

PHOTODYNAMIC INACTIVATION OF VERRUCAE VULGARES. I

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Abstract. Verrucae vulgares (v.v.) stained in vivo and in vitro with 0.1% and 1% proflavine in 100% dimethylsulphoxide (DMSO) and 0.1% and 1% neutral red in 100% DMSO were examined grossly and by fluorescence microscopy. Light transmission studies were made using both whole v.v. embedded in methacrylate and 5 μ m frozen sections. The dyes were seen to penetrate to the epidermal and dermal structures in the in vivo stained v.v. and accumulate in the cell nuclei. The average concentration of neutral red in the v.v. was estimated to be 2×10^{-5} M. The concentration of proflavine was lower than that, but exceeded 10^{-6} M. There was diffuse staining throughout the in vitro stained warts. From 400-600 nm the warts were penetrated by at least 1% of the light directed toward their surfaces.

Key words: Verrucae vulgares; Light transmission; Fluorescence microscopy; Proflavine; Neutral red

Photodynamic inactivation has been used in laboratory experiments to inactivate various DNA viruses (6, 16, 17, 18). The inactivation is thought to occur as a result of an irreversible binding of the dye, e.g. acridine dye to the guanine bases in viral DNA. When irradiated with visible light, the viral DNA is disrupted (5, 14).

This method has been proven effective in the treatment of herpes simplex infections in laboratory animals, and some studies indicate usefulness in the treatment of human herpes simplex infections (3, 10, 11, 13, 15).

Verrucae vulgares (v.v.) are benign tumours caused by human wart virus of the papovavirus group (7, 9). In order to determine the usefulness of photodynamic inactivation in the treatment of v.v. we have attempted to demonstrate that photodynamically active dyes, when applied to v.v. in vivo and in vitro, are capable of penetrating the v.v. We have also attempted to determine whether light of the quality used to elicit photodynamic action can penetrate warts.

MATERIALS AND METHODS

20 patients, each of whom had from 1 to 5 untreated warts which had been present for 3 weeks to 1 year, participated in this study. The v.v. of approximately half of these patients were dyed with one of the following: 1) 0.1% proflavine (3.6-Diaminoacridinum monohydrogen sulphate, May & Baker) in 100% dimethylsulphoxide (DMSO); 2) 1% proflavine in 100% DMSO; 3) 0.1% neutral red (Merck no. 1369) in 100% DMSO; 4) 1% neutral red in 100% DMSO. DMSO was used as the solvent because of its effect on the epidermal barrier (1, 8). 24 hours after application of the dye the v.v. were curetted carefully so as to preserve each as one piece of tissue. The anesthetic used was 1% carbocain injected subcutaneously. Following curettage, the v.v. were rinsed with saline to remove blood, which might alter the light absorption. The v.v. were stored at -70°C . 5 μ m frozen sections were used sequentially for fluorescence studies, transmission spectroscopy, and staining with hematoxylin-eosin. The sections were also stored at -70°C . Whole warts from 5 of the patients were embedded in methacrylate for light transmission studies.

A Zeiss microscope fitted with a HBO 200 lamp, a BG 12 primary filter, and a barrier filter no. 50 was used for the fluorescence studies. Prior to microscopy the sections were moistened with phosphate-buffered saline, pH 7.2.

The light transmission for whole warts and for 5 μ m wart sections was recorded on a Shimadzu MPS 50 L spectrophotometer, usually over the full wavelength range 350-900 nm. The whole warts were oriented in such a way that the light beam penetrated all the various layers of the wart from the surface through the basal layers of the wart. When investigating the sections a microspectrophotometry attachment was employed, and the field of view was 5 μ m in diameter. The absorption spectrum was scanned with the beam centred on various parts of the specimens.

The v.v. were left unstained in the remainder of the patients. These were also curetted using 1% carbocain as a local anesthetic. After rinsing with saline, approximately half of the unstained v.v. were placed in a chamber with the surface facing a filter paper disc moistened with either 0.1% proflavine in 100% DMSO or 0.1% neutral red in 100% DMSO. After 24 hours at 4°C the v.v. were frozen at -70°C ; frozen sections for

