The laser parameters chosen to treat and study the patients with plantar or mosaic verrucae reflect a standard therapeutic approach for the laser vaporization of these types of lesions and thus mimic the clinical setting. In two of seven patients, intact viral DNA was recovered from the laser vapor. It was anticipated that although in the BPV model viral DNA could be consistently demonstrated for all studied parameters, the HPV lesion would be a more difficult model for viral detection. This prediction was based on the presence of smaller amounts of viral DNA in the HPV lesions relative to the bovine fibropapillomas, which were specifically selected for large numbers of virions by antigen screening. As such, although viral DNA was demonstrated in lesional biopsy specimens from six of seven patients, there was a smaller number of cases with detectable viral DNA in the vapor. It is possible with the availability of more sensitive DNA detection techniques that laser vapor in the HPV model would also be consistently positive.

Although prior studies failed to demonstrate viable cellular material in laser vapor, it is apparent from our study that intact viral DNA is recovered over a wide range of laser parameters in both the in vitro and in vivo setting. The question of the potential infectivity of the recovered material remains unresolved; however, papillomavirus DNA alone has been demonstrated to be infectious.[16,17] Thus, the medical implications of recovering intact viral DNA in laser vapor requires serious consideration, especially in regard to risks for the patient and clinical personnel. When performing laser therapy on viral-infected processes or on patients with viral infections such as hepatitis or human immunodeficiency virus, it would be prudent to assume that the plume of smoke may be infectious. Care should be taken to maintain close approximation of the suction tip of the vacuum system to the area of laser exposure, gloves and appropriate masks should be worn throughout the procedure by the laser personnel, and the vacuum apparatus should have frequent filter changes to ensure a high suction flow rate.

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Clinical Investigation

Thomas P. Stossel, MD, *Section Editor*

Papillomavirus in the Vapor of Carbon Dioxide Laser–Treated Verrucae

Jerome M. Garden, MD; M. Kerry O'Banion, MD, PhD; Lori S. Shelnitz, MD; Kevin S. Pinski, MD; Abnoeal D. Bakus, PhD; M. E. Reichmann, PhD; John P. Sundberg, DVM, PhD

Vapor produced by the carbon dioxide laser during the vaporization of papillomavirus-infected verrucae was analyzed for viral DNA content. Two models were used for evaluation: an in vitro cutaneous bovine fibropapilloma and an in vivo human verruca model. Four bovine fibropapillomas were exposed to various laser parameters with power densities of 38 200 to 130 W/cm² and energy fluences of 3820 to 130 J/cm². The generated vapor was collected in a chamber in line with a vacuum system. Hybridization with bovine papillomavirus DNA probes revealed intact bovine papillomavirus DNA for all power densities and energy fluences used. The laser vapor from seven patients undergoing carbon dioxide laser therapy for plantar or mosaic verrucae was also collected. Laser parameter settings were similar to those usually chosen for clinical tissue vaporization. Intact human papillomavirus DNA was present in the vapor from two of seven patients. These studies indicate that intact viral DNA is liberated into the air with the vapor of laser-treated verrucae. It would be prudent for all practitioners who use the laser in treating patients with viral infections or conditions associated with viruses to practice extreme care and safety throughout the laser procedure.

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THE MEDICAL use of the laser has gained wide acceptance in multiple specialties. The carbon dioxide (CO_2) laser remains the most frequently used owing to physical parameters that allow it to operate as a precise cutting and vaporization instrument. The emission wavelength of the CO_2 laser is 10 600 nm, which corresponds to an absorption band of water. Since most tissues have a high water content, the incident photon energy is readily absorbed and converted to thermal energy. If sufficient energy is incorporated into the exposed area, a rapid vaporization of cellular water results, with subsequent cellular disruption. Focusing the emitted laser beam into a small area allows the development of high-energy densities that can produce fine tissue cutting with low thermal transmission to adjacent tissue areas. By defocusing the beam, a wider area of thermal effect is produced, with lower energy densities, producing a more diffuse vaporization. The CO_2 laser–tissue interaction accounts for the spatial confinement of the thermal damage that significantly increases the precision of therapeutic control.

Laser therapists have taken advantage of the relative control of thermal tissue damage for treating many processes. One area of intensive use is in the treatment of verrucae. The term *verruca* referred to in the text is used in its general sense to include condyloma acuminata, laryngeal papilloma, all forms of warts, and the bovine fibropapilloma. Multiple specialists, including the dermatologist, gynecologist, otolaryngologist, podiatrist, general practitioner, surgeon, and urologist,[1-7] treat verrucae with the CO_2 laser. The perception among the medical community regarding the use of the CO_2 laser in the treatment of verrucae is one of general effectiveness with low risk to the patient or laser personnel.

