

THE INHIBITORY EFFECT OF CERTAIN ARBOVIRUSES ON SHOPE FIBROMA FORMATION

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Received July 16, 1968

Summary. — The inhibitory effect of certain arboviruses on the production and development of Shope fibroma in the skin of rabbits was studied. When either Western equine encephalitis (WEE) or Japanese encephalitis (JE) virus was mixed with Shope fibroma virus (SFV) and immediately injected into rabbits, there was complete inhibition of tumor formation. When the arboviruses were inoculated 2 to 4 days prior to, or after, SFV inoculation, there was again a marked, but lesser inhibitory effect. The latent period of tumor formation was significantly prolonged. Type 1 dengue virus exerted a somewhat similar influence, but its potency was far less than that of WEE or JE virus, there being only slight prolongation of the latent period of tumor formation. When heated or UV-irradiated WEE and JE viruses were used, a clear inhibitory effect was again seen; however, it was not so complete as with the active viruses. Some of the inoculated arbovirus was found to persist for certain periods of time in “normal” rabbit skin and fibroma tissues. Although no underlying mechanism for the observed phenomena has as yet been unequivocally demonstrated, it seems most probable that the arboviruses interfered with SFV. Virological and oncogenic implications of the data are discussed.

Introduction

Inhibition of viral tumors by non-oncogenic viruses has been repeatedly reported (Ginder and Friedewald, 1951; Strandström *et al.*, 1962; Shirodkar, 1965). It is thought that some of such phenomena are due to “oncolytic action” resulting from viral multiplication in tumor cells, and subsequent suppression of tumor growth (Sharpless *et al.*, 1950; Ginder and Friedewald, 1951; Moore, 1952; Bernstein and Sigel, 1955). On the other hand, “interference” phenomena between oncogenic and non-oncogenic viruses appear to have a relationship to the inhibition of tumors (Strandström *et al.*, 1962). As to which of the mechanisms may be responsible for such anti-tumor actions remains at present an unresolved problem.

The present paper is an account of experiments designed to investigate the inhibitory effect of certain group B arboviruses on Shope fibroma formation in rabbits.

Materials and Methods

Shope fibroma virus (SFV). OA strain (kindly supplied by Dr. C. E. Schwerdt of the Department of Medical Microbiology, Stanford University, U.S.A.) was used. Stock virus suspensions were produced in primary or secondary rabbit kidney (RK) cell cultures grown in YLH medium, consisting 0.1% yeast extract, 0.5% lactalbumin hydrolysate, and 15 to 20% inactivated bovine serum in Hanks' balanced salt solution (BSS), supplemented with 100 u/ml penicillin and 100 μ g/ml streptomycin. After incubation at 37° C for 4 or 5 days, post infection, the infected cells were subjected to freezing and thawing 3 times, followed by ultra-sonic irradiation at 10 kilocycles/sec for 2 minutes (Takehara and Schwerdt, 1967). Viral activities of the fluids were usually in the range 2×10^5 to 2×10^6 RID₅₀/ml (50% rabbit infectious dose per ml), as calculated by the method of Reed and Muench. The materials were stored at -20° C until use.

Arboviruses. The following were used: Western equine encephalitis (WEE) virus, Rockefeller Institute standard stock strain, of the 15th mouse brain passage; Japanese encephalitis (JE) virus, G1 strain, of about the 200th mouse brain passage; and type 1 dengue (D1) virus, Mochizuki strain, of the 170th mouse brain passage. A 10% infected brain homogenate in Hanks' BSS was centrifuged at 10,000 rev/min. for 15 minutes and the supernatant used as starting inoculum. The mouse-intracerebral titers usually ranged from 10^6 to 10^8 LD₅₀/0.02 ml.

Inactivated arboviruses were prepared by heating or by UV-irradiation: (i) Viral suspensions in thin glass ampoules or in small rubber-stoppered glass tubes, containing 10^7 LD₅₀/0.02 ml, were immersed in a water bath at 60° C for 30 minutes; or (ii) one ml samples of the suspensions (of 10^7 LD₅₀/0.02 ml) were placed in Petri dishes of 55 mm diameter, and then irradiated under a UV-germicidal lamp ("National" Type 15 lamp, Matsushita Electric Co., Japan) at a distance of 20 cm for 15 minutes, with slow uninterrupted agitations. It was confirmed that the mouse-

