PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 569–571 (1978)

# Effect of Heparin on Cytomegalovirus Replication[1] (40098)

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Several studies have shown that heparin inhibits the infectivity of many members of the herpesvirus group. Inhibition of herpes simplex virus (HSV) infectivity was shown to increase as the concentration of heparin was increased (1, 2). It was also reported that heparin sensitivity can be of value in distinguishing HSV-1 from HSV-2 strains since most type 2 strains were found to be insensitive to treatment with this drug (3, 4). Other herpesviruses including pseudorabies virus, varicella virus and human cytomegalovirus (5, 6) were also found to be inhibited by this naturally occurring biological compound.

During the course of studies on the development of guinea pig cytomegalovirus (GPCMV) viremia, it was noted that loss of GPCMV infectivity occurred in the presence of heparin. Analyses of the effect of heparin on the replication of GPCMV, together with preliminary studies on human, simian and murine CMV are included in this report.

*Materials and methods. Virus stock.* The prototype guinea pig cytomegalovirus (GPCMV), strain 21222, originally isolated by Hartley *et al.* (7) was obtained from the American Type Culture Collection. Guinea pig herpeslike virus (GPHLV) strain LK40, was isolated in our laboratory (8). The human CMV strain AD169 and the green monkey CMV strain CSG, were obtained from Dr. Wallace Rowe, NIH, Bethesda, MD. The Smith strain of mouse CMV was obtained from Dr. John Booss, VA Hospital, West Haven, CT.

*Tissue culture and virus assay.* Primary guinea pig embryo (GPE) cell cultures were prepared from 20- to 40 day-old embryos and grown in Eagle's medium supplemented with 10% calf serum as previously described (9). Passaged GPE cell cultures were used for assaying the guinea pig virus. The human WI-38 cell line was used for assaying both human and monkey CMV and mouse embryo fibroblasts (ME) were used for mouse CMV assay. Laboratory grade sodium heparin powder was purchased from Fisher Scientific Company. A stock solution was prepared in 0.9% saline (1 U = 10 $\mu$g). For virus assay, both virus induced cytopathic effect (CPE) and virus induced intranuclear inclusions in hematoxylin-eosin stained preparations were used to determine end points.

*Results. Effect of heparin on cytomegalovirus replication.* The degree to which the presence of heparin in the culture medium affected replication of four different CMV types is summarized in Table I. Growth of all of the CMV types tested was inhibited in the presence of a concentration of heparin comparable to that used for blood collection. The guinea pig and monkey CMV types showed the greatest reduction in infectivity when subjected to this treatment.

*Comparison of inhibitory effect of heparin on GPCMV and GPHLV.* Two herpesviruses of guinea pigs, GPCMV and GPHLV, were tested in cell culture in both the presence and absence of heparin. Virus suspensions containing 100 $TCID_{50}$ were inoculated into cultures containing different concentrations of heparin. The results are summarized in Table II. The presence of as little as 0.1 U/ml of heparin resulted in a tenfold reduction in the infectivity titers of GPCMV. Increased concentrations of heparin resulted in increased losses in infectivity accompanied by a corresponding delay in appearance of CPE, demonstrating a close relationship between the virus concentration and the inhibitory action of heparin. However, complete inhibition could not be obtained even with 100 U of heparin. In contrast, virus infectivity titers

[1] This study was supported by U.S. Public Health Service Research Grant HD 10609 from the National Institute of Child Health and Human Development and by Medical Research Service of the Veterans Administration.

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0037-9727/78/1574-0569$01.00/0

TABLE I. EFFECT OF HEPARIN ON REPLICATION OF SELECTED CYTOMEGALOVIRUS STRAINS IN CELL CULTURE.

| Cytomegalovirus tested | Virus strain | Cell culture system | Average virus infectivity titers log $TCID_{50}$/ml | | Log reduction |
|---|---|---|---|---|---|
| | | | In heparin 100 U/ml | In absence of heparin | |
| Human | AD 169 | WI-38[a] | 2.0 | 3.5 | 1.5 |
| Green monkey | CSG | WI-38 | 1.5 | 5.0 | 3.5 |
| Guinea pig | 21222 | GPE[b] | 2.0 | 5.5 | 3.5 |
| Mouse | Smith | ME[c] | 2.0 | 3.5 | 1.5 |

[a] WI-38: Human fibroblast cell strain.
[b] GPE: Primary guinea pig embryo fibroblast.
[c] ME: Primary mouse embryo fibroblast.

TABLE II. COMPARISON OF HEPARIN EFFECT ON TWO HERPESVIRUSES OF GUINEA PIGS.

| Concentration heparin (unit/ml) | GPCMV | | GPHLV | |
|---|---|---|---|---|
| | Virus titer Log $TCID_{50}$/ml | Reduction (log) | Virus titer log $TCID_{50}$/ml | Reduction (log) |
| 0 | 5.5 | — | 4.0 | — |
| 0.1 | 4.5 | 1.0 | 4.5 | 0 |
| 1.0 | 4.5 | 1.0 | 3.7 | 0.3 |
| 10.0 | 3.5 | 2.0 | 3.8 | 0.2 |
| 100.0 | 2.0 | 3.5 | 4.0 | 0 |

were not significantly reduced when GPHLV was treated in a similar manner.

