Antiviral Research, 5 (1985) 335–343
Elsevier

AVR 00178

The inactivation of herpes simplex virus by some Solanaceae glycoalkaloids

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(Received 31 December 1984; accepted 15 March 1985)

Summary

The infectivity of herpes simplex virus Type I in tissue culture was inhibited by prior incubation with aqueous suspensions of glycoalkaloids in order of activity α-chaconine > α-tomatine > α-solasonine but not by the corresponding aglycones, solanidine, tomatidine and solasodine. However, inhibition was not only dependent on the presence of a sugar moiety since the glycone α-solanine was inactive under the conditions used.

The glycones, but not the aglycones, showed cytopathic effects on cellular membranes of Vero cells and erythrocytes; therefore, it is suggested that inactivation of virus results from insertion of the glycones into the viral envelope.

glycoalkaloids; antiviral activity; herpes simplex virus

Introduction

Steroidal alkaloid glycosides (glycoalkaloids) are found as constituents of many solanaceous plants including e.g., the nightshade family, the potato and the tomato, but are of largely unknown function. One suggested activity is conferring resistance to specific parasites such as the potato beetle [4]. Apart from fundamental structural chemical studies of a large number of these compounds [4] attention has been directed primarily towards toxicity, e.g., for humans and pigs in the greening of potatoes [6], teratogenicity [1] and antifungal and antibacterial activity [2]. We describe here the action of several of these substances and corresponding aglycones on the infectivity of herpes simplex virus Type I and demonstrate antiviral activities which may have both clinical and mechanistic interest.

Materials and Methods

Compounds

The glycones and aglycones tested were obtained from Sigma and Research Plus Laboratories, Denville, NJ, U.S.A. Structural formulae of the aglycones are shown in Fig. 1 and the compositions of the sugar moieties of the glycones in Table 1. (For further details of structural formulae see [4]). Stock aqueous suspensions 1% (w/v) for testing were prepared in normal saline and stored at -20°C.

Cell culture

Vero cell monolayers for use in plaque assay were cultured in medium 199 containing 5% fetal calf serum in 5-cm plastic petri dishes. HEp2 cells cultured as monolayers in Eagle's minimal essential medium containing 10% fetal calf serum were used for growth of virus.

Virus

Herpes simplex virus Type I (H.F.E.M. strain) was grown in cell cultures in maintenance medium (Eagle's MEM containing 1% fetal calf serum). The virus was harvested 28 h post-infection by discarding the supernatant medium and disrupting the cells, suspended in a small volume of maintenance medium, by three cycles of rapid freeze-thawing using liquid nitrogen. The suspension was centrifuged at 1000 × *g* for 30 minutes and aliquots of the supernatant stored at -70°C prior to use.

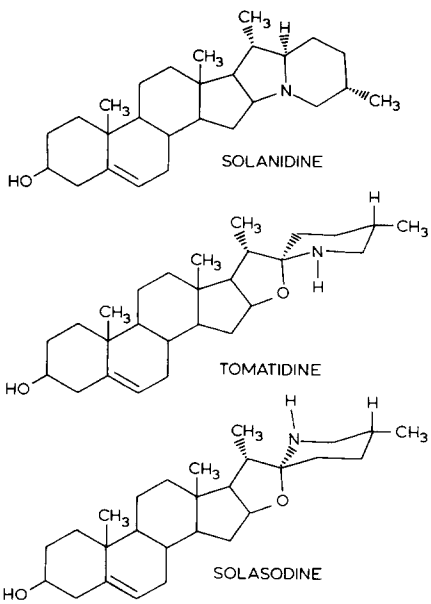

Fig. 1. Structural formulae of the aglycones, solanidine, tomatidine and solasodine.

TABLE 1

Glycoalkaloid	Aglycone	Sugars
α-Chaconine	Solanidine	-O-D-Glu < L-Rham, L-Rham
α-Solanine	Solanidine	-O-D-Gal < L-Rham, D-Glu
α-Solasonine	Solasodine	-O-D-Gal < L-Rham, D-Glu
α-Tomatine	Tomatidine	-O-D-Gal-D-Glu-D-Xyl, D-Glu

Plaque assay

The plaque assay method used (previously described [7]) employed a liquid overlay of medium 199 containing 1% fetal calf serum; plaques were counted at 48 h.

