pregnancy and progesterone on genital Herpes simplex virus (HSV) Type 2 infection in mice, with the aim of establishing whether elevated progesterone level, due either to pregnancy or parenteral administration, makes the vaginal HSV infection all the heavier. The investigation was carried out on three groups of mice, representing three test situations in connection with vaginal HSV infection: the first group consisted of non-pregnant experimental animals administered progesterone in the duration of 6 days and intravaginally infected with the virus on the 7th day; the other group consisted of pregnant mice infected on the 7th day of pregnancy in the same manner as the first group. Investigation in the scope of groups involved the monitoring of moratality in the duration of 20 days, and the rate thus registered was compared with that of the control-group mice which were neither pregnant nor administered progesterone, and which were infected in the same manner as those from the control group.

The results of experiments carried out on pregnant and progesterone-administered mice clearly show that experimental animals from these two groups are more susceptible to vaginal HSV Type 2 infection than those from the control group, which means that pregnancy and progesterone have produced the same effect on the in vivo replication of Herpes simplex virus Type 2.

Particular importance is attached to experiments on different animals in the study of infections caused by the Herpes simplex virus (HSV). The existence of a relevant animal model should provide for better understanding of the pathogenesis, and thereby also the application of suitable methods for the control of HSV infections. The experiments carried out on guinea pigs shosew that these experimental animals are suitable models for the study of latency and reactivity of this virus. The largest number of experiments carried out for the purpose of investigating the effect of genital HSV infection on the course and outcome of pregnancy involved rabbits, hamsters and cats, especially in connection with placental transfer of the virus ([1, 2]). The experiments carried out on mice showed that in pregnancy these animals show higher sussceptibility to virus infections such as: polyomelitis, encephalomyocarditis and genital herpes ([3]). A series of experiments carried out on pregnant and progesterone-treated mice was intended to contribute to better understanding of the hazards posed by genital HSV infection, both to the mother and fetus ([4, 5, 6]).

This project involved an investigation into the effect of pregnancy and progesterone on genital HSV Type 2 infection in mice, with the aim of establishing whether elevated progesterone level, due either to pregnancy or parenteral administration, makes the vaginal HSV infection all the heavier.

MATERIAL AND METHODS

Experimental animals: white laboratory mice, female, 6 to 7 weeks old, obtained from the Veteriary Farm of the Military Medical Academy in Belgrade.

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Virus: Herpes simplex virus Type 2 (strain ATCC VR-734) titre $10^{6.8}$ pfu/ml, used to infect the experimental animals by intravaginal inoculation of the virus suspension with the means of a polyethylene catheter, with a single 0.05 ml dose.

Progesterone: oil solution of progesterone (Inex-Hemofarm Vrsac) was administered to mice subcutaneously in a single dose of 0.1 ml (2.5 mg of progesterone).

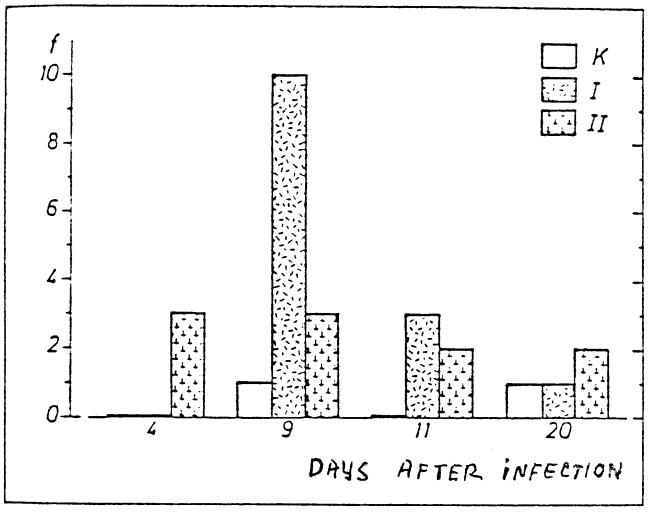
Description of experiment: The experiment included 100 female mice divided into three groups: I (25 non-pregnant mice administered progesterone for 6 days and intravaginally infected with the virus on the seventh day); II (20 pregnant mice infected on the seventh day of pregnancy in the same manner as Group I); K (35 non-pregnant mice administered no progesterone and infected in the same manner as the preceding two groups); and III (20 non-pregnant mice, 10 of which were administered progesterone in the same doses as Group I). All experimental animals from the latter group were kept under observation until the end of the experiment and were not infected.

Groups I, II and K represented three test situations in connection with vaginal HSV Type 2 infection, while Group III was the Control Group. The investigation within the groups involved the monitoring of mortality in the duration of 20 days.

RESULTS

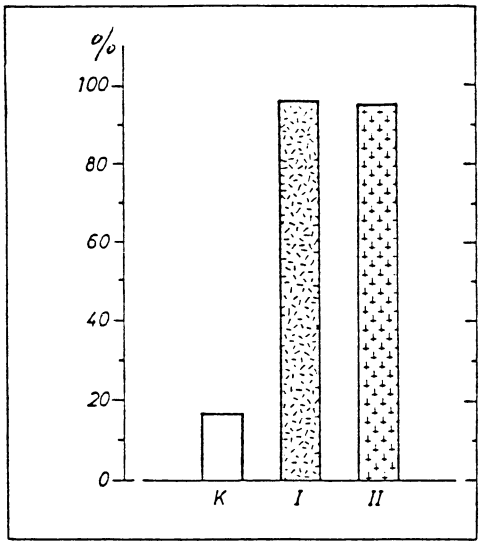
Figure 1 shows the number of dead experimental animals on the 4th, 9th, 11th and 20th day of the experiment, in groups I, II and K. The statistical significance of the difference in mortality by groups was established by the X^2-test, in order to establish the relationship between the number of dead mice in different groups.

