Antiviral Research, 15 (1991) 101–112

ADONIS 016635429100024V

AVR 00463

Antiviral activities of hypericin

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(Received 19 July 1990; accepted 15 October 1990)

Summary

Hypericin, a photodynamic plant quinone, readily inactivated murine cytomegalovirus (MCMV), Sindbis virus, and human immunodeficiency virus type 1 (HIV-1), especially on exposure to fluorescent light. Sindbis virus was significantly more sensitive than MCMV. The inactivated MCMV, when used to infect cells, was incapable of synthesizing early or late viral antigens. In addition to this direct virucidal effect, when hypericin was added to cells infected with viable MCMV, inhibition was also observed, particularly when the compound was added in the first two hours of infection. Again the antiviral effect was augmented by visible light. At effective antiviral concentrations, there were no discernible adverse effects on cultured cells. Thus hypericin appears to have two modes of antiviral activity: one directed at the virions, possibly on membrane components (although other virion targets cannot be ruled out), and the other directed at virus-infected cells. Both activities are substantially enhanced by light.

Other recent studies on the antiviral activities of hypericin have not considered the role of light, and it is conceivable that apparent discrepancies between their results may have reflected different conditions of light exposure.

Hypericin; Photosensitization

Introduction

Many plants contain compounds that have antiviral activities. Among the better characterized antiviral compounds are furocoumarins, alkaloids of various types, thiophenes, flavonoids, terpenoids, various phenolic derivatives, and polypeptides (Vanden Berg et al., 1986; Hudson and Towers, 1988; Hudson, 1989a,b). Many of them are also photosensitizers, i.e. their biological activities are dependent upon or

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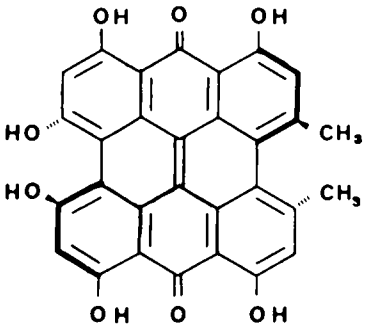

Fig. 1. Structural formula of hypericin.

are augmented by light of specific wavelengths.

A wide variety of mechanisms are shown by these compounds. Some of them (e.g. thiophenes, flavonoids) have direct antiviral effects, which may be specific for a few viruses, or may be generally effective against viruses with membranes. Others block virus replication, either by interfering with key components in replication or by blocking the maturation of virions (e.g. terpenoids, phenolic compounds, alkaloids). Still others work by an interferon-like mechanism (Andrei et al., 1988).

Quinones are widespread among plants, although relatively little attention has been given to their biological attributes. The condensed anthraquinone hypericin (structural formula, Fig. 1) has been known for many decades. It is a photosensitizer found in certain members of the genus *Hypericum*, and is particularly prevalent in *H. perforatum* (St. John's Wort). These plants have a long history in the traditional pharmacopoeia of many countries (Knox and Dodge, 1985; Satyavati et al., 1987).

Hypericin produces singlet oxygen on exposure to visible light (Duran and Song, 1986), and this is thought to be the property responsible for phototoxic symptoms observed in grazing animals that ingest large quantities of *Hypericum* plants (Giese, 1980). However, the compound can be administered safely in humans and other animals (Giese, 1980; Duran and Song, 1986).

Recently it was reported that hypericin could inhibit retrovirus replication in cell cultures, and furthermore could control the symptoms of retrovirus-induced disease in mice (Meruelo et al., 1988; Lavie et al., 1989). The underlying mechanisms were not clear, however, and a role for light in the apparent antiviral effect was not discussed.

We have investigated the effects of hypericin on three mammalian viruses: murine cytomegalovirus (MCMV), Sindbis virus (SV) and human immunodeficiency virus type 1 (HIV-1). We have shown that light has an important role to play in these antiviral activities, and that there may be more than one site of action.

Materials and Methods

Hypericin: extraction and purification

The aerial parts of *Hypericum perforatum* L. (St. John's Wort) were collected in Vancouver (voucher specimen is deposited at University of British Columbia) and dried, cut and milled. 216 g of the material were extracted first with methylene chloride (discarded), then with acetone until the solvent was colorless. The solution had a red fluorescence as described by Brockmann et al. (1957). Acetone was evaporated under reduced pressure to complete dryness of the residue, yielding 5 g. The crude extract was chromatographed on silica gel (70–230 mesh) and eluted first with methylene chloride until the solvent reached the column bottom, and then with solvent mixture methylene chloride/acetone/methanol (75:15:10 vol%, respectively).