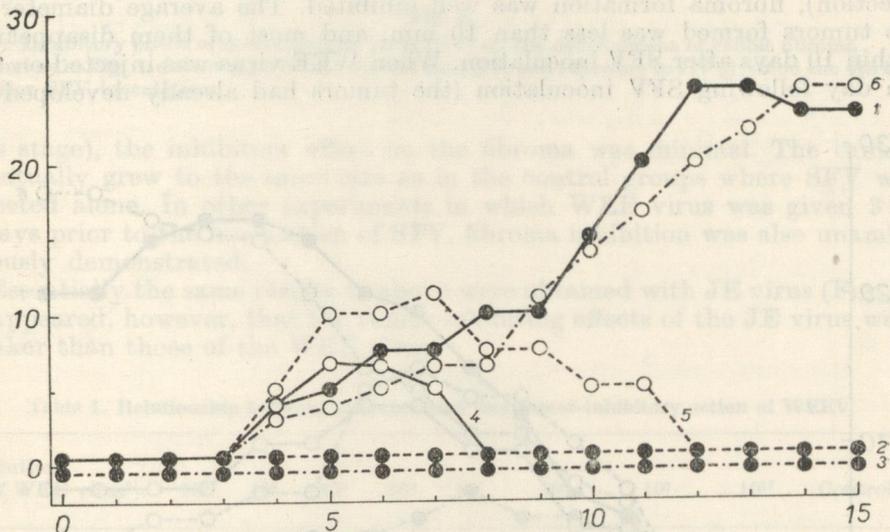


Fig. 1.

Inhibitory effect of active WEE virus on the development of rabbit fibroma

Ordinate: average diameter (mm) of tumors produced; abscissa: period in days after SFV inoculation.

Control: SFV alone injected intradermally (1); SFV and WEEV mixed and immediately injected (2); WEEV given on the 1st day (3), 2nd day (4), 4th day (5) and 5th day (6) after SFV inoculation.

Concentration of SFV: 10^6 RID₅₀/0.5 ml; WEEV: 10^5 LD₅₀/0.02 ml. All doses of SFV and WEEV: 0.25 ml.

infectivity of the viruses had been completely removed by such treatment. Two to 3-week old white mice, of an inbred strain, were used for the titration of arboviruses.

Inoculations. Domestic white rabbits, weighing from 1 to 2 kg, were used for inoculation of SFV and arboviruses. Prior to test, the fur was removed from the sides and back with electric scissors. Three different series of experiments were carried out: (i) one dose of 0.25 ml of each of the arboviruses, containing 10^5 LD₅₀/0.02 ml, was mixed with an equal volume of SFV (of 10^5 RID₅₀/0.5 ml) and the mixtures injected immediately into the skin of different rabbits; (ii) after a single inoculation of the above dose of SFV, one of each of the arboviruses, again of the same dose, was injected into different rabbits at the same sites and after various intervals; and (iii) after the inoculation of one of each of the arboviruses into different rabbits, SFV was injected at the same sites after various intervals. A similar series of tests was carried out with inactivated arboviruses. The inoculated rabbits were observed daily and the average diameter (in mm) of each of the resultant tumors measured.

Results

Inhibitory effect of active arboviruses on rabbit fibroma formation

The effects of WEE virus on the production and development of rabbit fibromas are illustrated in Fig. 1. When WEE virus was mixed with SFV and injected immediately into rabbits, the tumor-inhibitory effect was complete, no fibroma being produced. When WEE virus was given within 3 days after the inoculation of SFV (there was no tumor at this stage of the infection), fibroma formation was well inhibited. The average diameter of the tumors formed was less than 10 mm, and most of them disappeared within 10 days after SFV inoculation. When WEE virus was injected on the 5th day following SFV inoculation (the tumors had already developed at

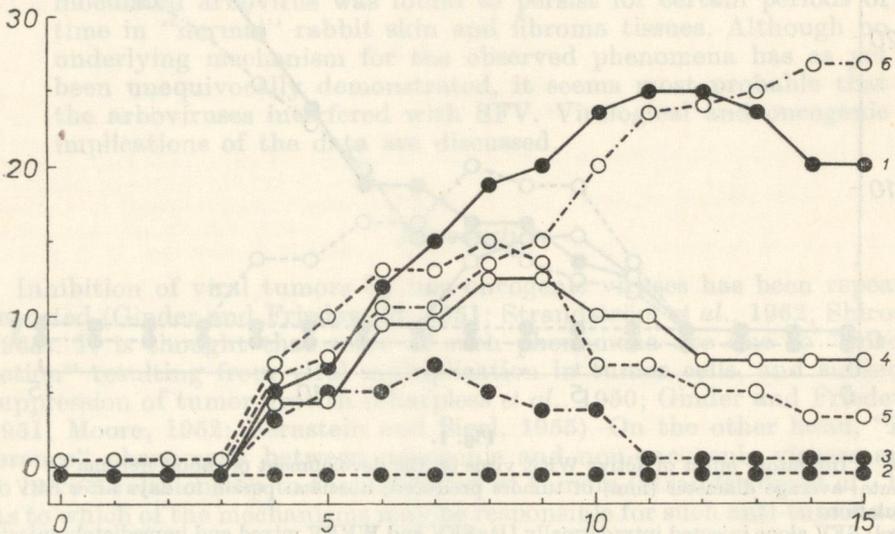


Fig. 2.

