

Glycyrrhizic acid inhibits virus growth and inactivates virus particles

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Screening investigations in antiviral action of plant extracts have revealed that a component of *Glycyrrhiza glabra* roots, found to be glycyrrhizic acid, is active against viruses. We report here that this drug inhibits growth and cytopathology of several unrelated DNA and RNA viruses, while not affecting cell activity and ability to replicate. In addition, glycyrrhizic acid inactivates herpes simplex virus particles irreversibly.

The effects of glycyrrhizic acid (ammonium salt, Fluka, Buchs) on growth of vaccinia, herpes simplex type 1, Newcastle disease, vesicular stomatitis (Indiana type) and polio type 1 viruses (all provided by NIH) in cultures of human aneuploid HEp2 cells (ATCC, Rockville) were studied. Twenty-four-hour-old cell monolayers (10^7 cells per sample) were infected with 5 infectious units per cell of each virus at 20 °C for 1 h, washed 3 times in Hank's balanced saline solution (BSS) and incubated at 37 °C for 18 h in Eagle's minimum essential medium (MEM) supplemented with 2% calf serum (pH 7.4). Infectious virus yield was determined by the Dulbecco and Vogt technique¹, slightly modified for vaccinia and herpes simplex type 1 viruses². Cytopathic effects were evidenced by light microscope observation of Giemsa-stained cells and by measuring spectrophotometrically at 530 nm the amount of neutral red incorporated by cell cultures ($100 \mu\text{g ml}^{-1}$, 1-h pulses in a drug-free medium) after solubilisation in 1% sodium deoxycholate in BSS (pH 7.4)³.

As shown in Table 1, addition of 8 mM glycyrrhizic acid to infected cell cultures soon after incubation at 37 °C completely inhibits both growth and cytopathic effects of vaccinia, herpes simplex type 1, Newcastle disease and vesicular stomatitis viruses. Protection of cell cultures from viral damage is so effective that, in spite of the total infection produced by the virus input used, differences can hardly be found at microscopic observation levels between drug-treated infected cells and uninfected controls. Later treatment (3 h post-infection) of cell cultures with 8 mM glycyrrhizic acid still suppresses virus replication and stops the progress of the cytopathic effect. Glycyrrhizic acid is also active on virus growth at 4 mM and at 2 mM, but not at 1 mM (data not shown). On the other hand, glycyrrhizic acid is without effect on poliovirus type 1.

Glycyrrhizic acid treatments that were inhibitory to virus growth were tolerated by cell cultures.

Uninfected cells incubated at 37 °C for 36 h in the presence of 8 mM glycyrrhizic acid remained unaltered both morphologically (evaluated as above) and in their ability to incorporate

Table 1 Effect of glycyrrhizic acid on virus growth and cytopathology

Virus strain input (5 i.u. per cell)	Virus growth and cell damage after 18 h at 37 °C in:								
	Drug-free medium			Medium supplemented with 8 mM glycyrrhizic acid:					
	i.u.	CPE	NR	Immediately after infection			3 h postinfection		
				i.u.	CPE	NR	i.u.	CPE	NR
Vaccinia	6.8×10^7	+++	45	2×10^4	-	99	3×10^4	+-	86
Herpes simplex type 1	3.4×10^7	+++	32	$<10^4$	-	97	$<10^4$	+-	84
Newcastle disease	1.3×10^7	+++	<5	6×10^4	-	102	9×10^4	+-	82
Vesicular stomatitis	1.2×10^8	+++	<5	6×10^4	-	96	1.3×10^5	+-	78
Polio type 1	7×10^7	+++	<5	4.6×10^7	+++	<5	5.2×10^7	+++	<5

i.u., Infectious units; CPE, cytopathic effect; +++, complete CPE, -, absence of CPE, evaluated microscopically; NR, neutral red uptake in % of untreated uninfected controls.

Table 2 Effects of glycyrrhizic acid on uninfected cell cultures

Glycyrrhizic acid in medium	¹⁴ C amino acid uptake* (c.p.m.)	No. of cells per ml of medium†
-	76.705 (74.534-80.212)	7.1×10^5 (6.2×10^5 - 7.9×10^5)
8 mM	73.952 (71.528-77.334)	5.2×10^5 (4.2×10^5 - 6.4×10^5)

Figures are mean values of six determinations. The range of values is given in parentheses.

* ¹⁴C amino acid uptake (0.3 μCi ml⁻¹, 1-h pulses) was measured after 35 h at 37°C in Eagle's MEM 2% serum.

† Initial inoculum, 2.5×10^5 cells ml⁻¹. Count made after 36 h at 37°C in Eagle's MEM 7% serum.

amino acids in acid-insoluble form*, as determined by 1-h pulses of ¹⁴C-protein hydrolysate (40 mCi mmol⁻¹, 0.3 μCi ml⁻¹, Amersham) (Table 2). Addition of 8 mM glycyrrhizic acid to 4-h-old cell cultures incubated in Eagle's MEM 7% calf serum had virtually no effect on cell growth. Thoma chamber counts of cells suspended by trypsin (0.25%, Difco) revealed a 28% decrease in cell growth in the respect of untreated controls, after 36 h at 37°C.

Taking into account that in antiviral tests glycyrrhizic acid was allowed to act on infected cultures for 18 h (the time required for growth of all viruses in untreated controls), these data indicate that the antiviral effect of this drug is not mediated by damaging the cells.

Apart from inhibiting growth of several viruses, glycyrrhizic acid also produces irreversible inactivation of herpes simplex virus type 1. Suspensions of this virus suffer a loss of infectivity of 10⁵ when incubated at 37°C with 8 mM glycyrrhizic acid for only 15 min. Similar treatments do not inactivate the other viruses (at least irreversibly), even if prolonged for 3h.

Table 3 Inactivating effect of glycyrrhizic acid on virus particles

Virus strain	Incubation mixture at 37°C	i.u. Evidenced*
Vaccinia	in MEM	5.3×10^7
	in 8 mM GA	5.6×10^7
Herpes simplex type 1	in MEM	1.2×10^7
	in 8 mM GA	1.3×10^2
Newcastle disease	in MEM	3.9×10^7
	in 8 mM GA	4.2×10^7
Vesicular stomatitis	in MEM	4.3×10^7
	in 8 mM GA	3.1×10^7
Polio type 1	in MEM	6.1×10^7
	in 8 mM GA	3.9×10^7

* By the Dulbecco and Vogt method¹ in incubation mixtures diluted in drug-free MEM.

5×10^7 i.u. of each virus was incubated for 180 min, except in the case of herpes simplex type I in 8 mM GA, which was for 15 min. GA, glycyrrhizic acid.

Data from current research had led us to hypothesise that glycyrrhizic acid interacts with sensitive virus proteins both at the virionic stage and later on, when these proteins are synthesised in host cells. This is, however, a working hypothesis that has yet to be verified experimentally.

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