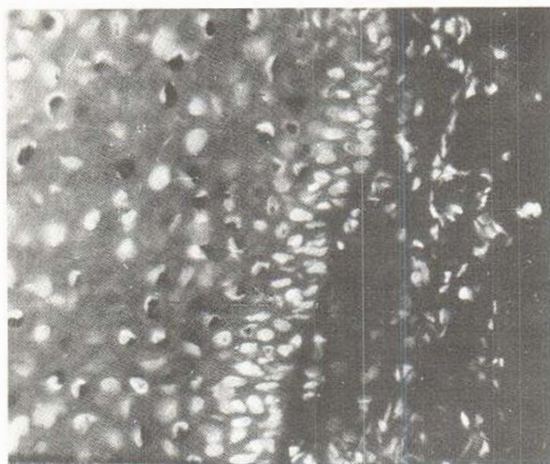


Fig. 1. Fluorescence microscopy of proflavine-dyed wart. The intense fluorescence of the cell nuclei in the basal sections of the wart is noted (left: epidermal layers; right: dermis) ($\times 225$).

fluorescence studies were prepared as described above.

The remainder of the unstained v.v. were frozen at -70°C immediately following curettage, and whole warts as well as frozen sections were prepared for use as controls.

RESULTS

The proflavine and neutral red stained surfaces of the v.v. were yellow and red, respectively, immediately following curettage, while the opposite sides were not visibly stained. Under black light (UV-A) the proflavine-stained warts showed a brilliant yellow fluorescence on all sides. This fluorescence could also be seen on the tissue beneath the original location of the v.v. Both the fluorescence and the visible staining were more intense when a 1% dye had been used, as opposed to a 0.1% dye, whereas the distribution of the dye was the same for both concentrations.

A section of a stained v.v. was characterized grossly by intense colouring of the superficial 1 mm, whereas there was no visible staining of the remainder of the v.v.

Fluorescence microscopy showed brilliant yellow-green fluorescence of the nuclei. The fluorescence was most intense in the superficial millimetre of each wart and in the suprabasal layers. These layers were identified by comparison with the hematoxylin-eosin stained sections. Weak fluorescence was also visible in the most superficial

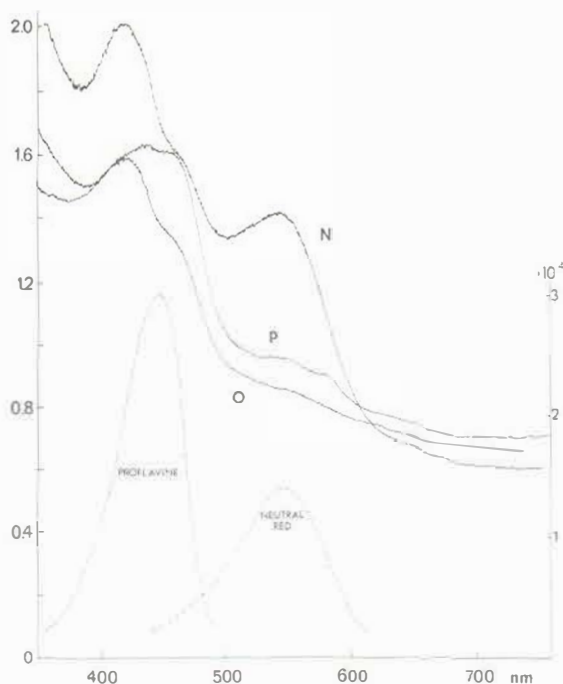


Fig. 2. Curves O, P and N: light absorption of whole warts (left ordinate in absorbance units). O: unstained wart; P: wart dyed with 0.1% proflavine; N: wart dyed with 0.1% neutral red. The curves marked proflavine and neutral red indicate the results of measurements of solutions of the pure compounds. The right ordinate applies and yields the absorption coefficient in $\text{M}^{-1} \times \text{cm}^{-1}$.

dermal nuclei (Fig. 1). The intensity and the distribution of the fluorescence did not vary with the age of the warts.

Evenly distributed fluorescence with no particular accumulation in the nuclei could be seen in the

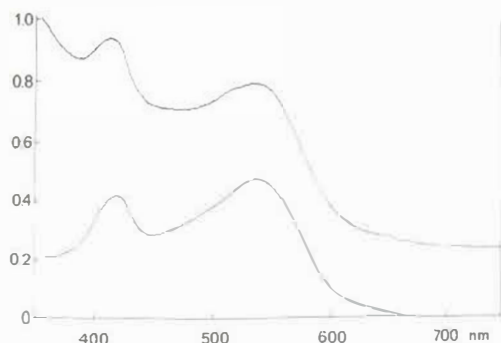


Fig. 3. Upper curve: experimental curve obtained when measuring the light absorption of a wart dyed with 1% neutral red. Lower curve: experimental curve corrected for light scattering (ordinate in absorbance units).

