In vivo Antiviral Activity of D-Glucosamine

Brief Report

By

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Summary

Intraperitoneal treatments with D-glucosamine, an inhibitor of the glycosylation of the viral envelope, decreased the growth rate of tumors induced in quails or in chicks by Rous sarcoma virus and increased the survival of mice inoculated with human influenza virus.

Several investigators have reported an inhibitory effect of D-glucosamine on the multiplication in cell cultures of a variety of enveloped animal viruses, including influenza (2, 5), Newcastle disease (9, 11), fowl plague (11), Sindbis and Semliki Forest (4, 11), vesicular stomatitis (11), respiratory syncytial (10), parainfluenza 3 (10) and avian sarcoma viruses (3, 6). It is commonly assumed that the inhibitory effect of glucosamine is the result of a selective interference with the glycosylation of the viral envelope polypeptides, causing an altered pattern of viral glycoprotein synthesis.

To our knowledge, no study has been devoted to the possible in vivo antiviral activity of D-glucosamine and we have therefore examined the effect of this inhibitor of glycosylation on the infection of quails and chicks with Rous sarcoma virus (RSV) and on the infection of mice with an A2 human influenza virus and with the Friend leukemia virus.

6- to 8-week old Coturnix coturnix quails, weighing between 150 and 200 g, were inoculated in the wing web with a dilution of the Bryan High Titer (RAV-1) strain of Rous sarcoma virus (VR-140, ATCC) causing discernible local tumor growth within 8 to 10 days and large, hemorrhagic and ruptured tumors within 15 to 18 days. Stock virus consisted of an homogenate of tumor tissue from leukosis-free chicks in which the strain had been propagated. D(+) -glucosamine hydrochloride (DG) (Sigma Chemical Co.) was dissolved in distilled water and injected intraperitoneally (i.p.) at various doses, once a day for 5 consecutive days,
beginning on the day of virus inoculation. 10 quails were used for each dose and in the control group. Beginning on the 8th day after virus inoculation tumors were scored individually every day according to diameter (1–2 mm = 2, 3–5 mm = 4, 6–8 mm = 6, 9–11 mm = 8, 12–14 mm = 10, ruptured = 12) and the sum of individual scores was determined for each group.

4- to 6-week old male CD-1 mice (Charles River France), weighing between 16 and 20 g and randomized as to weight were infected by aerosol with a dilution of mouse-adapted A2/Ann Arbor/2/62 influenza virus (infected lung tissue homogenate) causing 80 to 100 per cent mortality within 14 days in control animals.

DG was administered at various doses either as a single intranasal (i.n.) treatment immediately before infection or as 4 i.p. treatments (24 hours and 1 hour before and 24 and 48 hours after infection) or as combined (i.n. and i.p.) treatments. Each group consisted of 15 or 30 mice and mortality was determined daily for 14 days.

6- to 7-week old male BALB/c mice were inoculated intravenously with a dilution of the polycythemia-inducing strain of Friend leukemia virus (7) causing significant splenomegaly (3 to 4 times the average spleen weight of control uninoculated mice) within 10 days following inoculation. They were treated just before inoculation, and every day thereafter until the 8th day, with graded doses (1000, 300, 100 and 30 mg/kg i.p.) of DG. Each group consisted of 10 mice (20 for the inoculated controls). Animals were sacrificed on the 10th day following virus inoculation and average spleen weight was determined for each group.

1. Intraperitoneal treatments with DG of gSV-inoculated quails resulted in marked reduction in the tumor growth rate and this effect was observed over a wide range of doses, from 3 to 1000 mg/kg, without any clear cut dose-effect relationship. Although the extent of inhibition of tumor growth by DG as well as the rate of tumor growth in the control quails varied from one experiment to another, consistent inhibition at 10 and 30 mg/kg i.p. of glucosamine was seen in all 8 experiments performed (see Fig. 1). In several cases, while tumors continued to grow, became hemorrhagic and eventually ruptured in control quails within 15 to 18 days after virus inoculation, they remained stationary or even tended to regress in DG-treated birds. Up to 100 mg/kg i.p., glucosamine treatments did not alter the weight gain of the quails. Histological examination of the tumors 15 days after virus inoculation confirmed that the tumors were indeed smaller and more circumscribed in the DG-treated quails but did not indicate any difference, with respect to their microscopic appearance, from the tumors in the control animals: in both cases they were typical sarcomas.

It was still possible to obtain significant inhibition of tumor growth when DG treatment was started 2 days after virus inoculation and continued until the 4th day.

The activity of DG, under the same experimental conditions, was also demonstrated in quails inoculated with the non-defective Schmidt-Ruppin strain of RSV, the degree of inhibition of tumor growth being inversely proportional to the number of infectious units inoculated.

Finally, we tested the activity of DG in 8-day old Derco-109 chicks obtained from a non-leukosis-free flock and inoculated in the wing web with various dilutions of either the Bryan strain or the Schmidt-Ruppin strain: in both cases, 4 treatments at 30 mg/kg i.p. produced marked inhibition of tumor growth (see