Inhibitory effect of active JEV virus on the development of rabbit fibroma

Legend as in Fig. 1, except that WEEV should read JEV.

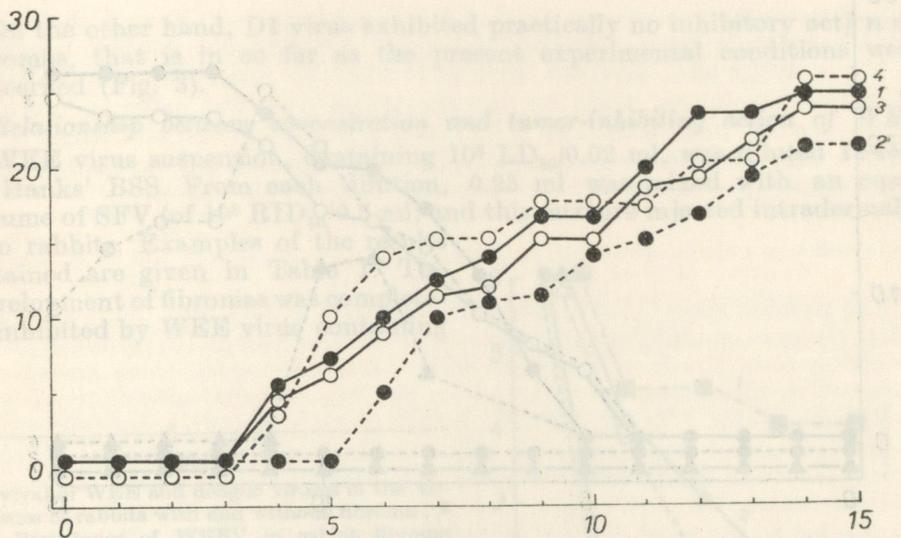


Fig. 3.

Inhibitory effect of active dengue virus (DV) on the development of rabbit fibroma. Legend as in Fig. 1, except that WEEV should read DV, and (4) refers to DV given on the 4th day after SFV inoculation.

this stage), the inhibitory effect on the fibroma was minimal. The tumors eventually grew to the same size as in the control groups where SFV was injected alone. In other experiments in which WEE virus was given 3 or 4 days prior to the inoculation of SFV, fibroma inhibition was also unambiguously demonstrated.

Essentially the same results as above were obtained with JE virus (Fig. 2). It appeared, however, that the tumor-inhibiting effects of the JE virus were weaker than those of the WEE virus.

Table 1. Relationship between concentration and tumor-inhibitory action of WEEV

Dilution of WEE virus ¹⁾ :	10^3	10^4	10^5	10^6	10^7	10^8	10^9	10^{10}	Control ²⁾
Rabbit fibroma formation ³⁾ :	-	-	+	++	++	+++	+++	+++	+++

1) Original virus titer: 10^8 LD₅₀/0.02 ml.

2) Control, SFV alone: 10^5 RID₅₀/0.5 ml.

3) Each dilution (0.25 ml) of WEEV was mixed with SFV in equal volumes and then injected into rabbits intradermally.

Average size of tumors produced was compared with that of the control on the 13th day after inoculation: - : no fibroma; + : 5-10 mm tumor; ++ : 10-20 mm tumor; +++ : 20-30 mm tumor.