Chromatography was monitored on thin-layer chromatoplates by observing the fluorescent spots under long-wave UV light while the chromatoplate was still wet with solvents. Development of the plates was done with the latter solvent mixture. Repeated chromatography was then done with silica gel (230–400 mesh), and solvent mixtures of methylene chloride/acetone combined in different proportions according to polarity of the products being eluted. Solutions yielding pure compound were then dried by slow evaporation; yield was 8.5 mg of hypericin. The compound was characterized by spectroscopy (^{1}H-NMR, mass and UV data), and comparison with a synthetic sample.

The compound was stored in the dark at 4°C as a 1.0 mg/ml solution in 95% ethanol.

Cells and viruses

Mouse embryo fibroblasts (MEF) were prepared and cultivated (in Dulbecco's modified MEM with 10% fetal bovine serum) as described elsewhere (Allan and Shellam, 1984; Hodgkins et al., 1988). Vero cells were propagated in the same medium. The CEM cells ($CD4^+$ human T-cell line) were propagated in RPMI medium supplemented with 2 mM glutamine and 10% fetal bovine serum.

Murine CMV and Sindbis virus were local laboratory strains propagated in Perth, Australia. They were provided by G. Shellam and J. MacKenzie respectively. Virus stocks were maintained in MEF cultures (Allan and Shellam, 1984). Virus plaque assays, on MEF, were performed in 24-well multiwell trays by the methyl cellulose overlay technique, followed by methylene blue staining to enumerate pfu (Hodgkins et al., 1988). Up to 500 plaques/well could be counted accurately by this technique.

The HIV-1 strain LAV-1 obtained from Dr. L. Montagnier, was propagated and assayed in CEM cells, as described (Schwartz et al., 1988).

Photochemical treatments

Virus-(MCMV,SV)-hypericin reactions were set up by mixing a small volume of hypericin (usually 5–20 μl) with 500–1000 pfu of virus in complete medium (1.0–2.0 ml), in sterile glass or plastic vials. These vials were then exposed to a fluorescent lamp (F48T12Cw/Ho, max. output at 700 nm, incident energy of approximately 0.5 mw/cm^2) for 60 min, or wrapped in aluminum foil, at a temperature of about 22°C. At the end of this incubation the mixtures were added to washed monolayers of MEF for 60 min to allow virus adsorption (in the dark). The cells were then washed and incubated in medium or overlaid with 1% methyl cellulose in medium containing 2% serum (Hodgkins et al., 1988). Virus incubated with a corresponding volume of ethanol in medium, and exposed to light, served as the control.

In the "time of addition" experiments, hypericin (final concentration 0.2 μg/ml) was added directly to quadruplicate cultures (2×10^5 cells in 1.0 ml medium) at various times relative to the virus inoculum. Thus all infections were performed simultaneously (500 pfu of MCMV per culture), and the hypericin was added in the form of "pulses" of 30 min in the dark, followed by 60 min of light exposure (or continued dark) and three rinses with medium to remove free hypericin. At 9 h p.i. all cultures were rinsed with medium, overlaid with methylcellulose in medium, and incubated further for plaque development (Hodgkins et al., 1988). Replicate plaque counts seldom differed by more than ± 5%.

In the cytotoxicity tests, preformed monolayers (2×10^5 cells) or freshly trypsinized cells (1×10^5 cells/ml) were exposed to hypericin, with or without light as indicated, for a total of 6 h, followed by cultivation in multiwell trays for 3 days. They were inspected twice daily for cytopathic effect (cpe).

In the case of HIV-1, various amounts of HIV-1 (expressed as TCD_{50} per ml) were reacted with hypericin in light or dark for 60 min in 24-well multiwell trays, following which 4×10^5 cells were added per well. Cultures were inspected daily under the microscope for cpe (syncytium formation).

