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Antiviral properties of extract of opuntia streptacantha

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Abstract

An extract of the cactus plant Opuntia streptacantha inhibited intracellular virus replication and inactivated extracellular virus. Inhibition of virus replication also occurred following pre-infection treatment — a favourable finding in terms of in-vivo limitation of virus disease. There was inhibition of both DNA and RNA virus replication, for example, herpes simplex virus, equine herpes virus, pseudorabies virus, influenza virus, respiratory syncytial virus and human immunodeficiency virus, with normal protein synthesis in uninfected cells at extract concentrations which were 15-fold in excess of 50% viral inhibitory concentrations (1 mg/ml). The active inhibitory component(s) of the extract appeared to be protein in nature and resided mainly in the wall of the plant rather than in the cuticle or inner sap. The extract was non-toxic on oral administration to mice, horses and human patients; the non-toxicity of intravenous administration of 70 mg to a mouse representing at least fifty tissue culture 50% viral inhibitory dosages encourages clinical trial of this extract in virus disease of human and veterinary species.

1. Introduction

It is well known that plants have been used in medicine for thousands of years. Of particular relevance to this study, an extract of the cactus plant, Opuntia streptacantha, has been used for some years in Mexico as an oral hypoglycaemic agent in the control of mild diabetes. A number of plants support virus growth without detriment or even with benefit to the plant; alternatively, virus infection can result in pathological damage or death to the plant (Delay, 1969; Koenig, 1972; Boiko et al., 1972; Nelson and Tremaine, 1975).

Certain plant extracts will inhibit virus replication and will inactivate extracellular viruses. As examples, inhibition of replication of herpes simplex virus type 1 by Geranium sanguineum L.VIII, influenza and human immunodeficiency virus by pine cone antitumour substance, murine cytomegalovirus by Chlorella vulgaris and poliovirus and herpes simplex virus type 1 by Ulex europaeus have been reported (Zgorniak-Nowosielska et al., 1989; Nagata et al., 1990; Ibusuki and Minamishima, 1990; De Rodriguez et al., 1990; Sakagami et al., 1991). In addition, a number of plant substances will neutralise virus infectivity; lectins, for example, Concanavalin A from Conovalia ensiformis, will neutralise herpes simplex virus, cytomegalovirus, Epstein-Barr virus and human immunodeficiency disease virus

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(Ito and Barron, 1974; Ito et al., 1978; Khelifa and Menezes, 1982; Lifson et al., 1986).

In 1990, a patient with mild diabetes reported cessation of frequent recurrences of herpes genitalis following self-medication with 2 g per day of extract of Opuntia streptacantha. The report motivated further exploration of the antiviral properties of the cactus plant. This initial study has examined inhibition of replication of DNA and RNA viruses by extracts of Opuntia streptacantha, the nature of the active components and preliminary evidence of safety in human and non-human species.

2. Materials and methods

2.1. Preparation of plant extract

Dried and powdered plant leaves from the cactus plant Opuntia streptacantha supplied by J. C. Alanis, supplier of herbal products within Mexico, Calle Condor 264, Irapuato, Mexico, were suspended in sterile water or Eagles minimum essential medium, Glasgow modification, supplemented with 10% tryptose phosphate broth and 10% newborn calf serum (ETC) at 60mg/ml and perturbated at 37°C for 1 h. Undissolved debris was discarded after centrifugation at 800 g for 15 min and the supernatant filtered first through a 1.2 μm pore-size glassfibre disc (Acrodisc, Gelman) followed by a 0.45 μm pore-size nitrocellulose filter (Minisart). The resulting solution, which contained the active constituents, was used immediately or within 24 h if stored at 4°C.

Dialysis was carried out using cellulose visking dialysis tubing-8/32 (The Scientific Instrument Centre Ltd, Eastleigh, Hampshire, UK) which had been boiled for 10 minutes in 0.001M ethylenediaminetetraacetic acid (EDTA) and was continued for 48 h at 4°C against phosphate buffered saline (PBS) with at least six changes of the buffer solution.

The effect of enzymes on efficacy was examined by incubating the plant extract for 2 h at 37°C with pronase (0.5 mg/ml) or for 10 min at 37°C with trypsin (120 mg/ml). Extracts were also mock incubated with PBS under identical conditions for control purposes. The effect of solvents was examined by treatment of the cactus extract for 15 min at room temperature with chloroform (5:3 v/v) or ether (5:3 v/v) and by precipitation with acetone at −20°C (10:1 v/v) followed by centrifugation (800 g).