When the laser interacts with tissue, a plume of smoke develops, which consists of vaporized material, steam, and particulate matter. The vapor is normally suctioned away from the therapy area by a vacuum system that passes through a filter. Study of the particulate matter after CO_2 laser tissue exposure has revealed mainly carbonized tissue and occasional intact cells.[8] Analyses of the cellular component for viability, using normal,[9] tumor,[8,10] and verrucae[11] tissue models, have all failed to show any cellular viability in the plume and debris that accompany CO_2 laser exposure. Only on rare occasions were bacteria or viable spores demonstrated in the vapor of CO_2 laser–exposed preinoculated tissues.[12,13] From these studies it would appear that the CO_2 laser vaporizes and devitalizes exposed tissue.

The verruca is a manifestation of tissue reaction to infection by the pap-

From the Department of Dermatology, Northwestern University Medical School (Drs Garden, Shelnitz, Pinski, and Bakus), and the Veterans Administration Lakeside Medical Center (Dr Garden), Chicago; and the Departments of Microbiology (Drs O'Banion and Reichmann) and Pathobiology (Dr Sundberg), University of Illinois, Urbana.

Reprint requests to Department of Dermatology, Northwestern University Medical School, 303 E Chicago Ave, Chicago, IL 60611 (Dr Garden).

Laser Parameter Values for the Bovine Fibropapilloma In Vitro Tumor Model

Parameters	Values 1	2	3	4
Power, W	12	12	4	4
Spot size, mm	0.2	2.0	0.2	2.0
Pulse, s	1/10	...	1/10	...
No. of pulses	500	...	500	...
Continuous, s	...	90	...	90
Power density, W/cm²	38 200	380	12 700	130
Energy fluence, J/cm²*	3820	400	1270	130

*The energy fluences for the pulsed exposures (Nos. 1 and 3) are calculated per pulse. The continuous exposures (Nos. 2 and 4) are spatially averaged energy fluences calculated, with the total irradiation area being 2.75 cm².

illomavirus.[14] An association of papillomaviruses with cancer in animals and humans[15] has accentuated the concern of the physician for effecting appropriate care. During CO_2 laser therapy, although intact or partially intact cells may be rendered permanently damaged and nonviable in the plume of smoke, there still remains the potential existence of intact virions or viral DNA. The intact virions or viral DNA may still be infectious,[16,17] and thus be hazardous to the patient as well as to the laser therapist and personnel.

To determine whether intact papillomavirus DNA exists in the plume of smoke during CO_2 laser treatment of verrucae, an in vitro tumor model was studied. Later a similar study of verrucae-infected patients treated in a clinical setting with the CO_2 laser was undertaken. These studies revealed that even with a variety of laser power densities and energy dosages, it was possible to demonstrate the presence of intact viral DNA in the plume of smoke obtained from either the in vitro or patient models.

MATERIALS AND METHODS

A CO_2 laser was used either in a defocused mode with a circular spot size of 2.0 mm or in a focused mode with a spot size of 0.2 mm. Power settings were either 12 or 4 W, emitted continuously or pulsed with a pulse duration of 0.1 s. An in-line phosphate-buffered saline (PBS) solution (pH 7.4) bubble chamber was assembled for vapor collection in the vacuum system (500 mm Hg).

In Vitro Tumor Model

Bovine cutaneous fibropapillomas were removed and analyzed for bovine papillomavirus (BPV) by the peroxidase-antiperoxidase technique.[18] Viral DNA was extracted from the fibropapillomas and typed after restriction endonuclease digestion.[19] Only tumors that were positive for papillomavirus group–specific antigens, and contained either BPV type 1 (BPV-1) or BPV type 2 (BPV-2) DNA, were used in the study. Intact viral DNA was obtained from purified BPV-2 virions as previously described.[19]

Four bovine fibropapillomas from four individual animals were studied. Two contained BPV-1, and the other two contained BPV-2. Normal bovine skin from an unaffected animal was used as a negative control. Each tumor received the following sets of laser parameter values directing the laser emission uniformly across the tumor: (1) 12 W, spot size of 0.2 mm, and a pulse duration of 0.1 s for 500 pulses (power density of 38 200 W/cm² and energy fluence of 3820 J/cm²); (2) 12 W, spot size of 2.0 mm, and continuous exposure for 90 s (power density of 380 W/cm² and a spatially averaged energy fluence of 400 J/cm²); (3) 4 W, spot size of 0.2 mm, and a pulse duration of 0.1 s for 500 pulses (power density of 12 700 W/cm² and energy fluence of 1270 J/cm²); and (4) 4 W, spot size of 2.0 mm, and continuous exposure for 90 s (power density of 130 W/cm² and a spatially averaged energy fluence of 130 J/cm²) (Table).