*Effect of heparin before or after virus adsorption.* In order to understand the mechanism by which GPCMV replication was inhibited by the heparin, GPE cells on coverslips were treated with various concentrations of heparin for 1 hr at 35° before inoculation of 100 $TCID_{50}$ of virus (Table III, Expt. A). When cultures without heparin showed visible CPE, all inoculated cultures were fixed, stained and examined for the number of infected versus noninfected cells, expressed as percent reduction in comparison with cultures without heparin. Again, a reduction of approximately 80% in the number of GPCMV induced inclusions was observed when 1 U of heparin was added to cultures before virus adsorption and reached nearly 100% at higher concentrations. In experiment B, cultures were treated as in experiment A except that medium containing various concentrations of heparin was added to cultures one hour after virus adsorption. Inclusion formation was reduced by 50% at 1 U/ml of heparin and at 100 U/ml, a 68% reduction was obtained. However, in a separate experiment, when the adsorption period was extended to 3½ hr before addition of medium containing heparin, there was no significant difference in number of GPCMV-induced inclusions in cultures with or without heparin.

*Comparison of different blood anticoagulants on the replication of CMV.* The infectivity titers of GPCMV and monkey CMV when grown in cell culture medium containing different anticoagulants, were studied. GPCMV infectivity was significantly reduced in the presence of heparin, but no effect was observed when Alsever's solution, sodium citrate solution (3.8% in saline), or ethylene–diamine–tetra acetic acid (EDTA) were tested in a similar manner. Essentially the same results were obtained with the monkey CMV strain, in that only heparin reduced the infectivity when it was present in the cell culture medium.

*Discussion.* Sensitivity to heparin, as measured by decreased infectivity, has been shown to be characteristic of a number of different viruses and in particular appears to be a property of certain members of the herpesvirus group (5, 6). Inhibition of virus replication by heparin has been used as a basis for differentiating HSV types 1 and 2, when used in conjunction with other biological characteristics (3, 4). In the present study, the heparin sensitivity of GPCMV but not GPHLV, provides a useful tool for differentiating between these two endogenous herpesviruses of guinea pigs, thus enabling an investigator to inhibit GPCMV replication in those instances when a mixed infection with both herpesviruses was present in the same animal.

In addition, data was obtained confirming previously published reports (5), showing that

TABLE III. EFFECT OF HEPARIN TREATMENT BEFORE OR AFTER GPCMV ADSORPTION.[a]

| Expt. | Method of assay | Heparin unit/ml | No. of inclusions per 1000 cells | Percent reduction |
|---|---|---|---|---|
| A | Heparin added before virus adsorption | 0 | 981 | — |
| | | 0.1 | 560 | 43 |
| | | 1.0 | 201 | 80 |
| | | 10.0 | 1 | 99.9 |
| | | 100.0 | 0.2 | 99.9 |
| B | Heparin added one hour after virus adsorption | 0 | 406 | — |
| | | 0.1 | 164 | 59.7 |
| | | 1.0 | 201 | 50.5 |
| | | 10.0 | 142 | 65.1 |
| | | 100.0 | 128 | 68.5 |

[a] Inoculum 100 $TCID_{50}$.

the inhibitory process occurred prior to virus attachment and penetration and that the heparin exerted no influence on virus replication when infected cells were exposed to the compound.

In earlier studies conducted in our laboratories on the pathogenesis of different herpesviruses in experimental animals, heparin was used as an anticoagulant for blood collected in studies of viremia. No discernible difficulty in recovering either GPHLV (9) or rabbit HLV (10) was observed. However, data obtained in the present report indicate varying degrees of sensitivity to heparin by GPCMV as well as the monkey, mouse and human CMV types. For this reason the heparin sensitivity of these virus types should be taken into account when studies of CMV viremia are carried out in these species. The cytomegaloviruses tested did not appear to be sensitive to any of the other commonly used anticoagulants including Alsever's solution, sodium citrate and EDTA. Furthermore, the data reported here show that the cytomegaloviruses isolated from the mouse, guinea pig and monkey should be added to the list of cytomegalovirus types previously reported to be sensitive to heparin.

*Summary.* The presence of heparin in cell culture medium was found to reduce the infectivity titers of cytomegaloviruses isolated from human, monkey, guinea pig and mouse. This inhibition was shown to increase as the heparin concentration was increased. When heparin was added to cultures after virus adsorption had taken place, the effect on virus infectivity was negligible. Other anticoagulants studied included Alsever's solution, sodium citrate and EDTA, none of which showed any inhibitory effect on cytomegalovirus replication in cell culture.

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Received November 9, 1977. P.S.E.B.M. 1978, Vol. 157.