2.2. Preparation of extract from different sections of the plant

It was important to exclude the possibility that the harvesting or subsequent processing of plants might have introduced substances with viral inhibitory activity. Thirty grams of fresh plant which had been transported from Mexico to the United Kingdom were suspended in 10 ml of sterile water, crushed and incubated at 25°C for 3 h. The liquid portion was withdrawn and subjected to water bath ultrasonication (Schuco International London Ltd, UK) for 4 min; this constituted the stock solution. The different parts of the plant, namely cuticle, wall and inner sap, were extracted in a similar manner. It should be noted that their concentrations were not equivalent to those of extracts of dried powder due to the water content of fresh plants.

2.3. Cells

Baby hamster kidney cells (BHK-21) (MacPherson and Stoker, 1962) were used for maintenance and assay of herpes simplex virus type 2 (HSV-2), pseudorabies virus (PRV), equine herpes virus type 1 (EHV-1), bovine mammilitis virus (BMV), influenza A and encephalomyocarditis virus (EMC). Human embryo lung cells (MRC-5) were used for cytomegalovirus (CMV) and varicella-zoster virus (VZV), HEp-2 cells for respiratory syncytial virus (RSV) and MT-4 cells for human immunodeficiency virus type 1 (HIV-1). Cell lines were maintained in Eagles minimum essential medium (Glasgow modification), supplemented with 10% tryptose phosphate broth and 10% newborn calf serum (ETC) for BHK-21 and HEp-2 cells or 10% foetal calf serum (ETF) for MRC-5 cells. MT-4 cells were maintained in RPM1 1640 medium supplemented with 10% foetal calf serum.

2.4. Viruses

2.4.1. DNA viruses

Strain 3345 was a prototype herpes simplex type 2 virus (HSV-2) isolated from a patient with penile herpes (Skinner et al., 1976a), strain AD169 was a classical laboratory adapted strain of cytomegalovirus (CMV) isolated from the adenoid of a young girl (Rowe et al., 1956), strain JH was a clinical varicella-zoster virus (VZV) isolate from a 30-year-old male patient and the Dekking strain was chosen as prototype for pseudorabies virus (PRV). Strain RAC-H of equine herpes virus type 1 (EHV-1) was obtained from Dr R Killington, Department of Microbiology, University of Leeds, UK, and strain Allerton was a laboratory strain of bovine mammalitis virus (BMV) from Professor D H Watson, University of Leeds, UK. The HSV-2 and pseudorabies were plaque purified and the other viral strains were not plaque purified following receipt in our laboratory.

2.4.2. RNA viruses

A laboratory strain (NWS) of influenza A virus which 'plaques' in BHK-21 cells, an isolate of respiratory syncytial virus (RSV) from a child with respiratory infection, and long established laboratory strains of encephalomyocarditis virus (EMC) and human immunodeficiency virus type 1 (HIV-1) strain III_B (Wain-Hobson et al., 1991) were used in the study. Influenza A virus was plaque purified but no information is available on the other viruses.

2.4.3. Virus assays

HSV-2, PRV, EHV-1, BMV and Influenza A were assayed by suspension plaque assay (Russell, 1962). Briefly, 2 ml of ten-fold serial dilutions of virus or virus infected cells were incubated in suspension with BHK-21 cells in ETC for 45 min at 37°C with shaking. Eagles medium containing 10% newborn calf serum and 0.6% carboxymethyl cellulose (CMC) was added and the dilutions plated out in 60 mm petri dishes at 37°C for 48 h before fixation with 10% formal saline. Plates were stained with carbol fuschin and plaques counted. Virus titre (plaque forming unit/ml) was determined from plaque counts from plates having 30–100 plaques per plate (i.e. per 2 ml of known dilution). EMC was assayed by monolayer assay using a 0.5% agarose overlay; virus dilutions as described were adsorbed to BHK-21 cell monolayers in 60 mm petri dishes for 1 h at 37°C before addition of the agarose overlay (ETC containing 0.5% agarose). CMV, VZV and RSV were similarly assayed on cell monolayers (MRC-5 for CMV and VZV and HEp-2 for RSV) but without CMC or agarose overlay. HIV-1 was assayed by syncytium formation in MT-4 cells in suspension (Baba et al., 1990).