The comparison made between Groups I and II did not result in a statistically significant difference ($X^2=0.16$; $p > 0.05$), which means that pregnancy and progesterone produced the same effect on the HSV Type 2 infection. On making a comparison between Group I and Group K, a highly significant difference was found ($X^2=18.86$; p 0.01), resulting from higher mortality in Group I than in Group K, which served as proof that progesterone makes the genital HSV infection all the heavier. The difference between the number of dead mice in Group II and K is highly significant ($X^2=14.63$; p 0.01) as



I progesterone treated non-pregnant mice
II pregnant mice
K control group

Fig. 1. – Mortality of experimental animals.



I progesterone treated non-pregnant mice
II pregnant mice
K control group

Fig. 2. – Mortality in individual groups of experimental animals.

the result of higher mortality in Group II than in Group K. We are taking this as proof that in-vivo cases progesterone has a stimulating effect on the replication of HSV Type 2.

A separate investigation was carried out with regard to the number of dead mice in all three Groups (I, II and K) with the aim of establishing the time interval between the onset of infection to the death of the experimental animals. By Fisher test, we established a significant difference ($P=0.04$) manifest in the fact that the progesterone-administered animals died in large numbers already on the 4th day. It can be concluded from the latter that pregnancy per se has a remarkably stimulating effect on the replication of HSV Type 2.

Mortality in Groups I, II and K is presented on the column diagram (fig. 2). The percentage of dead experimental animals in individual groups was as follows: 96 in Group I, 95 in Group II, and only 17 in Group K.

DISCUSSION

This project involves an investigation into the effect of pregnancy and progesterone on genital HSV Type 2 infection in mice, with the aim of establishing whether elevated progesterone level, due either to pregnancy or parenteral administration, makes the HSV infection all the heavier.

With reference to the well-known effect of progesterone and pregnancy on immune response, i.e. depression of the levels of humoral and cellular immunity, it is only logical to expect changes in the gravity of infection under such circumstances. This was investigated within the scope of this project by monitoring the mortality involved with experimental genital HSV Type 2 infection in pregnant and progesterone-administered mice.

The percentage of dead experimental animals in individual groups was as follows: 96% (progesterone-administered mice), 95% (pregnant animals) and 17% in the Control group. Such results and the existence of statistically highly significant differences established on making a comparison between the experimental groups and the Control Group, in addition to there being no difference between the progesterone-administered and pregnant mice, are indicative of markedly increased susceptibility these from the Control Group.

The differences in mortality between the mice administered progesterone and the pregnant ones are manifest on the level of statistical significance only when a comparison is made between the number of dead mice on the 4th and the 9th day. The largest number of deaths in the progesterone-administered mice was on the 9th day, as opposed to the group of pregnant mice where the highest number of deaths was registered already on the 4th day.

The investigation carried out by Baker and ass. ([6]) indicated the existence of a direct correlation between the dose of progesterone and the rate of mortality (0.04 to 2.0 mg daily in the duration of 6 days). In our experiment, the mice were administered 2.5 mg of progesterone in the duration of 6 days. Murr and ass ([7]) have shown that progesterone reaches a high level (100 ug/ml of plasma) within 48 hours in pregnant mice, which helps to explain the early death of pregnant animals, bearing in mind the effect of progesterone on cellular immune response. In the scope of their investigation into susceptibility of pregnant mice to HSV Type 2 infection, Young and Gomez ([3]) pointed out the importance of the different routes of virus inoculation, as well as the titre of the inoculated virus. The vaginal route of inocualiton used in the experiment imitates the natural route of genital HSV Type 2 infection in humans. When vaginal infection was caused by high-titre virus (10^5 pfu/ml), the mortality rate in the group of pregnant mice amounted to 95%, and 68% in the group of non-pregnant mice. If the titre of the inoculated virus is reduced to 10^4 pfu/ml, the mortality rate in pregnant mice amounts to 90%, and 63% in the Control Group. This goes to show that even in the case of a smaller quantity of virus used to cause experimental vaginal infection, the pregnant experimental animals showed higher susceptibility. The titre of the virus used in our experiment amounted to $10^{6,8}$ pfu/ml; mortality rate in the group of pregnant mice amounted to 95%, and 17% in the control group.

The discrepancy between such findings and those of the aforementioned Authors can be explained by different kinds of mice and different virus strains used in the experiment.

In response to virus infection during pregnancy, the increased susceptibility occurs not only due to the general condition of immunity, but also due to changes in the local immune response. Pregnancy makes the genital HSV Type 2 infection all the heavier also because its direct effect on the genital mucous membrane, which is largely dependent on the hormonal effect. The changes in local immunity, as well as a condition accompanying pregnancy (vascular changes and increased permeability of the cervicovaginal epithelium) may facilitate more voluminous penetration of the virus.

CONCLUSION

The results of experiments carried out on pregnant and progesterone-administered mice clearly show that these two groups of experimental animals are more susceptible to vaginal HSV Type 2 infection than those in the control group.

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