Inhibition of Experimental Vasospasm by Pretreatment with Ultraviolet Light Irradiation in a Rat Femoral Artery Model

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OBJECTIVE: Chronic cerebral vasospasm is resistant to conventional treatments despite recent advances in treatment modalities. We studied the preventive effect of ultraviolet (UV) irradiation on development of vasospasm and its mechanism in a rat femoral artery model.

METHODS: The rat femoral artery model for vasospasm was used in this investigation (n = 108). The femoral arteries were divided into four groups: empty and no irradiation (control), UV irradiation (UV group), blood placement (VS group), and blood placement after UV irradiation (VS + UV group). Luminal area was measured, and smooth muscle cell counts in the medial layer of the vessel wall were obtained. An immunohistochemical study was performed with cross sections of fixed femoral arteries at 12 hours and 1, 3, 5, 7, and 49 days after blood placement. The rings of femoral arteries on Day 7 were subjected to pharmacological study.

RESULTS: Pretreatment with UV irradiation (VS + UV group) resulted not only in significant inhibition of chronic vasospasm but also in a significant decrease in smooth muscle cells compared with the VS group on Days 5 and 7. The UV-treated arteries (UV and VS + UV groups) exhibited a significant number of Bax- and Bcl-2-positive cells on Days 5 and 7, but few CPP-32 positive cells were observed at the same time points. In the pharmacological study, contractile response to KCl or phenylephrine was reduced significantly in the UV-treated arteries.

CONCLUSION: These results imply that UV irradiation prevents chronic vasospasm and suggest that UV-induced cell death plays an important role in the preventive effect without causing complications during the chronic period. (Neurosurgery 48:1318–1327, 2001)

Key words: Animal models, Cell death, Subarachnoid hemorrhage, Ultraviolet therapy, Vasospasm

Chronic cerebral vasospasm is the major cause of morbidity and mortality in patients after aneurysmal subarachnoid hemorrhage (SAH). Cerebral vasospasm is resistant to conventional doses of vasodilatory agents. Although the pathogenesis of cerebral vasospasm has not been elucidated despite intensive investigations during recent years, several lines of evidence have revealed that inflammation and/or immunological response play important roles in the development of cerebral vasospasm after SAH. This evidence includes the presence of inflammatory cells coincident with chronic morphological changes in the walls of vasospastic vessels (17, 20), increased levels of several inflammatory mediators in the cerebrospinal fluid of patients with symptomatic vasospasm (10, 22), and the inhibition of experimental vasospasm with anti-inflammatory drugs, immunosuppressants, and complement depletion (5, 24, 27).

Because ultraviolet (UV) irradiation can induce anti-inflammation and/or immunosuppression, UV light is used in phototherapy for immunological skin disorders such as psoriasis vulgaris, atopic dermatitis, and others (29). These observations imply that UV irradiation may reduce development of vasospasm caused by anti-inflammatory and/or immunosuppressive effects. We reported previously that pretreatment irradiation with UV light prevents the development of chronic vasospasm of a rabbit common carotid artery (15). Although the histological findings suggest that apoptosis might be involved in the mechanism of this prophylactic effect, the precise mechanism remains obscure (15). Therefore, in this study we investigated a time course of the preventive effect of UV light for development of vasospasm, as well as the mechanism of this preventive effect, by use of immunohistochemical and histological examination.
associated with apoptosis in a rat femoral artery vasospasm model.