2.4.4. Inhibition of virus replication in cells

Except for HIV-1 where cells were in suspension, monolayers of appropriate cells were infected by adsorption of virus at a multiplicity of infection (MOI) of 1 plaque forming unit per cell (PFU/cell) for 1 h at 37°C, followed by removal of input virus and three washes in warm medium. A sample monolayer was stored at −70°C to provide a measure of residual input. Varying concentrations of extract in incubation medium were added immediately following adsorption, or 4 h after adsorption for HIV-1, to infected and uninfected cells (the latter for cytotoxicity controls). Incubation for 24 h at 37°C for HSV-2, PRV, EHV-1, BMV and Influenza A or 3–4 days for CMV, VZV and RSV and 5 days for HIV-1 was followed by removal of extract-containing medium and storage of cells at −70°C in sterile water for subsequent virus assay as described above ('Virus assay'). In the case of VZV, because of the highly cell-associated nature of the virus, cells were removed by washing with PBS and incubation with 0.02% versene for 5 min at 37°C and assayed immediately as described above. Prior to assay, all samples except for VZV were subjected to waterbath, ultrasonication for 1 min in order to disrupt infected cells. VZV samples were ultrasonicated for 10 s only, to achieve separation of cells without cell destruction.

Pre-treatment of cells was effected by pre-incubating cells in appropriate dilutions of extract in medium for 24 h at 37°C. The extract was then removed and cells washed twice before infection with virus and subsequent incubation in appropriate medium without plant extract.

2.4.5. Inhibition of protein synthesis

Monolayers of BHK-21 cells were infected with HSV-2 at a MOI of 10 PFU/cell, and incubated for 1 h at 37°C. After removal of virus input and washing, methionine-free medium containing varying concentrations of plant extract and 25 μCi ^{35}S labelled methionine (Amersham, UK) was applied to virus-infected monolayers and uninfected controls. After incubation for 24 h at 37°C, monolayers were washed twice in PBS, boiled in disruption mix (0.05m TRIS-HCl pH7 containing 2% sodium-dodecyl sulphate, 5% mercaptoethanol and 3% sucrose) and equivalent loadings in terms of cell numbers were subjected to polyacrylamide gel electrophoresis using 10% gel based on the method of Laemmli (1970). The ^{35}S methionine incorporation was visualised by autoradiography using Kodak X-OMAT S-film.

2.4.6. Virus neutralisation assay

These were carried out using the general method of Skinner et al. (1976b). Varying concentrations of extract or control medium alone were incubated with approximately 5 x 10^5 PFU of HSV-2, PRV or EHV-1 at room temperature and at 37°C for 1 h and 24 h after which residual virus was assayed as described above ('Virus assays'). Reaction mixture was assayed at time zero to provide a measure of initial virus titre.

2.4.7. Inhibition of virus replication in human cervix

Explants of normal human cervix were infected with HSV-2 as previously described (Cowan, 1984; Birch et al., 1976) and placed in organ culture for 2 days in ETC medium containing 2 mg/ml of plant extract or ETC alone. Explants were harvested into 0.5 ml sterile water, frozen at −70°C, then thawed and subjected to water bath ultrasonication for 1 min. The supernatant fractions were then assayed for infectious virus as described above ('Virus assays'). A measure of residual input virus was obtained by assay of one explant immediately after virus infection.

2.5. Toxicity studies

Adult CBA mice (Harlan UK Ltd, Blackthorn, Bicester, Oxon, UK) of average weight 30 g were inoculated with 370 mg cactus extract in 0.5 ml water by the intraperitoneal and subcutaneous route or 70 mg cactus extract in 0.1 ml water by the intravenous route under fluothane anaesthesia. Adult Balb/c mice (Harlan UK Ltd, Blackthorn, Bicester, Oxon, UK) of average weight 21 g were given cactus extract at a concentration of 120 mg/ml in their drinking water. The weight of the mice was recorded daily and the following clinical signs recorded at least twice daily; the rectal temperature, diarrhoea, slit eyes, ruffled fur, hunching and reduced mobility (Soothill et al., 1992).