Fluorescent antibody staining

Cultures of MEF were grown on glass cover slips in the multiwell trays until confluency. They were infected by the centrifugal inoculation technique (MCMV) to ensure a high MOI (multiplicity of infection; 30–50 pfu/cell; Hudson, 1988). The virus had been either untreated, or incubated with hypericin (1–2.5 μg/ml), with or without light exposure. Unadsorbed virus and hypericin were washed out of the cultures, following centrifugal infection, by two rinses in medium; mock-infected cells similarly exposed to hypericin did not show cytotoxicity. Cultures were inspected periodically for cpe, and were fixed by air drying and 85% acetone, at either 4 h p.i or 22 h p.i. They were subsequently reacted with a rabbit polyclonal anti-MCMV serum and a commercial (Tago) FITC-labelled goat anti-rabbit IgG (Farrell and Shellam, 1989).

HIV-antigen detection

Infected cell cultures, cells and supernatants, were assayed for p24 antigen content by means of the Abbott HIVAG antigen-capture kit. Cultures were diluted 10- or 100-fold in the diluent buffer supplied and the values were expressed as A_{420} units per ml of original culture. Readings between 2.0 and 0.2 units were proportional to antigen content.

Results

The effect of light on antiviral activity

Fig. 2 shows survival curves for MCMV and SV treated with various concentrations of hypericin, in the presence and absence of light.

Sindbis was significantly more sensitive than MCMV. More than 99% of the infectivity of SV was lost upon exposure to 0.12 μg/ml of hypericin in light, whereas 0.25 μg/ml was required to inactivate 99% of MCMV. In the absence of light exposure the virucidal effect was reduced considerably – by more than two orders of magnitude for a given concentration of hypericin. Therefore light is

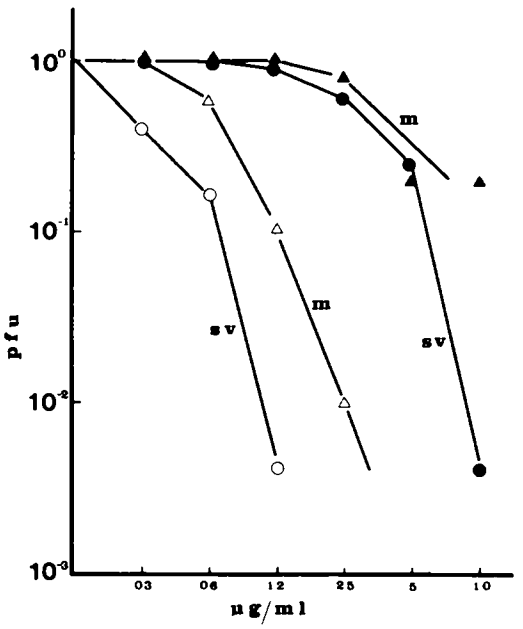

Fig. 2. Inactivation of MCMV (m) and Sindbis (sv) by various concentrations of hypericin. Open symbols: + light; closed symbols: dark. pfu values at each concentration were subtracted from controls (10°) with no hypericin. Control pfu values were MCMV: dark 420, light 465; sv: dark 153, light 140. All values ± 5% between quadruplicates.

essential for the optimum antiviral effect, although a certain amount of activity is manifest in the dark (see Discussion).

In these experiments, a standard light exposure time of 60 min was used (see Materials and Methods), although longer exposure times resulted in proportionately more inactivation of virus (data not shown). Unfortunately, the viruses themselves were intrinsically unstable at ambient temperatures and the reaction medium gradually lost buffering capacity; consequently a 60 min incubation time was the most practical. The 100% values represented control viruses exposed to light in the absence of hypericin.

Time of addition of hypericin

Hypericin was added at various times before, during, or after MCMV infection (as described in Materials and Methods), in order to determine if the compound could affect the virus indirectly in addition to its virucidal effect.

When cells were incubated with hypericin for 2 h (Fig. 3), or up to 16 h (not shown), before virus infection, with or without light exposure, there was no effect on subsequent plaque formation, provided the compound was washed out prior to the addition of virus. Thus hypericin did not induce an "antiviral state" in the cells.

When virus and hypericin were added simultaneously to the cells, plaque formation was inhibited more than 90% in the light exposed cultures, and by 65% in the dark cultures (Fig. 3). Since MCMV adsorption to cells is a rapid process (Hodgkins et al., 1989), then it is likely that some virions would attach to the cells (and possibly penetrate) before hypericin could bind to them.