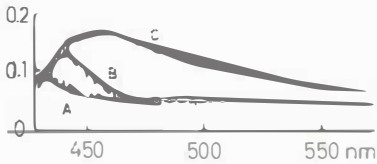


Fig. 4. Microspectrophotometry of a 5 μm section of wart dyed with 0.1% proflavine. Field of view: 5 μm . (A) Beam centred outside the specimens. (B) Beam centred on the cytoplasm of a cell. (C) Beam centred on the nucleus of a cell. (Ordinate in absorbance units.)

in vitro stained v.v. and in the adjacent connective tissue.

Neutral red stained v.v. showed no fluorescence in vivo or in vitro, regardless of variations in the pH of the buffer.

Fig. 2 shows typical results from transmission measurements of in vivo dyed whole warts (solid lines, left ordinate). The absorption spectra for aqueous solutions of the two pure dyes proflavine and neutral red are given in absolute units (dashed curves, right ordinate). The warts show intense light scattering. As a result of light scattering and absorption, only 1–2% of blue and near-ultraviolet light is transmitted. When correcting for the light scattering (using the Rayleigh λ^{-4} dependence) the absorption spectrum will correspond to that of hemoglobin and the dye. This is indicated in Fig. 3 where the experimental curve for a neutral red dyed wart is given, together with a curve obtained after correction for Rayleigh scattering. It is assumed that the absorbance at longer wavelengths is determined solely by the light scattering and these absorbance data are used to fit a λ^{-4} dependence. The peak at 420 nm is that of hemoglobin and that at 540 nm, of neutral red. From the absorption spectrum of the pure dye (Fig. 2) it may be estimated that the average concentration of the dye (neutral red) in the warts is of the order 2×10^{-5} M. This is true both for warts dyed with 1% and with 0.1% dye solution. Proflavine-dyed warts contain much less dye, and as hemoglobin has an absorption peak close to that of proflavine (450 nm) it is more difficult to obtain a fair idea of the dye concentration. A rough estimate indicates an average concentration of at least 10^{-6} M.

The microspectrophotometry of the 5 μm sections from neutral red dyed warts showed strong absorption at 550 nm in small granules located in the superficial layers of the wart. The recorded

absorption spectrum corresponded to the absorption of neutral red, and the granules were 10–20 μm in size. No other structure was found which had any significant absorption in the accessible wavelength region of 400–600 nm.

Microspectrophotometry of proflavine-dyed sections showed absorption at 450 nm (corresponding to the absorption maximum of proflavine) for structures which morphologically correspond to the cell nuclei (Fig. 4). The concentration of dye in these structures must be very high, approximately 0.05 M.

DISCUSSION

Photodynamic inactivation of virus may be a valuable therapy for cutaneous eruptions of herpes simplex infections (3, 11). Successful photodynamic inactivation of cutaneous disorders caused by viruses is attributable to the affinity of the dye for the virus and upon the ability of light to penetrate to the tissues harbouring the virus.

A study employing immunofluorescence microscopy showed a high concentration of wart virus in the suprabasal layers of the v.v. (4). It has also been shown that 0.1% proflavine dissolved in 10% DMSO and 90% water, when applied to the skin of guinea pigs, penetrates to the epidermal and dermal nuclei (2). Our findings of intense fluorescence in the suprabasal nuclei following in vivo application of proflavine to v.v., and fluorescence in dermal structures, are in agreement with these studies.

The high concentration of dye in the superficial layers of the v.v. may act as a light filter, and it seems likely that paring of the v.v. before irradiation will increase the amount of light penetrating them.

The dye concentration in the warts determined by the light transmission studies indicates that the cells are saturated with dye even when a 0.1% dye concentration is used, and that the excess dye disappears from the warts in less than 24 hours.

The light transmission studies of stained whole v.v. embedded in metacrylate showed a penetration of at least 1% of the amount of light delivered to the surface of the v.v., indicating that light will reach the layers of the v.v. where virus is accumulated.

The results of light transmission studies of 5 μm sections of v.v. suggest an accumulation of proflavine in the cell nuclei. This is in agreement with

the results of fluorescence microscopy. The dye granules observed in neutral red dyed warts could not be assigned to any specific structure of the cells, but may represent pycnotic cell nuclei. Light absorption at 450 nm for the proflavine-stained sections and at 540 nm for the neutral red sections supports the assumption that neutral red is distributed in the v.v. in a manner similar to proflavine. Light absorption at 420 nm corresponds to an absorption maximum of hemoglobin and indicates that blood in the v.v. may filter out some of the light (12).

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Received August 24, 1976

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