Acta Medica Okayama

Volume 34, *Issue* 6 1980 *Article* 1

DECEMBER 1980

Effect of glucosamine on phenotype mixing of vesicular stomatitis virus with avian sarcoma virus

Hajime Ogura* Tazuko Fujiwara†

*Okayama University,

†Okayama University,

Effect of glucosamine on phenotype mixing of vesicular stomatitis virus with avian sarcoma virus*

Hajime Ogura and Tazuko Fujiwara

Abstract

The effect of glucosamine on phenotypic mixing between vesicular stomatitis virus (VSV) and avian sarcoma virus (ASV) was studied. Phenotypic mixing decreased with increase in glucosamine concentration, and, in the presence of 20 mM glucosamine, was no longer detectable. In the presence of 20 mM glucosamine, cells still produced 10(2)–10(3) focus forming units (FFU) of ASV and 10(6) plaque forming units (PFU) of VSV per milliliter. These results suggest that cells producing a relatively large amount of ASV (more than 10(3) FFU/ml) are essential for phenotypic mixing of VSV with ASV.

KEYWORDS: glucosamine, phenotopic mixing, vesicular stomatitis virus, avian sarcoma virus

---

*PMID: 6258397 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **34**, (6), 355—359 (1980)

EFFECT OF GLUCOSAMINE ON PHENOTYPIC MIXING OF VESICULAR STOMATITIS VIRUS WITH AVIAN SARCOMA VIRUS

Hajime OGURA and Tazuko FUJIWARA

Department of Virology, Cancer Institute Okayama University Medical School, Okayama 700, Japan (Director: Prof. Y. Yabe)

Received August 15, 1980

Abstract. The effect of glucosamine on phenotypic mixing between vesicular stomatitis virus (VSV) and avian sarcoma virus (ASV) was studied. Phenotypic mixing decreased with increase in glucosamine concentration, and, in the presence of 20 mM glucosamine, was no longer detectable. In the presence of 20 mM glucosamine, cells still produced 10^2-10^3 focus forming units (FFU) of ASV and 10^6 plaque forming units (PFU) of VSV per milliliter. These results suggest that cells producing a relatively large amount of ASV (more than 10^3 FFU/ml) are essential for phenotypic mixing of VSV with ASV.

Key words: glucosamine, phenotopic mixing, vesicular stomatitis virus, avian sarcoma virus.

VSV mixes phenotypically not only with avian retrovirus envelope (1), but also with chick helper factor (*chf*), a product of the envelope gene of endogenous virus present in avian cells (2). This phenomenon has been utilized to detect expression of the viral envelope gene in avian cells (2-4). To utilize this phenomenon widely, however, it is necessary to know its sensitivity. Glucosamine blocks retrovirus replication, but hardly affects replication of VSV (5-8). Therefore, in the present study, we investigated the effect of glucosamine on the phenotypic mixing of VSV with ASV and estimated the sensitivity of the phenotypic mixing assay for the detection of envelope gene product of avian retrovirus.

MATERIALS AND METHODS

Viruses. The Bratislava 77 strain of ASV (ASV B77) was obtained from Dr. R. R. Friis, Giessen, West Germany. The mutant *tl*-17 of VSV (VSV *tl*-17) was obtained from Dr. J. Zavada, Bratislava, Czechoslovakia, and imported with the permission of the Ministry of Agriculture of Japan. VSV *tl*-17 has thermolabile envelope components, and its infectivity rapidly decreases when incubated at 45°C (1). Chick embryo cells (CEC) were inoculated with VSV *tl*-17 at room temperature for 30 min, washed twice with phosphate-buffered saline (PBS) to remove non-adsorbed virus, and then incubated at 32°C (permissive temperature for VSV *tl*-17) for about 18 h till the appearance of cytopathogenic effects (CPE).

The culture supernatants were stored at −80°C and thawed before use.

Chick embryo cells. The primary CEC were prepared from 11-day-old C/B phenotype, *chf* negative chick embryos obtained from the Research Institute for Microbial Diseases of Osaka University, Kanonji, Japan. The cultures were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% tryptose phosphate broth and 5% heat-inactivated calf serum.

ASV B77-transformed cells. CEC were transformed *in vitro* by ASV B77 and maintained in the same way as CEC. These ASV B77-transformed cells produced a large amount of ASV.