Fifteen horses in three different stables were given a daily dose of 27 g cactus extract mixed with the horse feed and were monitored at daily intervals by experienced horse veterinary specialists for clinical signs: urine and blood samples were taken and analysed.

Human patients were given 6 g per day (2 g thrice daily) for 1 month or 3 g per day (1 g thrice daily) for 6 months by the oral route in the form of capsules containing 300 mg of extract each.

3. Results

3.1. Inhibition of virus replication

Virus-infected cells were incubated with varying concentrations of extract in the incubation medium. Replication of HSV-2 was reduced by over 2 $\log_{10}$ at a concentration of 3.5 mg/ml and to input levels (where there is no virus replication) at 15 mg/ml of extract. There was significant inhibition of replication of other herpes viruses, namely, PRV, BMV and EHV-1, and of the more cell-associated human herpesviruses CMV and VZV although to lower levels (Table 1). The replication of the RNA viruses, influenza A, RSV and HIV-1, was reduced although this was more striking following pre-infection incubation of cells in extract-containing medium (Table 1). The replication of the picornavirus EMC was unaffected by extract concentration as high as 30 mg/ml.

The efficacy of extract in virus-infected human tissue was explored by measuring replication of HSV-2 in explants of human cervix in organ culture. Virus replication was inhibited by 3.5 $\log_{10}$ following addition of 15 mg/ml of extract to the incubation medium.

Inhibition of virus replication by incubation of cells with extract prior to infection was examined using varying concentrations of extract.

3.1.1. Type 2 herpes simplex virus; 24-h pre-infection treatment

There was a significant reduction in replication of intracellular virus which correlated with increased concentrations of extract in the medium; reduction in virus titres ranged from 3.6 $\log_{10}$ at 15 mg/ml to 1.3 $\log_{10}$ at 1.8 mg/ml. Extracellular virus in the supernatant medium was also reduced by approximately 1.6 $\log_{10}$, irrespective of extract concentration.

Table 1
Inhibition of replication of DNA and RNA viruses with 3.5mg/ml of cactus extract

DNA viruses	$\log_{10}$ reduction in virus titre Treatment of cells	
	Pre-infection[2]	Post-infection[2]
Herpes viruses		
Herpes simplex type 2	2.6	2.3
Cytomegalovirus	0.5	0.9
Varicella-zoster	0.3	1.3
Pseudorabies	0.6	0.5
Equine herpesvirus I	NT[1]	1.1
Bovine mammilitis	1.4	1.3
RNA viruses		
Influenza A	2.3	0.1
Respiratory syncytial disease virus	NT[1]	1.0
HIV-1	2.3	0.9
Encephalomycarditis virus	0	0

[1]NT not tested.
[2]Extract was added at 24 h and removed immediately prior to addition of virus (pre-infection) or added following absorption of virus to the cells for appropriate times of incubation as indicated in Methods.
These data were based on six independent testings for HSV-2, two for the remaining viruses except RSV and HIV-1 which were only tested on one occasion.

3.1.2. Type 2 herpes simplex virus, influenza A virus; 48-h pre-infection treatment

Following 48-h pre-infection treatment of cells in extract-containing medium, there were similar reductions in replication of both viruses with no replication over input levels at concentrations greater than 3.5 mg/ml (Fig. 1). Comparison with reduction in virus titres of HSV-2 following only 24-h pre-treatment with extract indicated little difference, a favourable finding suggesting that prolonged pre-incubation of cells in the extract may not be necessary towards significant inhibition of virus replication. As it is known that certain constituents of plants, for example, lectins, are capable of virus neutralisation, BHK-21 cells which had been pre-incubated in extract were examined as whole cells and as ultrasonically-disrupted preparations for neutralising activity against HSV-2. There was no evidence of virus neutralisation (data not shown) which, therefore, did not contribute to the aforesaid reduction in intracellular virus replication.

3.2. Specificity of inhibition of virus replication

There were minor morphological alterations consisting of a slight roughness in cell outline with cytoplasmic granularity leading to rounding of cells and detachment at higher concentration of the extract in BHK-21, MRC-5, HEp-2 and MT-4 cells following 24-h incubation at 3.8 mg/ml of extract which appeared in general to decrease by 3–4 days, but these morphological alterations were still evident at 7.5 mg/ml of extract and remained for longer times of incubation.