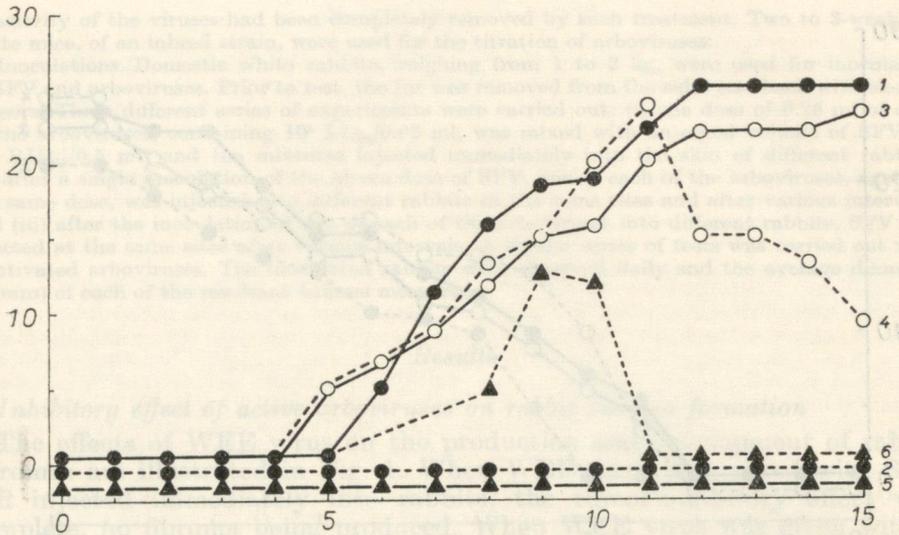


Fig. 4.

Inhibitory effect of heated and UV-irradiated WEE virus on the development of rabbit fibroma. Control; SFV alone injected intradermally (1); SFV and active WEEV mixed and immediately injected (2); heated WEEV (10^5 LD₅₀ before heating) given immediately (3) and on the 1st day after SFV inoculation (4); UV-irradiated WEEV (10^5 LD₅₀ before irradiation) given immediately (5) and on the 1st day after SFV inoculation (6). Other details as in Fig. 1.

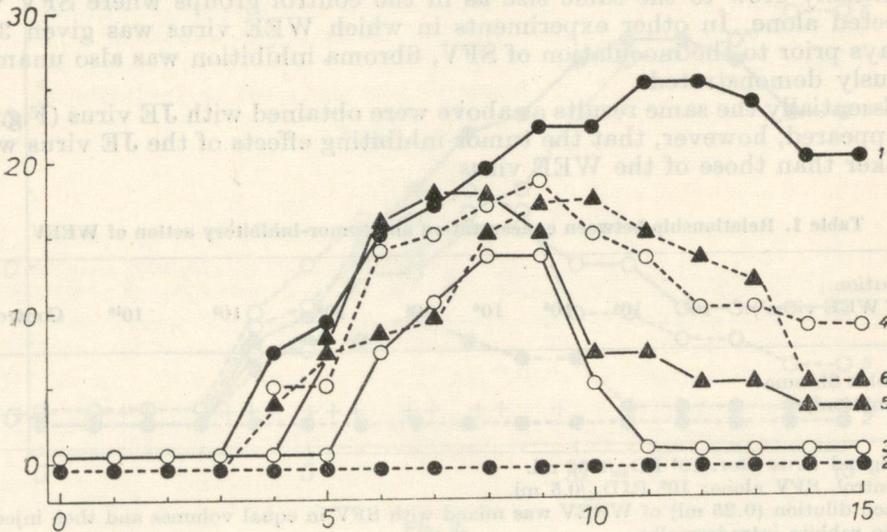


Fig. 5.

Inhibitory effect of heated and UV-irradiated JE virus on the development of rabbit fibroma. Legend as in Fig. 4, except that WEEV should read JEV.

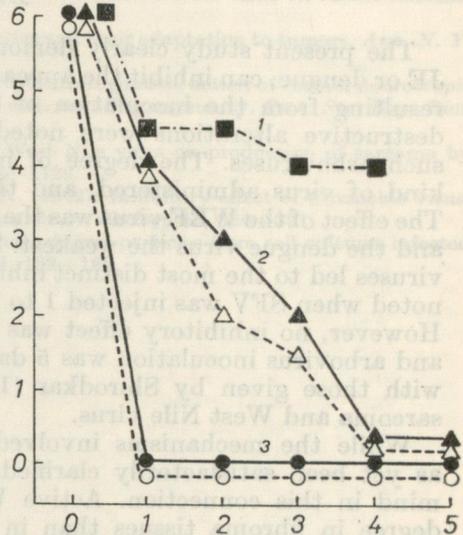
On the other hand, D1 virus exhibited practically no inhibitory action on fibromas, that is in so far as the present experimental conditions were concerned (Fig. 3).

Relationship between concentration and tumor-inhibiting action of WEE

WEE virus suspension, containing 10^8 LD₅₀/0.02 ml, was diluted 10-fold in Hanks' BSS. From each dilution, 0.25 ml was mixed with an equal volume of SFV (of 10^5 RID₅₀/0.5 ml) and this mixture injected intradermally into rabbits. Examples of the results obtained are given in Table 1. The development of fibromas was completely inhibited by WEE virus containing

Fig. 6.