Assay for antiviral activity

90 µl of virus suspension ($\approx 10^6$ to 10^7 pfu) in maintenance medium were mixed with 10 µl of the compounds suspended in normal saline (final concentrations: 0.1, 0.01 and 0.001%, w/v) and incubated at 37°C for 1 h. Controls containing the same concentration of virus in 1 : 9 normal saline/maintenance medium lacking compounds were similarly treated. Serial 10-fold dilutions in medium 199 containing 1% fetal calf serum were assayed for virus on duplicate monolayers. The surviving fraction of infectivity of virus treated with compound was calculated as a fraction of the incubated control virus titre.

Electron microscopy

Untreated and treated virus suspensions were applied to Formvar-coated copper grids, negatively stained with 1% phosphotungstic acid and examined using a Philips Transmission Electron Microscope, Model EM301.

Results

Effects of compounds on virus infectivity

The results in Table 2 demonstrate significant inactivation of the virus by the glycones, α-chaconine at 0.1 and 0.01% (w/v) and by α-solasonine and α-tomatine at 0.1% (w/v). α-Chaconine was the most active and α-tomatine somewhat more active than α-solasonine. The corresponding aglycones solanidine, solasodine and tomatidine were inactive. These results would appear to suggest that the presence of sugar

TABLE 2

Effect of compounds on infectivity of herpes simplex Type I after 1 h at 37°C

	Concn. (%, w/v)	No. of assays	Assay infectivities (pfu/ml)			Mean surviving fraction (%)	Range (%)
Glycone							
α-Chaconine	0.1	3	$<1 \times 10^3$ (1.9×10^8)*	7.2×10^3 (9.0×10^6)	1.6×10^4 (1.4×10^8)	< 0.03	< 0.0005–0.08
	0.01	3	$<1 \times 10^5$ (3.8×10^7)	1.2×10^5 (9.0×10^6)	$<1 \times 10^4$ (6.0×10^5)	< 1	< 0.27–< 1.7
	0.001	2	1.9×10^7 (3.8×10^7)	6.5×10^6 (9.0×10^6)		60	49–72
α-Solanine	0.1	2	1.1×10^7 (3.8×10^7)	8.3×10^6 (9.0×10^6)		60	28–92
	0.01	2	1.8×10^7 (3.8×10^7)	6.5×10^6 (9.0×10^6)		60	48–72
	0.001	2	3.4×10^7 (3.8×10^7)	7.6×10^6 (9.0×10^6)		88	84–91
α-Solasonine	0.1	3	8.0×10^4 (6.4×10^5)	7.0×10^4 (1.6×10^4)	4.0×10^4 (6.0×10^5)	8	5–13
	0.01	3	4.2×10^5 (6.4×10^5)	7.0×10^5 (1.6×10^6)	7.0×10^4 (6.0×10^5)	40	12–65
	0.001	2	4.7×10^5 (6.4×10^5)	1.5×10^6 (1.6×10^6)		83	73–93

α-Tomatine	0.1	3	$<1 \times 10^4$ (3.0×10^7)	$<1 \times 10^4$ (1.3×10^8)	2.1×10^5 (1.9×10^8)		<0.05	<0.008–0.11
	0.01	2	1.2×10^7 (3.0×10^7)	7.9×10^5 (1.2×10^6)			54	40–68
	0.001	1	2.9×10^7 (3.0×10^7)				97	
Aglycone								
Solanidine	0.1	4	8.5×10^5 (6.4×10^5)	1.4×10^6 (1.6×10^6)	5.5×10^5 (6.0×10^6)	1.4×10^8 (1.9×10^8)	98	76–132
	0.01	3	3.5×10^5 (6.4×10^5)	1.5×10^6 (1.6×10^6)	9.5×10^5 (6.0×10^5)		103	55–160
	0.001	2	6.0×10^5 (6.4×10^5)	1.0×10^6 (1.6×10^6)			81	66–94
Solasodine	0.1	1	6.9×10^5 (6.4×10^5)				108	
	0.01	1	7.0×10^5 (6.4×10^5)				109	
	0.001	1	6.3×10^5 (6.4×10^5)				98	
Tomatidine	0.1	4	3.2×10^7 (3.0×10^7)	1.2×10^8 (1.3×10^8)	4.5×10^8 (5.1×10^8)	5.5×10^7 (5.8×10^7)	95	88–107
	0.01	2	3.3×10^7 (3.0×10^7)	1.1×10^8 (1.3×10^8)			100	90–110
	0.001	1	3.3×10^7 (3.0×10^7)				110	