MATERIALS AND METHODS

The rat femoral artery model for vasospasm developed by Okada et al. (17) was modified in the present study (7, 11, 13, 19, 20, 25, 27). One hundred eight male Sprague-Dawley rats weighing 300 to 400 g were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and allowed to breathe spontaneously on Day 0. By use of sterile microsurgical technique, 10-mm lengths of bilateral femoral arteries were exposed in the inguinal region and cleaned of adherent perivascular tissue. The following conditions were applied randomly before each femoral artery was wrapped in a 6-mm long Silastic cuff (Dow-Corning, Auburn, MI) that had a 3-mm inner diameter (n = 216): 1) empty and no irradiation (control group); 2) empty and UV light irradiation (UV group); 3) autologous whole blood placement and no irradiation (VS group); 4) autologous whole blood placement after UV light irradiation (VS + UV group). The autologous whole blood was obtained from the adjacent femoral vein and allowed to clot spontaneously. A 300-μl amount of blood clot was placed within the Silastic cuff. Animals were allowed to recover, and the vessels were fixed in situ by perfusion-fixation technique 12 hours (n = 20) and at Day 1 (n = 24), Day 3 (n = 24), Day 5 (n = 22), Day 7 (n = 66), or Day 49 (n = 60) as described below. All procedures were performed according to protocols approved by the animal care committee of National Defense Medical College. Perfusion of animals, imaging analysis for vasospasm, cell counts in the medial layer, histopathological studies, pharmacological experiments, and data analysis were blinded.

UV irradiation

The exposed femoral arteries were isolated from the surrounding tissue by use of aluminum foil and irradiated with UV (wavelength, 313 nm) with a spot 7 mm in diameter at 0.85 mW/mm² for 30 seconds. The 313 nm of UV irradiation were obtained by use of a 200 W Hg-Xe L5662-01 lamp (Hamamatsu Photonics, Shizuoka, Japan) coupled with a VPF-25C-10-15-31300 narrow-band-pass filter (peak, 313 nm; full-width half maximum, 2 nm) (Sigma Koki, Saitama, Japan) coupled with a 200 W Hg-Xe L5662-01 lamp (Hamamatsu Photonics, Shizuoka, Japan) coupled with a VPF-25C-10-15-31300 narrow-band-pass filter (peak, 313 nm; full-width half maximum, 2 nm) (Sigma Koki, Saitama, Japan). The UV irradiance was measured by a Model LM-10 power meter (Coherent, Auburn, CA).

Preparation of vessels for histological examination

At 12 hours and on Days 1, 3, 5, 7, or 49, animals were anesthetized deeply with intraperitoneal sodium pentobarbital (100 mg/kg), and vessels were perfusion-fixed via intracardiac infusion at physiological mean arterial pressure with 50 ml of saline, followed by 150 ml of 4% paraformaldehyde in phosphate buffer. Perfusion was performed at a standard height of 100 cm from the chest. After perfusion-fixation, 6-mm segments of both femoral arteries encompassed by the Silastic sheet were removed and stored overnight in cold (4°C) perfusion fixative. The femoral artery was cut into two segments and embedded in paraffin. The specimens were cross sectioned at a thickness of 3 μm and stained with hematoxylin and eosin.

Immunohistochemistry and deoxyribonucleic acid (DNA) in situ end labeling

To examine involvement of apoptosis in the preventive effect of UV irradiation, sections were studied for expression of apoptosis-related proteins and DNA fragmentation at the following time points: 12 hours and Days 1, 3, 5, and 7. The following primary polyclonal antibodies were used: Bcl-2 (N-19, no. sc 492); Bax (P-19, no. sc 526); and CPP-32 (H-277, no. sc 7148) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunohistochemistry was performed with a standard, indirect streptavidin-biotin immunoreaction technique by use of the Dako LSAB2 Kit (Dako, Carpinteria, CA). Paraffin-embedded 3-μm-thick tissue sections were cut and mounted on Silan-coated glass slides, and deparaffinization and rehydration were performed. Degraffinized tissue sections were placed in a glass jar filled with 10-mmol/L citrate buffer, pH 6.0, and then placed in a 500-W microwave oven twice for 5 minutes. The sections were dipped in 3% H₂O₂ solution for 10 minutes to inactivate endogenous peroxidase. The primary antibodies against Bcl-2, Bax, and CPP-32 were diluted 1:500, 1:200, respectively, and incubated overnight at 4°C. Automated immunohistochemistry was performed with a standard, indirect streptavidin-biotin kit. The final reaction product was visualized by use of 0.05% 3,3′-diaminobenzidine as the chromogen in the presence of 0.02% hydrogen peroxide for 5 minutes. Sections were counterstained with hematoxylin.