When virus was incubated with compound prior to inoculation onto the cells, considerable inhibition in plaque numbers resulted. When hypericin was added 1

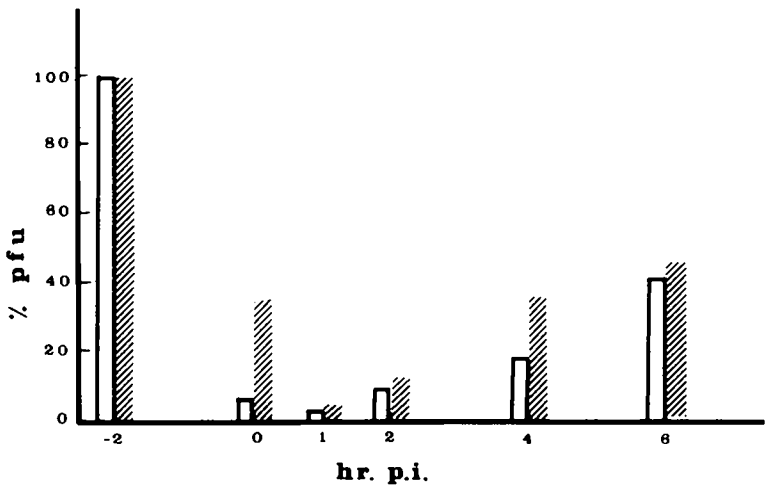

Fig. 3. Time of addition of hypericin relative to MCMV infection (0 h p.i.). Open bars: hypericin + light; shaded bars: hypericin without light. 100% value represents virus with no hypericin (dark, 396 pfu; light, 376 pfu). All values ± 5%.

or 2 h after virus inoculation, the reduction in plaque formation was more dramatic (Fig. 3). In both the presence and absence of light, virus titers were reduced 90% or more, although the effect was always more evident in the presence of light.

As the addition of hypericin was delayed, the inhibitory effect became less pronounced, suggesting that an early stage in the replication cycle was sensitive to the compound.

Viral antigen production

Cells were infected with hypericin-treated virus (2.5 μg/ml hypericin in the presence or absence of light), or untreated virus, and samples of the infected cells were fixed at 4 h p.i. or 22 h p.i., for fluorescent antibody staining. The anti-MCMV antibody used in these experiments was known to react with the dominant immediate-early proteins (i.e. the 100 kDa, 96 kDa, 89 kDa and 86 kDa proteins; giving intranuclear and perinuclear staining; Walker and Hudson, 1988) and with the late proteins (giving mainly cytoplasmic staining; Farrell and Shellam, 1989; Farrell, 1990).

A high MOI was used (50 pfu/cell) so that characteristic early cpe could be observed as well as late cpe. We have shown previously that certain antiviral compounds could selectively inhibit late cpe but not early cpe (Hudson et al., 1986).

Table 1 summarizes the results. As expected, untreated virus gave rise to early cpe in which more than 90% of the cells showed rounding or retraction (from adjacent cells), and 90% contained viral antigens. By late times, all of these cells were rounded (though still attached to the glass) and contained substantial quantities of cytoplasmic antigens.

Cultures infected with hypericin (dark) treated virus showed reduced amounts of cpe (both early and late), and a small percentage of weakly antigen-positive cells. In contrast virus treated with hypericin in light gave rise to no discernible cpe, and only rare cells (fewer than one in a thousand) with late antigens (Table 1).

TABLE 1

MCMV antigen production and cytopathic effects

Treatment of virus	Early (4 h p.i.)		Late (22 h p.i.)	
	cpe[1]	% Ag. pos. cells[2]	cpe[1]	% Ag. pos. cells[2]
None	pronounced >90% cells	90	100% cells involved	100
Hypericin (dark)	rare foci of retracting cells	10 (weak)	few foci of rounded cells	40 (weak)
Hypericin (light)	None	<0.1	None	~0.1 (weak)

Hypericin concentration 2.5 μg/ml; MOI 50 pfu/cell.

[1]Early cytopathic effect (cpe): cell retraction, occasional rounding; late cpe: rounded cytomegalic and fused cells.

[2]Early antigen fluorescence: nuclear and perinuclear; late antigen fluorescence: predominantly cytoplasmic.

In other experiments it was found that delays in hypericin addition (after virus infection) resulted only in a decrease in fluorescence intensity without affecting the number of positive cells (data not shown).

This confirms the sensitivity of the very early part of the replication cycle.

Cytotoxicity of hypericin

Preformed monolayers of MEF cells, and MEF cells in suspension, were exposed to various concentrations of hypericin in the dark, or in the presence of one or three 60 min pulses of light. Both types of culture were then incubated in multiwell plates for 3 days, with periodic microscopic examination. The final set of observations is recorded in Table 2.