Glucosamine treatment. Glucosamine (Katayama Kagaku, Japan) solution was neutralized with sodium hydroxide, and added at 32°C for 6 h to 1) ASV B77-transformed CEC, 2) normal CEC after inoculation of VSV *tl*-17 and 3) ASV B77-transformed CEC after inoculation of VSV *tl*-17 at a multiplicity of 2 PFU per cell. Then the cultures were washed three times with PBS to remove virus produced during the initial 6 h, and fresh medium containing the same amount of glucosamine was added and further incubated at 32°C. When CPE appeared in VSV *tl*-17-inoculated cultures, the culture supernatants were harvested. About the same time, the culture supernatants of glucosamine-treated ASV B77-transformed CEC were also harvested.

Titration of viruses. FFU of ASV B77 and PFU of VSV *tl*-17 were determined by routine methods on CEC (5, 8).

RESULTS

Effect of glucosamine on the production of ASV B77 and VSV tl-17. In the presence of glucosamine, the replication of ASV B77 in ASV-transformed CEC decreased with increase in the concentration of glucosamine (Fig, 1). The production of ASV B77 in the absence of glucosamine was 10^6 FFU/ml, while that in the presence of 20 mM glucosamine was 10^2–10^3 FFU/ml. The production of VSV *tl*-17 in the absence of glucosamine was 10^7 PFU/ml and in the presence of 20 mM glucosamine was $1\text{–}4 \times 10^6$ PFU/ml. The production of VSV *tl*-17 was slightly inhibited by the addition of glucosamine (Fig. 1).

Effect of glucosamine on the phenotypic mixing of VSV with ASV. As shown in Fig. 2, VSV *tl*-17 propagated in normal CEC in either the absence of glucosamine or the presence of 20 mM glucosamine was strongly inactivated by heating at 45°C for 60 min. VSV *tl*-17 propagated in ASV B77-transformed CEC in the presence of 20 mM glucosamine was also strongly inactivated by heating at 45°C for 60 min (Fig. 2). However, VSV *tl*-17 propagated in ASV B77-transformed CEC in the absence of glucosamine was rather heat-stable and was only weakly inactivated by heating at 45°C for 60 min (Fig. 2). The production of heat-stable virions of VSV *tl*-17 in ASV B77-transformed CEC decreased with increase in the added glucosamine (Table 1).

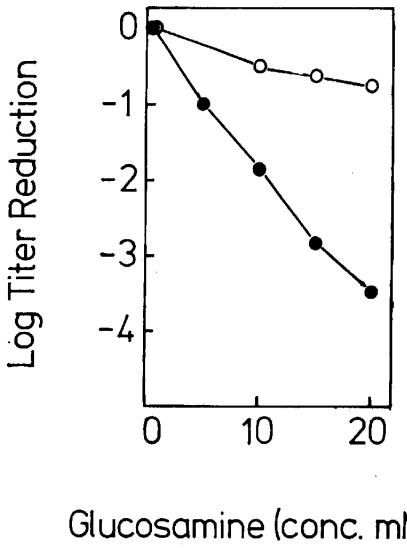

Fig. 1

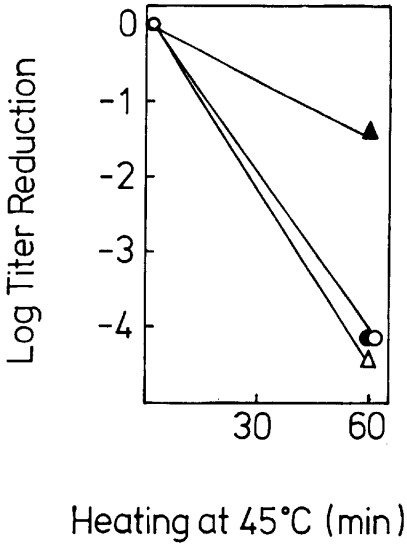

Fig. 2

Fig. 1. Effect of glucosamine on the production of ASV B77 and VSV *tl*-17. Glucosamine was added to normal CEC inoculated with VSV *tl*-17 and to ASV B77-transformed CEC. About 18 h later, the titers of VSV *tl*-17 and ASV B77 were determined.

○—○ VSV *tl*-17

●—● ASV B77

Fig. 2. Thermal inactivation kinetics of VSV *tl*-17 propagated in normal and ASV B77-transformed CEC in the presence and absence of 20 mM glucosamine. Virus fluids were diluted to 1 : 10 with MEM (pH 7.6), heated at 45°C for 60 min and titrated.

○—● VSV *tl*-17 harvested from normal CEC without glucosamine.

○—○ VSV *tl*-17 harvested from normal CEC with 20 mM glucosamine.

○—▲ VSV *tl*-17 harvested from ASV B77-transformed GEC without glucosamine.