We examined DNA fragmentation by a method for in situ labeling of DNA breaks in nuclei. The method is based on the specific binding of terminal deoxynucleotidyl transferase to 3'-OH ends of DNA (TUNEL) (4). We used the TACS in situ apoptosis detection kit (Genzyme, Cambridge, MA) for this purpose. The procedure was performed in accordance with the manufacturer’s instructions. In brief, the deparaffinized sections were digested by 20 μg/ml protein kinase K for 15 minutes, rinsed with phosphate-buffered saline and quenched with 2% H₂O₂, then incubated with terminal deoxynucleotidyl transferase and biotinylated deoxyuridine 5-triphosphate solution. The DNA nicks labeled with terminal deoxynucleotidyl transferase-deoxyuridine 5-triphosphate were reacted with streptavidin-horseradish peroxidase solution and detected with diaminobenzidine tetrahydrochloride. Finally, the sections were counterstained with methyl green, dehydrated, coverslipped, and observed under a light microscope. As positive controls for staining, we used a tissue in which the DNA of every cell was fragmented with TACS-nuclease.
DNAase (Genzyme) for the TUNEL staining and a rat thymus in the immunohistochemical study.

Cross sectional area measurements and cell counts in the medial layer

The lumen cross sectional area in the hematoxylin and eosin-stained sections was calculated by computerized image analysis using the public domain NIH Image program (developed at the National Institutes of Health and available on the Internet by anonymous file transfer protocol from zippy.nimh.nih.gov). An actual circumference of the vessel lumen was measured, then a radius \( r \) of a generalized circle was calculated by the equation \( r = \frac{\text{measured circumference}}{2\pi} \). An area of the generalized circle corresponding to \( \pi r^2 \) was defined as a lumen cross sectional area, thus correcting for vessel deformation and off-transverse sections (20, 27). For each vessel, two separate sections at least 200 \( \mu m \) apart were measured and averaged.

Proliferation or reduction of smooth muscle cells was scored quantitatively. The digital images of the hematoxylin and eosin-stained sections were analyzed by enhancing their blue component for the determination of nuclei (hematoxylin positive) with Photoshop version 5.0 software (Adobe Systems, Mountain View, CA). The nuclei of smooth muscle cells, lying between the internal elastic lamina and the external elastic lamina, were counted manually.

Pharmacological analysis

For the pharmacological study, 40 additional femoral arteries (20 rats) were obtained on Day 7 and cut into rings 1.2 mm in length. The vascular rings were suspended at a tension of 400 mg between stainless steel hooks (0.08 mm in diameter) in 5-ml water-jacketed tissue baths (Ufer; Medical Kishimoto, Kyoto, Japan) filled with Krebs-Ringer bicarbonate solution (millimolar composition: NaCl, 118; KCl, 4.8; CaCl\(_2\), 2.5; KH\(_2\)PO\(_4\), 1.2; MgSO\(_4\), 1.2; NaHCO\(_3\), 24; and glucose, 11) bubbled with 95\% O\(_2\)/5\% CO\(_2\) at pH 7.4 and 33.5°C. The 33.5°C temperature, rather than 37°C, was adopted because of longer tissue viability and much slower rundown of the preparations (16); we first confirmed that contractile responses for phenylephrine and KCl at 33.5°C were similar to those at 37°C. The rings were equilibrated for 1 hour, and the bath solution was changed every 20 minutes. The response to 80 mmol/L KCl was recorded, and preparations were washed until the resting tension again was obtained. Cumulative dose-response curves for phenylephrine (10\(^{-8}\) to 10\(^{-5}\) mol/L) were then recorded for each arterial ring. At the end of the experiments, the tissues were tested again with 80 mmol/L KCl for recovery. Only arterial rings with a recovery response of 85 to 115% of the initial stable KCl response were included.