Following the exposures to hypericin it was evident that 100 μg/ml was toxic in all cases, and no subsequent cell proliferation was observed. In addition 20 μg/ml was toxic in the presence of one or three light exposures; although some cells survived, there was no apparent proliferation during the next 3 days.

A concentration of 4 μg/ml eventually proved to be toxic in all the light-exposed cultures, although the effect was noticeably quicker in the cells exposed to three light pulses. Also the suspended cells tended to be slightly more susceptible than the preformed monolayers, but no attempt was made to quantitate this effect.

In addition Vero cells, which were used on occasion as the host cells for SV, showed a level of sensitivity to hypericin and light similar to MEF cells (data not shown).

Effects of hypericin on HIV-1

In preliminary experiments we found that concentrations of 1.0 or 0.1 μg/ml of hypericin could completely inactivate 100 TCD_{50} of HIV-1 when virus plus compound were exposed to light before adding the virus to CEM cells. In the absence of light only partial inactivation resulted.

Table 3 shows the results of a more detailed experiment in which aliquots of 10^5 TCD_{50} were incubated with or without 1.0 μg/ml hypericin, in the presence or absence of light exposure, and then serially diluted and plated onto CEM cultures.

TABLE 2

Cytotoxic effects of hypericin on MEF cells

Light treatment[1]	Maximum non-toxic conc. hypericin (μg/ml)[2]	
	Preformed monolayer	Suspended cells
None	20	4 (20±)[3]
1 × 60 min	0.8 (4±)[3]	0.8
3 × 60 min	0.8	0.16 (0.8±)[3]

[1]Exposure to light for 1 or 3 × 60 min pulses with intermittent dark periods, followed by washing the cells, incubation for 3 days in the dark, and periodic microscopic inspection. Total exposure to hypericin was 6 h.

[2]Concentrations of hypericin: 0, 0.16, 0.8, 4, 20, 100 μg/ml.

[3]Concentrations in parentheses: some replicate cultures looked normal, some showed cytopathic effect.

TABLE 3

Effects of hypericin on HIV-1 infectivity

Virus dilution	No hypericin		+hypericin (dark)		+hypericin (light)	
	cpe[a]	Ag.[b]	cpe	Ag.	cpe	Ag.
10^{-1}	4+	>200	4+	>200	2+	13
10^{-2}	3+	86	3+	93	+	4.9
10^{-3}	2+	15.3	2+	9.2	±	~0.15
10^{-4}	2+	13.6	±	~0	−	0
10^{-5}	±	~0.15	−	0	−	0

[a]cpe = degree of cytopathic effects in CEM cells, from + to 4+ (complete involvement of culture), read at 6 days p.i. ±; some culture wells positive, some negative.

[b]Ag. = relative amount of p24 antigen produced (at 6 days p.i.) expressed as absorbancy units (420 nm) in the undiluted culture.

The first column of the results (Table 3) show that untreated virus could be diluted to 10^{-5} with retention of viral cpe and a low level of p24 antigen production in some of the replicate test cultures, as anticipated on the basis of the known virus titre.

In contrast, virus treated with hypericin in the dark (column two) could only be diluted between 10^{-3} and 10^{-4} with retention of virus production, i.e. 90% or more of the virus had been inactivated. Hypericin plus light (column three) resulted in a 99% decrease in infectivity.

Since the HIV-1 experiments were necessarily carried out in a different facility from the other viral studies (for biosafety reasons), it is not possible to compare their relative sensitivities to hypericin directly, although the conditions of treatment were similar.

Discussion

These results have shown that hypericin has impressive light-mediated antiviral activities against the three viruses MCMV, SV, and HIV-1. In the absence of light the activities were diminished, though still significant.

Hypericin is known to be a photosensitizer which, in the presence of light in the 650–700 nm range, generates singlet oxygen and possibly other reactive species (Giese, 1980; Knox and Dodge, 1985; Duran and Song, 1986). These in turn could conceivably damage viral membranes (and possibly proteins and nucleic acids), and thus account for the loss in infectivity and for the consequent inability of the treated virus to code for viral antigens in infected cells. The compound is also active, however, at higher concentrations, in the absence of visible light. This suggests an alternative mode of action.