More detailed analysis was carried out on BHK-21 cells. Cell replication was reduced at 7.5 mg/ml of extract and totally inhibited at 30 mg/ml. At 7.5 mg extract/ml concentration, there was reduced HSV-2 virus protein synthesis with virtual disappearance of virus polypeptides at 15 mg/ml of extract (Fig. 2). For example, the major and other capsid proteins at molecular weight 159 kD and 29 kD, respectively, the gA/gB glyco-

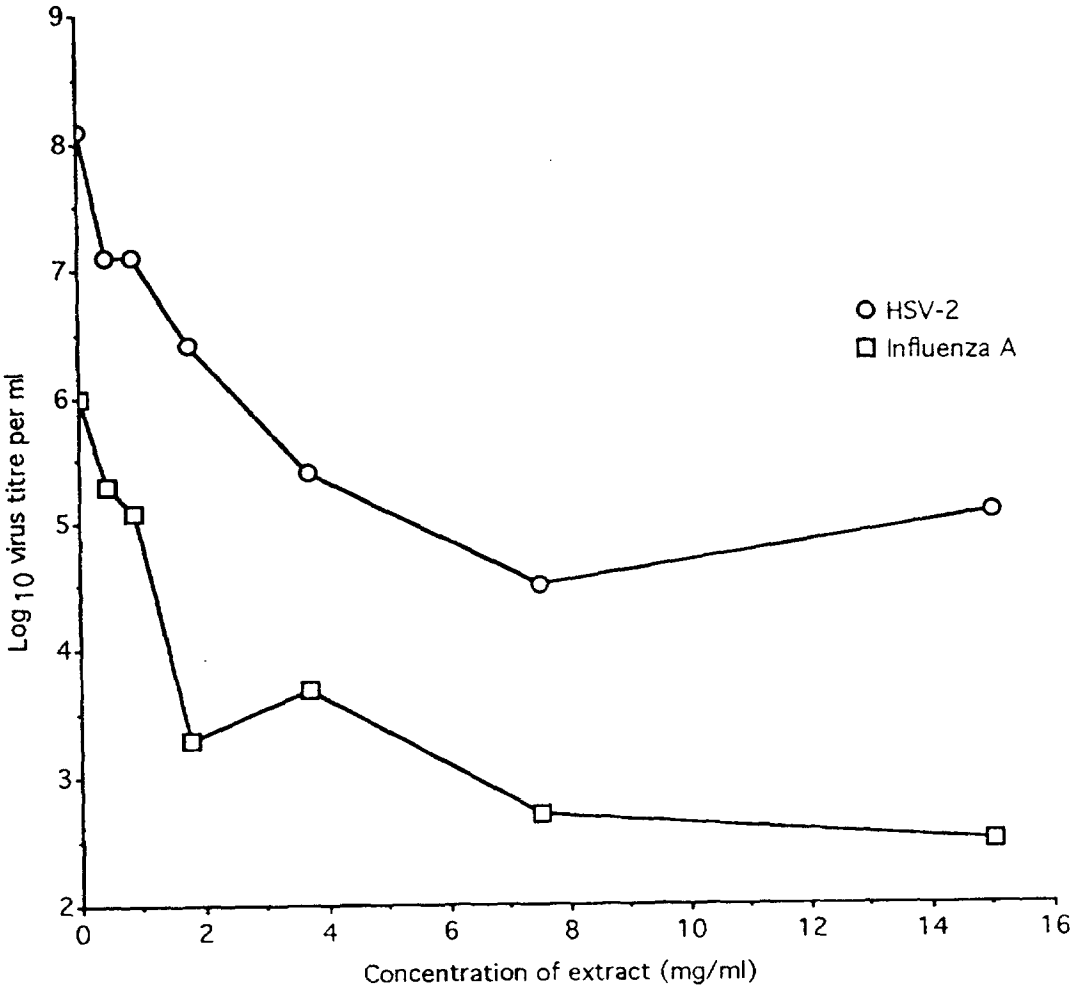

Fig. 1. Inhibition of replication of HSV-2 (○) and influenza A (□) by by pre-infection treatment of cells with extract of Opuntia streptacantha for 24 h. Extract was added for 24 h and removed immediately prior to addition of virus.