Data and statistical analysis

All data are presented as means \( \pm \) standard error of the mean. Statistical analysis was performed using a Kruskal-Wallis nonparametric analysis of variance followed by a Scheffé’s test multiple comparison if a significant probability was reached. A level of \( P < 0.05 \) was considered significant.

RESULTS

Effect of UV irradiation on development of vasospasm

Cross sectional areas of the femoral arteries are shown in Figure 1A. On Day 7, the luminal areas for the VS group (n = 14) and control group (n = 22) were 0.149 \( \pm \) 0.013 mm\(^2\) and 0.208 \( \pm \) 0.011 mm\(^2\), respectively. Because there was a significant reduction in the luminal area in the VS group on Day 7 (72% of the control group; \( P = 0.007 \)), it was judged that vasospasm was induced successfully in this model. Although the luminal areas in the groups with placement of autologous blood around vessels (VS and VS + UV groups) were smaller than in the non-VS groups (control and UV groups) at 12 hours and Day 1, there were no significant differences among all groups on Day 3. Luminal areas in the UV and VS + UV groups were significantly larger than in the VS group on Days 5 and 7, and compared with the control group on Day 7 (A). In contrast, the cell counts of the UV-treated groups (UV and VS + UV groups) were significantly decreased compared with the VS group on Days 3, 5, and 7 and compared with the control group on Day 7 (B). *, \( P < 0.05 \) compared with the VS group; **, \( P < 0.01 \) compared with the VS group; #, \( P < 0.01 \) compared with the control group.

![FIGURE 1. Graphs showing the time course during 7 days of changes in measured cross sectional areas of lumen (A) and smooth muscle cell counts in the medial layer (B) in rat femoral arteries for each of the vessel categories. A cross sectional luminal area of UV-treated groups (UV and VS + UV groups) was significantly larger compared with the VS group on Days 5 and 7, and compared with the control group on Day 7 (A). In contrast, the cell counts of the UV-treated groups (UV and VS + UV groups) were significantly decreased compared with the VS group on Days 3, 5, and 7 and compared with the control group.](image-url)
5 (P < 0.05) and 7 (P < 0.01). On Day 7, luminal areas of vessels in the UV group (n = 8) and VS + UV group (n = 11) were larger than in the control group, reaching 146% and 134% of the control group (n = 22), respectively. These results revealed that pretreatment with UV irradiation resulted in a significant inhibition of chronic vasospasm. Smooth muscle cell counts in the medial layer in UV-treated vessels (UV and VS + UV groups) were decreased significantly compared with untreated vessels (control and VS groups) on Days 3, 5, and 7 (Fig. 1B). On Day 7, cell counts of the VS + UV group reached their lowest value, which was 54% of the cell counts of the VS group. There were no significant differences between the control group and the VS group at all time points of 12 hours and Days 3, 5, and 7 (Fig. 1B).

On Day 49, UV-treated arteries remained slightly larger (UV group [n = 10], 0.413 ± 0.033 mm²; VS + UV group [n = 9], 0.388 ± 0.036 mm²) than untreated arteries (control group [n = 20], 0.305 ± 0.027 mm²; VS group [n = 19], 0.292 ± 0.028 mm²), but the results did not reach statistical significance (Fig. 2A). There were no significant differences in the smooth muscle cell counts among all groups on Day 49 (Fig. 2B).