After collection of the vapor material in the PBS bubble-chamber apparatus, equal amounts of each sample were adjusted to a final concentration of 10-mmol/L ethylenediaminetetraacetic acid (EDTA) and 0.6% sodium dodecyl sulfate. Proteinase K (50 μg/mL) was added and the solutions were incubated for one hour at 55°C. After incubation, extraction was performed twice with equal volumes of phenol-chloroform (1:1) saturated with 0.1-mol/L TRIS hydrochloride buffer (pH 7.5) and a final time with only chloroform. Nucleic acids were precipitated overnight at −20°C with 2½ volumes of 100% ethanol in the presence of 0.3-mol/L sodium acetate. After centrifugation, rinsing with 70% ethanol, and drying, the nucleic acid was resuspended in 100 μL of 10-mmol/L TRIS hydrochloride, pH 8.0, and 1-mmol/L EDTA (TE buffer) containing 20 μg/mL of RNAse A for 30 minutes at 37°C. The sample was precipitated with ethanol, dried, and resuspended in 30 μL of TE buffer. Electrophoresis of half of the material was performed in 0.8% agarose gel in parallel with undigested BPV-2 virion and digested lambda DNA markers (Eco R1 and Hind III). The DNAs were later transferred to nylon membranes.[20] The membranes were subjected to hybridization using a BPV-2 virion DNA probe labeled to a specific activity of approximately 1×10^8 cpm/μg by nick translation[21] using [α–phosphorus 32]–deoxycytidine triphosphate (specific activity, 3000 Ci/mmole). The hybridization was carried out in 50% formamide as described previously.[19] Signals were detected by autoradiography with intensifying screens at −70°C.

Fig 1.—Plume of smoke generated during treatment of verruca of right large toe. Vacuum system not in use for vapor demonstration purposes only.

In Vivo Patient Study

Seven patients with plantar or mosaic verrucae were entered into the study. The patients were drawn from the regular laser therapy population that had plantar or mosaic verrucae. In general, these patients have either extensive verrucae or lesions that have been recalcitrant to other therapies. Prior to laser therapy a 2-mm lesional area from each patient underwent biopsy and the specimen was frozen immediately in liquid nitrogen. Afterward, each lesion was treated with the CO_2 laser set at 12 W, a spot size of 2.0 mm, and a power density of 380 W/cm² in a continuous mode until the bulk of the tumor was vaporized. The remainder of the lesion was then treated with the CO_2 laser at 4 W, a spot size of 2.0 mm, and a power density of 130 W/cm² in a continuous mode until the lesion was inapparent (Fig 1). The exposure time, area treated, and energy fluences varied with each patient, depending on lesional size and keratotic thickness. The vapor throughout the treatment session was collected into one PBS bubble chamber for each patient.

The hybridization techniques used to analyze the lesional biopsy specimens and laser vapor for human papillomavirus (HPV) DNA were similar to those described for the in vitro study, except that a cloned HPV-2a DNA probe was used. Hybridization testing with cloned HPV-1a or HPV-4 DNA failed to detect the viral DNA present in the lesional biopsy specimens.

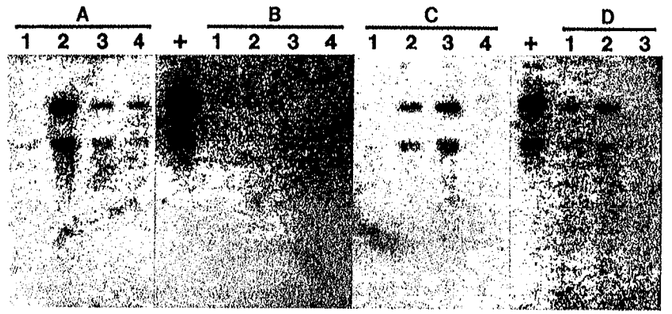

Fig 2.—Bovine papillomavirus (BPV) DNA in plume of laser-vaporized bovine fibropapillomas demonstrated by Southern blot hybridization with BPV-2 virion DNA probe (specific activity, 1×10^8 cpm/μg, exposed for one week with intensifying screens). Letters correspond to individual tumors, while numbers correspond to different sets of laser parameters as summarized in text and Table. Control lanes designated by plus sign contain 0.6 ng of BPV-2 virion DNA.