protein complex at 129 kD and the viral enzymes ribonucleotide reductase and the DNA polymerase-associated proteins which were ascribed molecular weights of approximately 150 kD and 55 kD, respectively, (Killington and Powell, 1985) were notably reduced in the presence of extract. On the other hand, there was no reduction in polypeptide synthesis in uninfected BHK-21 cells at 15 mg of extract/ml of medium except perhaps a slight lower representation of a polypeptide at 104 kD at the higher concentration of 15 mg/ml. Loading of uninfected and virus-infected samples were directly comparable in terms of cell numbers. Thus, there were normal levels of cell protein synthesis at a concentration of 15 mg/ml (Fig. 2), which was approximately five times the concentration of 3.5 mg/ml which reduced the replication of herpes simplex virus by 2.5 $\log_{10}$ (Table 1); of particular interest, a picornavirus (EMC) replicated at 60 mg/ml of extract at which concentration there was gross drug-induced cytopathic effect, yet EMC replicated from an input titre of 4.5 $\log_{10}$/ml to 8.1 $\log_{10}$/ml, a 4 $\log_{10}$ increase and only 0.4 $\log_{10}$ less than control levels in extract-free medium.

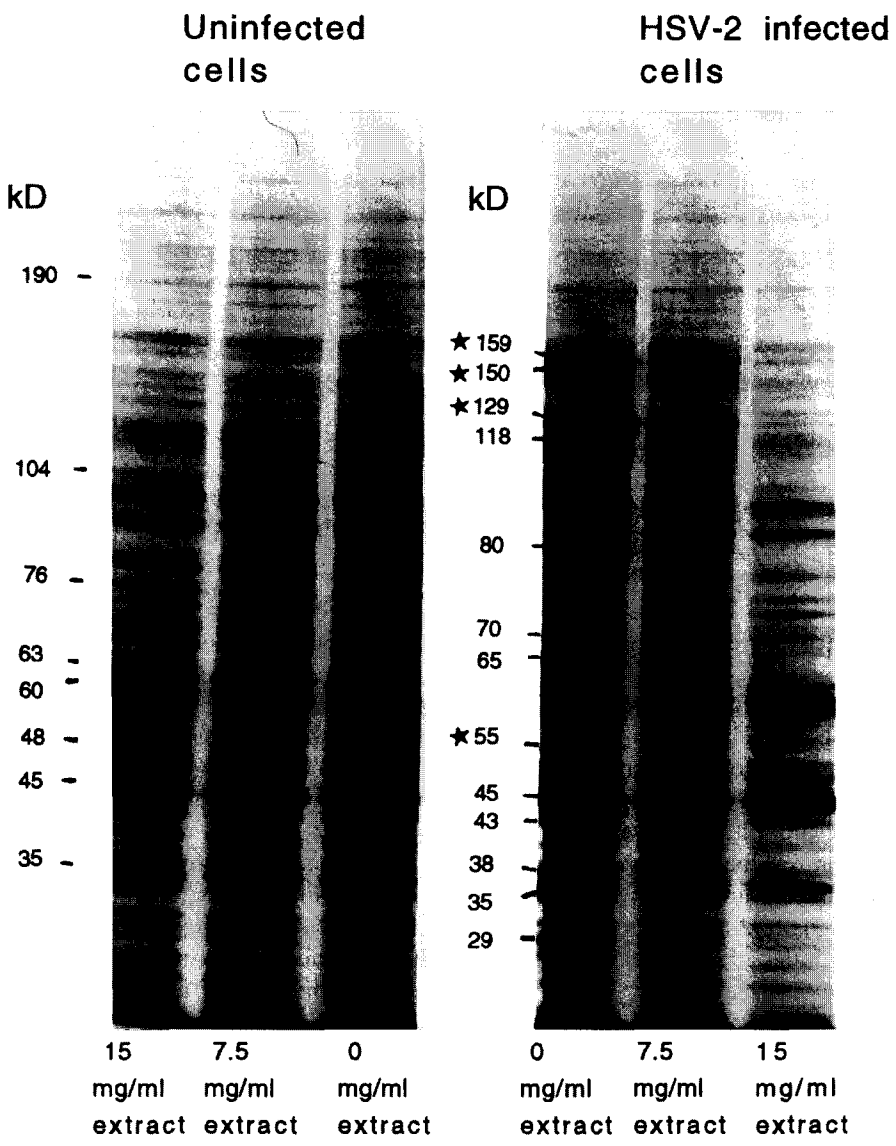

Fig. 2. Inhibition of synthesis of viral proteins (HSV-2) by extract of Opuntia streptacantha. HSV-2-infected BHK-21 cells and BHK-21 cells alone were incubated overnight with varying concentrations of extract in the presence of ^{35}S-methionine. Synthesised proteins were visualised by autoradiography after separation by polyacrylamide gel electrophoresis. * Virus-specific proteins.