Histological studies

In the vessels of the control group, no histological damage was observed at any time point. At Day 7, the femoral arteries exposed to periadventitial whole blood (VS group) demonstrated morphological changes throughout the vessel wall. Endothelial cells appeared to be contiguous, but they were distorted by convolutions of the adjacent internal elastic lamina and protruded into the lumen. Smooth muscle cells in the media exhibited a shorter and thicker configuration (Fig. 3). These features are comparable with a spectrum of histological changes associated with vasospasm reported in previous studies (13, 17, 19). The UV effect (UV and VS + UV groups) was associated with loss of smooth muscle cells in the media; however, neither the internal elastic lamina nor the external elastic lamina were torn (Fig. 3). The loss was prominent on Days 5 and 7 (Fig. 1B). Furthermore, on Day 7, the arteries of the VS + UV group exhibited thinning of the vessel wall and extension of the internal elastic lamina compared with the arteries of the VS group (Fig. 3). At all time points, no obvious signs of necrotic cellular changes such as edema, eosinophilic staining enhancement, or inflammatory infiltration were present in the UV-treated arteries (UV and VS + UV groups). The cross sections on Day 49 demonstrated no histological abnormality in the vessels of all four groups (Fig. 3). Neither obstruction nor perforation were observed in any of the groups at any time point.

Time course of expression of apoptosis-related protein and DNA fragmentation

In the UV-treated groups (UV and VS + UV groups), few Bcl-2-positive cells were observed 12 hours or 1 day after blood placement in the perivascular space, but the appearance of Bcl-2-positive cells increased on Days 3, 5, and 7 (Figs. 4 and 5). In the UV-treated groups (UV and VS + UV groups), Bax-positive cells appeared remarkably on Days 5 and 7 (Figs. 4 and 5). Conversely, Bax or Bcl-2-positive cells were scarcely observed in untreated groups (control and VS groups) at any time point (Fig. 4). Although CPP-32-positive immunoreactivity was observed occasionally in the vessels of the UV-treated groups (UV and VS + UV groups) on Days 5 and 7 (Fig. 5), the population of its positive cells was not different from that in vessels of untreated groups (control and VS groups) (data not shown). On Day 7 in UV-treated groups (UV and VS + UV groups), there were few TUNEL-positive cells (Fig. 5), and the population was comparable to that of the untreated groups (control and VS groups) (data not shown).

Pharmacological study

Response to a single dose of 80 mmol/L KCl and cumulative dose-response curves for phenylephrine were recorded for the four groups of vessels (Fig. 6). The contractile response of arteries to KCl was comparable between the VS group and the control group (0.43 ± 0.07 g versus 0.51 ± 0.06 g, respectively). UV-treated vessels (UV and VS + UV groups) demonstrated significantly diminished contractile responses to 80 mmol/L KCl by approximately 60% compared with vessels of untreated groups (control and VS groups; P < 0.01) (Fig. 6A). Furthermore, in comparison with the untreated vessels (con-
trol and VS groups), the UV-treated vessels (UV and VS + UV groups) did not respond to the phenylephrine (Fig. 6B). However, there was no difference in the cumulative dose-response curve of the vessels between the UV group and the VS + UV group. In summary, UV irradiation significantly reduced contractile responses of both normal and vasospastic arteries.

RESULTS

Presumed mechanism of inhibition of vasospasm development

The results of the present investigation indicate that pretreatment with UV irradiation can prevent development of chronic vasospasm in a rat femoral artery model. The reduction in the number of vascular smooth muscle cells in the media was observed when the preventive effect of UV pretreatment for vasospasm was obvious. This implies that UV-induced cell death in the vessel wall may be involved in preventing the development of chronic vasospasm. The UV-induced cell death in this study seems to be different from typical apoptotic cell death, because DNA fragmentation was rarely observed in the UV-treated arteries. Expression of caspase-3 (CPP-32), which is an effector to induce apoptosis, was almost absent in these results. However, Bax and Bcl-2-positive cells were frequently observed in the UV-treated vessels. This UV-induced cell death also differs from typical necrotic cell death because of the absence of histological signs implying necrotic features such as edema, eosinophilic staining enhancement, and inflammatory infiltration. These findings seem to agree with characteristics of caspase-independent cell death, which has been revealed by recent reports (1, 14, 23). This type of cell death demonstrates the cytoplasmic features of apoptosis (cell shrinkage, decrease in mitochondrial transmembrane potential, and phosphatidylserine externalization) without the nuclear features (chromatin condensation, appearance of single-stranded DNA, DNA fragmentation, and cleavage of polyadenosine diphosphate-ribose polymerase) (14). The caspase-independent cell death occurs in the absence of caspase activation and is not inhibited by the overexpression of antiapoptotic Bcl-2 protein (2). Bax is well known as a proapoptotic molecule in a typical apoptosis, and Bax-induced alterations in mitochondrial function lead to and enhance the caspase-independent cell death (18, 30). In the present study, the cell death in the medial layer occurred 3 days after the UV irradiation. The onset was much later than that of typical apoptotic cell death, which usually develops within 24 hours after exposure to triggers. Delayed onset might be one of the characteristics of the caspase-independent cell death. Stefanis et al. (26) demonstrated that delayed cell
death, not apoptosis, of embryonic cortical neurons progresses in a caspase-independent manner.