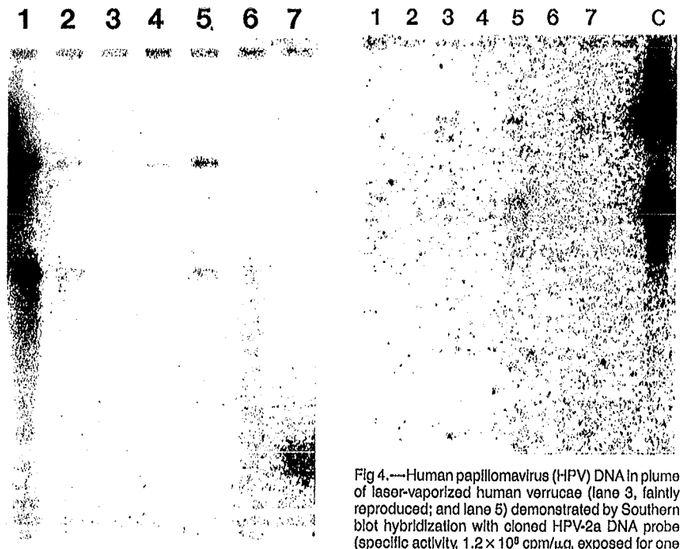

Fig 3.—Human papillomavirus (HPV) DNA in biopsy specimens of mosaic or plantar verrucae from seven patients demonstrated by Southern blot hybridization with cloned HPV-2a DNA probe (specific activity, 1.2×10^8 cpm/μg, exposed for one week with intensifying screens). Each lane contains 1 μg of DNA extracted from biopsy samples. (Faint bands present in lane 3 did not reproduce well; no viral-specific bands were detected from biopsy sample 7.)

Fig 4.—Human papillomavirus (HPV) DNA in plume of laser-vaporized human verrucae (lane 3, faintly reproduced; and lane 5) demonstrated by Southern blot hybridization with cloned HPV-2a DNA probe (specific activity, 1.2×10^9 cpm/μg, exposed for one week with intensifying screens). The lane designated as "C" contains 1.5 ng of BPV-2 virion DNA demonstrated by Southern blot hybridization with BPV-2 virion DNA probe.

RESULTS

In vitro tumor assays by dot-blot hybridization revealed BPV-specific DNA sequences present in the plume of smoke collected during laser therapy in all four treated fibropapillomas (data not shown). Intact BPV DNA was detected in the plume of smoke in three of the four treated fibropapillomas (Fig 2; parts A, C, and D). In evaluating the tested power densities and energy fluences, each set of laser parameters yielded detectable intact BPV DNA in at least one, and in some cases three, of the fibropapillomas (Table, Fig 2). Normal bovine skin was negative for the virus. Two forms of the DNA, supercoiled and circular, were present in the vapor samples, with the position of the bands corresponding to those obtained with DNA extracted from BPV-2 virions. The direct correlation between the vapor and control samples is evidence that the vapor-collected viral DNA was intact.

Analysis of the in vivo patient study demonstrated HPV DNA in six of the seven patient biopsy specimens (Fig 3). Viral DNA was detected in the collected laser vapor from two of seven patients (Fig 4, lanes 3 and 5). The position of the viral DNA bands of the laser vapor corresponded to the bands obtained with a control papillomavirus DNA preparation electrophoresed in parallel and to the viral DNAs observed in the tissue biopsy specimens. These results indicated that intact HPV DNA was present in the laser vapor.

COMMENT

The popularity of the CO_2 laser in the treatment of verrucae has increased dramatically over the last several years. The greater accessibility of the laser in the surgical and outpatient clinical area has allowed multiple specialists the opportunity to treat patients. Patients with laryngeal, cervical, and cutaneous verrucae may be significantly benefited by the CO_2 laser.[1-3,6,7]

Smoke evacuation is necessary during CO_2 laser therapy since copious amounts of vaporized material are generated. It has been demonstrated that when the end of the vacuum apparatus is moved only 2 cm from the exposed tissue, up to 50% of the particulate matter escaped into the air.[22] Therefore, the amount of matter escaping into the air can be significantly increased if the suction apparatus is not closely approximated to the exposed tissue or has a reduced negative pressure and flow secondary to mechanical or filter problems.

Viable cells have not been demonstrated in the CO_2 laser vapor by tissue-culture techniques,[8-10] vital dye-exclusion studies,[8] in vivo vapor inoculum reimplantation,[8] or metabolic activity studies,[11] although morphologically intact cells were occasionally observed.[8] According to some investigators,[13] the loss of cell viability in these studies was due to the use of power densities greater than 705 W/cm². It was suggested from the data that there may be a greater chance for dissemination of viable material if lower power densities are used. After inoculating bacterial spores intradermally, a few viable spores were recovered in the vapor material with power densities less than 500 W/cm².[13]

In this study, intact BPV DNA was detected in the laser vapor of exposed bovine fibropapilloma using power densities ranging from 38 200 to 130 W/cm², with accompanying energy fluences of 3820 to 130 J/cm². It is apparent from the in vitro tumor data that regardless of wide variations in power density, whether the laser is pulsed or continuous, focused or defocused, intact viral DNA may still be liberated in the plume of laser smoke.