The mean drop in infectivity of saline controls in 1 h at 37°C compared to unincubated virus from 14 determinations was 0.29 log units, range 0–0.82.
*Incubated saline control titres in parentheses.

residues was of prime importance in determining the antiviral activity. However, α-solanine which has the attached sugar residues —Gal$<^{Rham}_{Glu}$ was found inactive whilst α-solasonine which has the same sugar component but differs in its sterol was weakly active.

The kinetics of inactivation by α-chaconine at 0.01% (w/v) at 37°C are shown in Fig. 2; a uniform fall in infectivity of ≈2.5 log units in 1–1.5 h was followed by a slower subsequent decline. Similar results were obtained with 0.1% (w/v) tomatine.

In aqueous media the compounds were only sparingly soluble, however, centrifugation of the suspensions of the active glycones at 10000 × *g* for 30 min and assay of supernatant and pellet activities showed substantial activity in dispersed and micellar forms present in the supernatant (Table 3).

The solubility of the glycones and aglycones was much greater in dimethyl sulphoxide (DMSO) than in salt solutions but DMSO alone at concentrations of 10% (w/v) inactivated virus and at the possible concentrations tested (0.1% and 1.0%) no substantial increases in activity of the glycones were observed (Table 4).

Effect of saccharides

None of the monosaccharides galactose, glucose or xylose present in α-tomatine, added first at a concentration of 10 mM to the virus suspension, inhibited the action of α-tomatine on virus infectivity, neither did the disaccharides lactose, maltose, trehalose or the trisaccharide fructose similarly applied. Since none of these simulates the structure of the sugar moiety this is perhaps not surprising. Prior incubation with any of the saccharides at 10 mM before addition of virus did not confer activity on tomatidine.

Cytotoxicity

All of the glycones, but not the aglycones, at concentrations of 0.01% (w/v) or

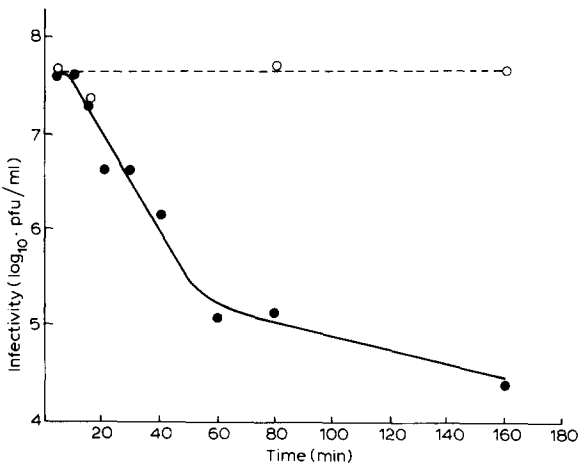

Fig. 2. Kinetics of inactivation of herpes simplex virus by α-chaconine at 37°C (●——●, α-chaconine 0.01% (w/v); o---o, normal saline).

TABLE 3

Effect of centrifugation on activity of suspensions of compounds at 37°C

	Concn. (% w/v)	Surviving fraction after 1 h (%)		
		Whole suspension	Supernatant	Resuspended pellet
α-Solasonine	0.1	(4.0×10^4)*[a] 7 (6.0×10^5) [b]	$(< 1 \times 10^4)$ < 1.5 (6.0×10^5)	ND
α-Tomatine	0.1	$(< 1 \times 10^6)$ < 1 (4.6×10^8)	(4.0×10^6) 1 (4.6×10^8)	$(< 1 \times 10^6)$ < 1 (4.6×10^8)
Tomatidine	0.1	(4.9×10^8) 105 (4.6×10^8)	(4.8×10^8) 105 (4.6×10^8)	(3.4×10^8) 75 (4.6×10^8)

*Assay infectivities (pfu/ml): [a] compound; [b] incubated saline control.
ND = not done.