3.3. Location of virus inhibitory activity

There was significant inhibition of virus replication by the extract from fresh cactus plant. Virus inhibitory activity resided in the wall of the plant but not in the cuticle or inner sap. Possible antiviral activity in relevant environmental substances was examined. Samples of soil from the plant bed, the relevant fertiliser and insecticide did not inhibit virus replication on testing at concentrations in significant excess of cultivation concentrations in the field (data not shown).

Table 2
Effect of lipid solvents on inhibitory activity of extract on HSV-2

Treatment; concentration of extract (per ml medium)	Log_{10} virus titre per ml	Log_{10} reduction in virus titre
No solvent added		
12mg	4.4	2.8
6mg	4.7	2.5
Ether		
12mg	3.7	3.5
6mg	4.8	2.4
Chloroform		
12mg	4.2	3.0
6mg	4.7	2.5
Acetone precipitation		
12mg	4.3	2.9
6mg	4.7	2.5
0mg	7.2	—
Input virus	4.3	—

3.4. Nature of active components of extract

The extract was treated with pronase or trypsin and tested against HSV-2. There was elimination of virus inhibitory activity of the extract at 1.2 mg/ml by pronase at 0.5 mg/ml for 2 h at 37°C and by trypsin at 1.2 mg/ml for 10 min at 37°C. Virocidal activity from residual pronase or trypsin was excluded by testing an uninfected cell extract (BHK-21 cells) which had been treated with pronase and trypsin under identical experimental conditions.

Virus inhibitory activity was also eliminated by heating to 60°C for 10 min but was stable at room temperature. On the other hand, liquid phase extraction with ether or chloroform did not reduce viral inhibitory activity and precipitation with acetone and reconstitution to the same concentration maintained virus inhibitory activity (Table 2). Finally, there was no significant reduction in virus inhibitory activity following prolonged extraction filtration or dialysis of the extract. Therefore, it seems likely that the active components were protein in nature.

3.5. Inactivation of extracellular virus (HSV-2) by extract

Cell-free HSV-2 was incubated at 37°C for 1 h and 24 h with varying concentrations of extract in Eagles medium. There was significant reduction of greater than 0.5 log_{10} and 2.5 log_{10} at 1 h and 24 h, respectively, using 30 mg/ml of extract.

3.6. Safety of extract

Safety studies were carried out in mice, horses and human patients. In mice, there were no adverse effects following a single intravenous dose of 70 mg and subcutaneous dose of 370 mg; an intraperitoneal dose of 370 mg gave slight symptoms consisting of ruffled fur, mild diarrhoea and slight reduction in mobility which disappeared by day 2 after inoculation. The mice were monitored for clinical signs, weight and rectal temperatures at regular intervals (Soothill et al., 1992) and subsequently observed for 6 weeks with no evidence of adverse effects. Six Balb/c mice which drank an average of 5 mls of cactus extract at a concentration of 120 mg/ml per day in their drinking water for 3 weeks showed no adverse effects with normal weight and rectal temperature for 3 weeks.

In horses which were given oral daily doses of 27 g of cactus extract for 2–4 weeks, there was no evidence of adverse clinical effect or of laboratory abnormalities of liver, kidney or haematological functions.

There were no general adverse effects or specific adverse effects on the cardiovascular or extrapyramidal system as have been reported for other hypoglycaemic agents, for example, diazoxide on human patients who ingested 6 g cactus extract per day for 1 month or 3 g for 6 months.