Survival of WEE and dengue viruses in the tissues of rabbits with and without fibroma:
 1—Persistence of WEEV in rabbit fibroma tissue: virus inoculated into the tumor produced on the 5th day after SFV inoculation.
 2—Titers of WEEV in rabbit skin tissue: virus inoculated simultaneously with SFV (Δ), or without SFV (\blacktriangle).
 3—Titers of DV in rabbit skin tissue: virus inoculated simultaneously with SFV (\circ), or without SFV (\bullet).
 Abscissa: days after inoculation; ordinate: virus titers in log mouse LD₅₀/0.02 ml values.



more than 1.26×10^6 LD₅₀. Partial inhibition of tumor development was brought about with the addition to the SFV of WEE virus containing 1.25×10^3 to 1.25×10^5 LD₅₀.

Inhibitory effect of inactivated arboviruses on rabbit fibroma formation

Examples of the data obtained are given in Figs 4 and 5. Considerable inhibition of fibroma production and development was observed when heat- or UV-inactivated WEE or JE virus was injected either simultaneously or one day after inoculation of SFV. The effects, however, were apparently less marked than those of the active viruses. Moreover, a tendency was noted, in the case of WEE virus, for the effect of the UV-inactivated materials to be more marked than that of the heat-inactivated ones.

Survival of arboviruses in rabbit skin tissues

The following series of experiments was carried out: (i) Active arbovirus of a given infective dose was injected into each of several fibromas on the 5th day after SFV inoculation. Every day thereafter, each tumor was excised and its arbovirus content titrated. (ii) Arbovirus, either alone or mixed with SFV, was injected into several sites of the skin of rabbits. Every day after

injection, tissue pieces were excised from each injected site and their arbovirus contents measured. Typical examples of the results obtained are given in Fig. 6. WEE virus survived fairly well in fibroma tissues, at least for 4 days after the inoculation. A similar, but shorter survival was noted in "normal" rabbit tissues, both when the WEE virus was given alone and when it was mixed with SFV. However, active DI virus, inoculated under the same conditions, disappeared rapidly from the sites of inoculation.

Discussion

The present study clearly demonstrates that arboviruses, such as WEE, JE or dengue, can inhibit the appearance and development of rabbit fibromas resulting from the inoculation of SFV. At the same time, no necrotic or destructive alterations were noted in the tumor cells administered with such arboviruses. The degree of inhibition was different according to the kind of virus administered, and the procedure of administration adopted. The effect of the WEE virus was the strongest; that of the JE virus the second; and the dengue virus the weakest. Simultaneous injection of SFV and WEE viruses led to the most distinct inhibition of tumors. A similar effect was also noted when SFV was injected 1 to 4 days after the inoculation of arbovirus. However, no inhibitory effect was evident when the interval between SFV and arbovirus inoculation was 5 days or more. These results are compatible with those given by Shirodkar (1965) for the combination Rous chicken sarcoma and West Nile virus.

While the mechanisms involved in the observed phenomena have not as yet been satisfactorily clarified, the following data should be borne in mind in this connection. Active WEE virus was maintained to a higher degree in fibroma tissues than in "normal" rabbit skin. Further, dengue virus similarly inoculated disappeared within a much shorter period of time. It seems, therefore, that the tumor-inhibiting effect depends upon the survival or persistence of arbovirus at the inoculated sites. Moreover, heated or UV-irradiated WEE and JE viruses showed similar, though somewhat weaker, effects in comparison with those of the active viruses. These data suggest that the tumor-inhibiting action observed in the present study was due to "interference" between arbovirus and SFV, rather than to "oncolysis" by the arboviruses.

The question as to whether or not interferon or interferon-like substances (Baron and Levy, 1966) are produced in such cases is worthy of investigation. Furthermore, the possibility that the mechanisms of tumor inhibition by nononcogenic viruses may perhaps be different among different tumor-virus combinations must be considered. It has been reported that inhibition of Rous sarcoma by influenza virus is apparently due to interferon, while similar phenomena revealed in the case of Coxsackie virus might be of different origin, since the Coxsackie virus does not produce interferon(s) in certain experimental systems (Strandström *et al.*, 1962). Further studies, and especially those applying tissue culture techniques, are required to solve these and related problems.

Acknowledgments. We are deeply indebted to Dr. C. E. Schwerdt of Stanford University School of Medicine, for his kindness in supplying the fibroma virus strain tested. Thanks are also due to Mr. Andrew Smith for his help in preparing the manuscript.

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