On Day 7, both normal arteries and vasospastic arteries subjected to UV (UV and VS + UV groups) demonstrated markedly attenuated contractile responses to high potassium or phenylephrine compared with arteries that did not receive UV irradiation (control and VS groups). Given the results from both the pharmacological and the pathological experiments, the attenuated contractile response in UV-treated arteries may be reasonably attributed to the reduced numbers of smooth muscle cells. This hypothesis is reinforced by MacDonald et al. (12), who suggested that contractile tension change of a vascular ring could be positively correlated to numbers of smooth muscle cells. The attenuated contractility may diminish the arterial tone, resulting in dilation of the artery. This can be explained by the observation that the luminal area in UV-treated arteries was larger than that in the arteries without UV irradiation. The reduced contractility also

FIGURE 5. Photomicrographs of immunohistochemistry and TUNEL staining of cross sections of UV-irradiated arteries before placement of perivascular space (VS + UV group) on Days 3, 5, and 7. Bax- and Bcl-2-positive cells were observed in the medial layer on Day 3 and were apparent at Days 5 and 7. CPP-32-positive cells were observed rarely on Days 5 and 7, and TUNEL-positive cells were scarcely present on Day 7. As a result, the incidence of CPP-32-positive cells or TUNEL-positive cells is small and comparable to that in untreated groups (control and VS groups). Scale bar, 5 μm.

FIGURE 6. Concentration-response curves of the four vessel groups (control, VS, UV, and VS + UV; n = 6 each) to the two vasoconstrictors: KCl (A), and phenylephrine (B). One concentration of KCl (80 mmol/L) was tested. For KCl at 80 mmol/L concentration, the UV-treated vessels (UV and VS + UV groups) demonstrated a significant reduction in vasoconstriction compared with the untreated vessels (control and VS groups). The trend was present for phenylephrine at higher concentrations. M, vasoconstrictor concentration (molar); $, P < 0.05$ compared with the control group; **, $P < 0.01$ compared with the VS group.
may be associated with the inhibition of vasospasm, as most investigators agree that a contractile process, which occurs either in smooth muscle cells or involves myofibroblasts, is responsible for vasospasm (9, 31). Therefore, we think that the reduced contractility, which resulted from the delayed cell death of the smooth muscle cells, is a dominant mechanism of the preventive effect of UV irradiation on development of delayed vasospasm.

In contrast, neither the preventive effect of UV on the development of early vasospasm nor the death of vascular smooth muscle cells were observed at 12 hours and Day 1. These findings imply that cell death might play an important role in the preventive effect of UV on vasospasm. The initiation of the UV-induced cell death precedent to the onset of vasospasm seems to be necessary for the execution of the preventive effect. This hypothesis could explain why UV irradiation was not effective for early vasospasm.

**Limitation of the rat femoral artery model**

Although an animal model for cerebral vasospasm should be created in cerebral arteries, we used a rat femoral artery in this study for technical reasons. The intracranial location of cerebral arteries makes it difficult to irradiate them with UV light. The femoral artery model has been validated by Okada et al. (17), who documented that the rat femoral artery shares many morphological and physiological features of the cerebral arteries. Because histological changes associated with vessel narrowing in the rat femoral artery model closely parallel those observed in intracranial vessels in human SAH and animal models of SAH, these findings suggest an inflammatory mechanism that is preserved across both vessel types (6, 17). However, the results of this study must be replicated in an intracranial model of chronic posthemorrhagic vasospasm before they are generalized to chronic vasospasm after SAH.