4. Discussion

The study has indicated that extract of the cactus plant Opuntia streptacantha will inhibit replication of a number of DNA and RNA viruses. Virus replication was also inhibited by pre-treatment of cells with removal of extract; this was most notable for RNA viruses influenza A and HIV which might be simplistically attributed to their cytoplasmic site of virus replication where there is likely to be a higher concentration of extract. Pre-infection efficacy is a favourable finding whereby uninfected cells in-vivo will synthesise less virus if they become infected in the course of disease. The extract also neutralised extracellular HSV-2, but at higher concentrations than required for inhibition of virus replication. It was interesting that the virocidal but not the inhibitory function of the extract resisted boiling, suggesting that the two functions are mediated at least in part by different component(s); identification and characterisation of the virocidal factor will form the subject of a separate communication.

Inhibition of virus replication was 'specific' in that there was reduced virus and virus protein synthesis at 1–2 mg/ml while cell protein synthesis was unaffected at 7.5 mg/ml and EMC virus replicated to virtually control titres at 30 mg/ml of extract. These findings are pertinent to changes in cell morphology at 7.5 mg/ml and indicate that the compound is notably better tolerated in vivo than might be predicted by cell culture assay. In addition, we question the contention that breadth of efficacy of an antiviral agent embracing DNA, RNA and retroviruses indicates 'non-specificity', as it is quite conceivable that an antiviral agent will operate on a specific macromolecular event which is general to viruses of different groups. Indeed, it has been known for many years that a number of antiviral agents, for example N',N'-anhydrobis-(b-hydroxyethyl) biguanide (Ishida et al., 1962; Rada et al., 1964) and forscarnet (Price et al., 1986; Chrisp and Clissod, 1991; Sandstrom et al., 1985) have efficacy against both DNA and RNA viruses. Secondly, breadth of efficacy is essentially a favourable attribute, penicillin perhaps representing a relevant precedent.

The mechanism of action of the active components of the extract is presently unknown. While preliminary and unpublished data suggests that inhibition of primary cytopathic foci, for example, plaques on cell monolayers, can be demonstrated in the presence of extract, the largest effect was evident by measuring intracellular virus replication hinting that the mechanism of action, at least for herpes viruses and HIV, is not an early event in that the mediators of virus cytopathic effect are not inhibited while synthesis of infectious virus is significantly inhibited. A number of potentially useful antiviral compounds remain undiscovered when measurement of virus synthesis is not included in screening protocols.

A number of plant components have been reported to inhibit virus replication. Two antiviral proteins from Phytolacca americana (pokeweed), PAP I and PAP II, inhibited replication of a number of viruses (Irvin, 1975; Irvin et al., 1980; Barbieri et al., 1982), including herpes simplex virus type 1, although the precise contribution of virus neutralisation at absorption or post-harvesting was unclear in this brief report (Aron and Irvin, 1980). In contrast to Opuntia streptacantha, pokeweed proteins appear to be potent inhibitors of protein synthesis both in-vivo and in reticulocyte translation systems with cellular toxicity (Hela cells) at 1 mg/ml and a 7-day intraperitoneal LD50 of 3 mg/Kg for Swiss mice; this contrasts with Opuntia streptacantha where mice received 2.3 g/Kg or 70 mg in total of crude extract by the intravenous route without adverse effect. The plant Trichosanthes kirilowii contains trichosanthin and its non toxic active principle of molecular weight 29 kD designated TAP29 which inhibits replication of human immunodeficiency virus by inhibiting polypeptide chain elongation (Lee-Huang et al., 1991). While it is not possible to identify the active principle in the Opuntia

streptacantha or its relationship to other recognised antiviral components until it has been isolated and characterised, our data, particularly the proteinase sensitivity of the inhibitory activity, suggests that the active component(s) is protein and is thus distinct from the alkaloid-flavanoid group of viral inhibitors. It is intended to thoroughly characterise the antiviral component by biochemical analysis.

The safety of a putative therapeutic agent is of paramount importance. To date, we have been unable to identify adverse effect following oral administration of 27 g/day for 14–28 days in horses, 6 g/day for 1 month or 3 g/day for 6 months in human patients and it seems virtually impossible to induce toxic or adverse reaction by oral administration, although the survival of the cactus or any putatively toxic component following passage through the stomach and duodenum is, as yet, unknown. It was also encouraging that mice tolerated an intravenous bolus dose of 70 mg of extract which corresponds to approximately fifty 50% tissue culture viral-inhibiting doses or a human dose of approximately 140 g on a weight for weight basis.

In summary, the breadth of in vitro reactivity in association with a high index of clinical safety in uncontrolled studies encourage the institution of studies of in vivo efficacy in animal models.

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