Several other groups have recently studied hypericin-virus interactions (Meruelo et al., 1988; Lavie et al., 1989; Schinazi et al., 1990; Tang et al., 1990). All have demonstrated some degree of virus inactivation by hypericin and certain other anthraquinones with similar structures (pseudohypericin and others, Schinazi et al., 1990); but in none of these studies was the role of light discussed, and in fact it is

not at all clear if these workers even considered light exposure in their experimental designs. Yet this is an important factor. If virus hypericin mixtures are exposed to normal room lights, or the typical fluorescent lamps found in biosafety cabinets, then hypericin will be photoactivated, with consequent production of singlet oxygen (i.e. type II photosensitization) and possibly radicals (type I photosensitization; Knox and Dodge, 1985; Duran and Song, 1986).

This may explain some of the apparent discrepancies between those studies referred to above. If, for example, virus-hypericin mixtures are inoculated immediately into mice without exposure to light, then the virus may retain its infectivity, apart from a possible "dark-effect". In contrast, similar mixtures exposed to light would undoubtedly suffer some inactivation of the virus. Furthermore, experiments designed to examine the effects of temperature of incubation of virus-hypericin mixtures (Tang et al., 1990) could be misleading if some of the mixtures were incubated in the dark, e.g. in a 4°C refrigerator or a 37°C culture incubator. Therefore effects ascribed to temperature might in fact be due to the presence or absence of light exposure. In the absence of specified conditions for each experiment described, however, we can only speculate on the possible alternative explanations.

Hypericin did show, in the present studies, a significant antiviral effect in the dark. Whether this could be due to leakage of stray light into the reaction mixtures or a distinct mechanism independent of singlet oxygen, remains to be investigated. An antiviral effect of hypericin and pseudohypericin has been reported for mice exposed to the compound some hours before or after virus inoculation (Meruelo et al., 1988; Tang et al., 1990). This suggests that light is not necessary in vivo. However, most of these results were expressed as relative mortalities in the different groups of mice, which is not necessarily a reflection of virus titres in individual tissues. Consequently it is not known if animal survival was due to a direct antiviral effect of the compound in vivo, or an indirect effect due to chemical stimulation of a protective response of some kind.

The fact that non-enveloped viruses were resistant to hypericin (Tang et al., 1990), under conditions that readily inactivated membrane-containing viruses, suggests that a membrane component could be a principal target. This would agree with the conclusions derived from studies with cells (Duran and Song, 1986). However, the fact that other plant photosensitizers can interact with various viral macromolecules (Hudson, 1989a) suggests that there might be alternative targets for hypericin too. In this connection it is interesting that hypericin and pseudohypericin were reported to have specific inactivating effects on protein kinase C, whereas several other enzymes were unaffected (Takahashi et al., 1989). Effective concentrations were relatively high, however, and light was not considered.

Since the half-life of singlet oxygen, and its path length, are both relatively short, then the type of photodamage sustained by a virion may depend on what macromolecules are in proximity to the hypericin molecules.

In addition to the direct antiviral effect, hypericin inhibited an early stage in the MCMV replication cycle, and this effect was also augmented by light, although the distinction between light and dark in this case was not so marked. This implies that the compound can selectively impair virus replication without damaging the cells.

It is possible that the intracellular inhibition was due to binding of hypericin to residual virion components in the infected cell, i.e. by the same basic mechanism as the virucidal effect. However, immediate-early MCMV gene transcription commences within 1 h p.i. (Keil et al., 1987; Walker and Hudson, 1988). This observation, together with the fact that most of the hypericin was probably removed by the washing steps after the 90 min hypericin treatment, suggest that an early step in the replication cycle was the most likely intracellular target.

In conclusion, hypericin shows a light-enhanced direct antiviral effect (virucidal-effect) towards viruses with membranes, as well as a light-enhanced intracellular antiviral effect, but dark activities are also significant. It remains to be seen if all these effects represent a common mechanism caused by singlet oxygen or radical-induced damage, or distinct mechanisms in different situations.

Regardless of the specific mechanisms involved, it is clearly important that future antiviral studies with hypericin must be conducted under carefully controlled and specified conditions of light exposure, in order to avoid possible ambiguities in interpretation.

Acknowledgements

This work was supported by the National Sciences and Engineering Research Council, Ottawa and by Health and Welfare, Ottawa (Federal Centre for AIDS). Some of the experiments were carried out by J.B.H. while on leave in the Department of Microbiology, University of Western Australia. The authors thank Professor G. Shellam and associates for providing the facilities.