○—△ VSV *tl*-17 harvested from ASV B77-transformed CEC with 20 mM glucosaminc.

TABLE 1. EFFECT OF GLUCOSAMINE ON THE PRODUCTION OF HEAT-STABLE VSV TL-17[a]

Glucosamine concentration (mM)	Log titer reduction[b]
0	1.5
5	2.9
10	3.2
15	3.5
20	4.3

a VSV *tl*-17 was inoculated on to ASV B77-transformed CEC in the presence of various concentrations of glucosamine, and the harvests were heated at 45°C for 60 min.

b The log titer reduction in PFU after heating was listed.

DISCUSSION

It has been reported that glucosamine inhibits the replication of ASV by blocking the synthesis of viral glycoproteins, whereas VSV has a different pathway for synthesis of viral glycoprotein and its replication is only weakly inhibited by glucosamine (5–10). In the present study also, 20 mM glucosamine decreased the replication of ASV B77 from 10^6 FFU to 10^2–10^3 FFU/ml, but had little effect on the replication of VSV *tl*-17.

When ASV B77-transformed CEC were infected with heat-labile VSV *tl*-17, VSV harvested was heat-resistant, indicating that the virions of VSV *tl*-17 produced were phenotypically mixed with ASV B77. In the same experiment done in the presence of 20 mM glucosamine, however, only heat-labile VSV was harvested, indicating that phenotypic mixing between VSV *tl*-17 and ASV B77 was only weakly induced, or not induced at all. As described above, 20 mM glucosamine showed little effect on the replication of VSV *tl*-17, but decreased the production of ASV B77 to a level of 10^2–10^3 FFU/ml. Therefore, it is suggested that, in cells producing a small amount of ASV, VSV *tl*-17 dose not mix phenotypically with ASV.

Phenotypic mixing assay with VSV has been used recently for detection of the production of ASV or *chf* expressed on the surface of CEC (1–4, 11). However, the results of the present study suggest that this method is applicable only to cells producing a large amount of either retrovirus or its envelope glycoproteins.

Acknowledgment. The authers are very grateful to Dr. Y. Yabe for his encouragement and critical reading of the manuscript. This work was supported by a Grant-in-Aid for Cancer Research from the ministry of Education, Science and Culture of Japan.

REFERENCES

1. Zavada, J.: VSV pseudotype particles with the coat of avian myeloblastosis virus. *Nature (New Biol.)* **240**, 122–124, 1972.
2. Love, D. N. and Weiss, R. A.: Pseudotypes of vesicular stomatitis virus determined by exogenous and endogenous avian RNA tumor viruses. *Virology* **57**, 271–278, 1974.
3. Ogura, H. and Friis, R.: Further evidence for the existence of a viral envelope protein defect in the Bryan high titer strain of Rous sarcoma virus. *J. Virol.* **16**, 443–446, 1975.
4. Ogura, H. and Bauer, H.: Biological and electron microscopic studies on the phenotypic mixing of the thermolabile mutant of vesicular stomatitis virus, *tl*-17, with avian RNA tumor viruses. *Arch. Virol.* **52**, 233–242, 1976.
5. Hunter, E., Friis, R. and Vogt, P. K.: Inhibition of avian sarcoma virus replication by glucosamine. *Virology* **58**, 449–456, 1974.
6. Hayman, M. J., Hunter, E. and Vogt, P. K.: Inhibition of avian sarcoma virus replication by glucosamine: A specific effect on the synthesis and processing of viral proteins. *Virology* **71**, 402–411, 1976.
7. Lewandowski, L. J., Bolognesi, D. P., Smith, R. E. and Halpern, M. S.: Viral protein synthesis under conditions of glucosamine block in cells transformed by avian sarcoma

viruses. *Virology* **66**, 347-355, 1975.

8. Kaluza, G., Scholtissek, C. and Rott, R.: Inhibition of the multiplication of enveloped viruses by glucosamine and 2-deoxy-D-glucose. *J. Gen. Virol.* **14**, 251-259, 1972.
9. Klenk, D. H.: Viral envelopes and their relationship to cellular membranes. *Current Topics in Microbiol. Immunol.* **68**, 29-58, 1974.
10. Scholtissek, C., Rott, R. and Klenk, H. D.: Two different mechanisms of the inhibition of the multiplication of enveloped viruses by glucosamine. *Virology* **6**', 191-200, 1975.
11. Weiss, R. A. and Bennett, P. L. P.: Assembly of membrane glycoproteins studied by phenotypic mixing between mutants of vesicular stomatitis virus and retroviruses. *Virology* **100**, 252-274, 1980.