TABLE 4

Effect of dimethyl sulphoxide (DMSO) on activity of compounds at 37°C

	Concn. (% w/v)	Surviving fraction after 1 h (%): DMSO concn. (%, w/v)		
		0	0.1	1.0
α-Chaconine	0.01	$(< 1 \times 10^4)$*[a] < 1 (1.6×10^6) [b]	$(< 1 \times 10^4)$ < 1 (1.6×10^6)	$(< 1 \times 10^4)$ < 1 (1.6×10^6)
α-Tomatine	0.1	(3.9×10^5) < 1 (1.9×10^8)	ND	(4.1×10^5) < 1 (1.9×10^8)
α-Tomatine	0.01	(1.3×10^8) 70 (1.9×10^8)	(6.0×10^7) 30 (1.9×10^8)	(1.4×10^8) 75 (1.9×10^8)
Tomatidine	0.1	(1.3×10^8) 70 (1.9×10^8)	ND	(1.8×10^8) 100 (1.9×10^8)

See footnote Table 3.

greater, were found to be cytotoxic when incubated in maintenance medium for 20 min at 37°C with Vero cell culture monolayers as used for plaque assay. α-Tomatine was more fully investigated, using suspensions of Vero cells in medium 199 containing 5% fetal calf serum and trypan blue staining to assess cell viability. Concentrations of

0.00125%, 0.0025% and 0.025% in suspensions containing 5×10^5 cells/ml incubated for 20 min at 37°C resulted in 25, 50 and 100% trypan blue-stained cells, respectively, with obvious cytopathic effects, such as crenation of the plasma membrane and condensation and vacuolisation of the cytoplasm, whereas tomatidine had no effects at concentrations up to at least 0.1% (w/v). Sheep erythrocytes at 0.5% (v/v) in normal saline were lysed by concentrations of α-tomatine in excess of 0.001% (w/v) after 30 min at 20°C but were unaffected by tomatidine at concentrations up to at least 0.1% (w/v).

The concentrations of the compounds present during the plaque assay were at least 100 times lower than the observed cytotoxic concentrations during the 1-h adsorption stage ($<0.0001\%$ w/v) and a further factor of 50 lower subsequently.

Discussion

The principal result obtained in this work demonstrating the inactivity of aglycones compared to glycones favours the importance of the role of sugar residues in the inactivation of herpes virus by glycoalkaloids. However, the full significance of the roles of the hydrophilic sugar moiety and the planar hydrophobic steroidal component remains to be investigated. It is of interest in this context that the cardiotonic activity of a range of glycoalkaloids was found to be dependent on the number of sugar residues as well as on the steroidal structure [3]. The inactivity of the glycone α-solanine against virus may result from presently unknown effects of secondary properties.

The mechanism of the loss of infectivity is unknown, however, the membrane-directed cytopathic effects of the glycoalkaloids and the reported use of α-tomatine as a cholesterol probe [5] would suggest that virus inactivation results from interaction of the glycone with the viral envelope presumably by insertion of the steroidal moiety. It may be that the sugar component modifies the interaction of the virus with cell surface viral receptors or has a more direct effect on the properties of the viral envelope. In preliminary electron-microscopic observations of virus suspensions treated with 0.1% (w/v) α-tomatine or tomatidine for 1 h at 37°C viral envelopes appeared normally associated with capsid.

From general considerations, it can be anticipated that the results obtained with herpes simplex virus Type I would be duplicated with other membranous viruses and in accordance with this we have shown (Thorne, H.V. and Clarke, G.F., unpublished results) that under the same conditions 0.1% (w/v) α-chaconine inactivated measles virus and, interestingly, 0.1% (w/v) α-solanine was again inactive.

Although the antiviral activities reported are probably too weak to warrant immediate in vivo experiments e.g., in virus-induced skin infections of laboratory animals, the suggested specific action of the sugar moiety would seem to warrant a more extensive examination of a wider range of glycoalkaloids, or possibly synthetic modification of the sugar composition, to produce enhanced antiviral activity and/or a greater differential between antiviral and anticellular activity. Such an investigation might profitably be coupled with one in which specific reactions of the compounds with cellular surface components were studied for possible therapeutic and immunological applications in other fields.

Acknowledgements

We acknowledge support of the Multiple Sclerosis Society of Great Britain and Northern Ireland in some of this work and thank Mr. Garth Fenning for technical assistance.

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