References

Allan, J.E. and Shellam, G.R. (1984) Genetic control of murine cytomegalovirus infection: virus titres in resistant and susceptible strains of mice. Arch. Virol. 81, 139–150.

Andrei, G.M., Damonte, E.B., Torres, R.A. and Coto, C.E. (1988) Induction of a refractory state to viral infection in mammalian cells by a plant inhibitor isolated from leaves of *Melia azedarach* L. Antiviral Res. 9, 57–65.

Brockmann, H.H., Kluge, F. and Muxfeldt, H. (1957) Total synthese des hypericins. Chem. Ber. 90, 2302–2318.

Duran, N. and Song, P.-S. (1986) Hypericin and its photodynamic action. Photochem. Photobiol. 43, 477–480.

Farrell, H.E. (1990) Ph.D. Thesis. University of Western Australia.

Farrell, H.E. and Shellam, G.R. (1989) Immunoblot analysis of the antibody response to murine cytomegalovirus in genetically resistant and susceptible mice. J. Gen. Virol. 70, 2574–2586.

Giese, A.C. (1980) Hypericism. Photochem. Photobiol. Rev. 5, 229–255.

Hodgkins, P.D., Scalzo, A.A., Swaminathan, N., Price, P. and Shellam, G.R. (1988) Murine cytomegalovirus binds reversibly to mouse embryo fibroblasts: implications from quantitation and explanation of centrifugal enhancement. J. Virol. Meth. 22, 215–230.

Hudson, J.B. (1988) Further studies on the mechanism of centrifugal enhancement of cytomegalovirus infectivity. J. Virol. Meth. 19, 97–108.

Hudson, J.B. (1989a) Plant photosensitizers with antiviral properties. Antiviral Res. 12, 55–74.

Hudson, J.B. (1989b) Antiviral compounds from plants. CRC Press, Florida.

Hudson, J.B. and Towers, G.H.N. (1988) Antiviral properties of photosensitizers. Photochem. Photobiol. 48, 289–296.

Hudson, J.B., Graham, E.A., Fong, R., Hudson, L.L. and Towers, G.H.N. (1986) Further studies on the antiviral activity of harmine, a photoactive β-carboline alkaloid. Photochem. Photobiol. 44, 483–487.

Keil, G.M., Ebeling-Keil, A. and Koszinowski, U.H. (1987) Immediate-early genes of murine cytomegalovirus: location, transcription, and translation products. J. Virol. 61, 526–533.

Knox, J.P. and Dodge, A.D. (1985) Isolation and activity of the photodynamic pigment hypericin. Plant Cell Environment 8, 19–25.

Lavie, G., Valentine, F., Levin, B., Mazur, Y., Gallo, G., Lavie, D., Weiner, D. and Meruelo, D. (1989) Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA 86, 5963–5967.

Meruelo, D., Lavie, G. and Lavie, D. (1988) Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc. Natl. Acad. Sci. USA 85, 5230 5234.

Satyavati, G.V., Gupta, A.K. and Tandon, U. (Eds.) (1987) Medicinal plants of India Vol. 2. Indian Council of Medical Research, New Delhi.

Schinazi, R.F., Chu, C.K., Babu, J.R., Oswald, B.J., Saalmann, V., Cannon, D.L., Eriksson, B.F.H. and Nasr, M. (1990) Anthraquinones as a new class of antiviral agents against human immunodeficiency virus. Antiviral Res. 13, 265–272.

Schwartz, O., Henin, Y., Marechal, V. and Montagnier, L. (1988) A rapid and simple colorimetric test for the study of anti-HIV agents. AIDS Res. Hum. Retrov. 4, 441 448.

Takahashi, I., Nakanishi, S., Kobayashi, E., Nakano, H., Suzuki, K. and Tamaoki, T. (1989) Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their anti-retroviral activity. Biochem. Biophys. Res. Commun. 165, 1209 1212.

Tang, J., Colacino, J.M., Larsen, S.H. and Spitzer, W. (1990) Virucidal activity of hypericin against enveloped and non-enveloped DNA and RNA viruses. Antiviral Res. 13, 313–326.

Vanden Berghe, D.A., Vlietinck, A.J. and Van Hoof, L. (1986) Plant products as potential antiviral agents. Bull. Inst. Pasteur 84, 101–147.

Walker, D.G. and Hudson, J.B. (1988) Further characterization of the murine cytomegalovirus induced early proteins in permissive and non-permissive cells. Arch. Virol. 101, 143–154.