**Suitable UV range and penetration of UV into the femoral artery**

We expected that narrow-band UVB (313 nm) would be appropriate for the present study because it has a greater immunosuppressive effect and a lesser carcinogenic effect. Narrow-band UVB (wavelength, 311 nm) phototherapy without a component less than 290 nm has been demonstrated more effective for the treatment of psoriasis than conventional broad-band UVB phototherapy (wavelength; 270–350 nm) (3, 21). In addition, the narrow-band UVB source can lessen the risk of late-onset impairment of the tissue; a study with a comet assay has revealed that the narrow-band UVB lamp has a lower carcinogenic potential than the broad-band UVB lamp (28).

We made preliminary measurements of the transmittance of 313 nm light into the rat femoral artery including three layers (adventitial, media, and intima) and found that approximately 15% of the incident light penetrated the wall of the femoral artery, which was usually 100- to 120-μm thick. As a result, the light energy seems to be sufficiently absorbed in the layers of the artery, whereas the energy does not seem to reach the opposite side of the artery because the blood inside the artery absorbs UV light. Therefore, in the present study, the UV effect may be limited to the exposed side. One of the methods to irradiate an artery in a cylindrical pattern would be through endovascular transluminal irradiation and use of an optical fiber with a diffuser tip.

**Clinical application and possible complication**

In clinical applications for the treatment of chronic vasospasm, considerations of a therapeutic window and its possible complications are important. After UV irradiation, dilation of vessels was observed, and chronic vasospasm did not develop. Because UV light does not penetrate the blood clot, we irradiated a vessel with UV light externally before blood immersion. Although as yet the procedure might not be clinically acceptable, the persistence of the UV-induced dilation of vessels might lead to prospective clinical value after SAH. Intraoperative UV irradiation on the cerebral arteries could be useful in the prevention of chronic vasospasm. The development of less invasive endovascular techniques may allow us to apply UV irradiation as an adjuvant therapy to coil embolization. UV-induced cell injury included only cell death, not immediate vascular damage such as perforation, disruption, and necrosis, because the UV power was quite low in the present experiment. The extent of UV-induced injury seems to be much less than that caused by percutaneous transluminal angioplasty, which induces endothelial damage, extension, disruption of the smooth muscle cell arrangement, and perforation of the vascular wall (8, 9, 32). There were no sustained differences in pathological findings at Day 49 between UV-treated vessels (UV and VS + UV groups) and untreated vessels (control and VS groups). These results imply that UV irradiation could be a potential therapeutic strategy without the complications caused by severe vascular injury.

**CONCLUSION**

The results from the present study imply that UV irradiation prevents the development of chronic vasospasm, and that UV-induced cell death might play an important role in the preventive effect. Although these findings are preliminary, they provide encouragement that UV irradiation may open a novel avenue for the treatment of chronic cerebral vasospasm.

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REFERENCES


COMMENTS

This article is informative regarding the mechanism of vascular damage by ultraviolet (UV) light at the power dose used, and the impact of this damage on the ability of the blood vessels to develop chronic vasospasm response in an experimental model. UV irradiation seems to result in nonapoptotic (noncaspase-related) and non-necrotic cell death in the vessel wall. This damage seems to inhibit the chronic vasospasm response observed in control vessels not subjected to UV irradiation. Although the authors explain their rationale for selecting the power range for irradiation, the dose-response of this injury is not well studied, and its relationship to inhibition of vasospasm is not clearly defined. It is possible that

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lower ranges of UV irradiation, and possibly exposure to other, less-harmful light spectra, may or may not result in similar injury or similar protection from vasospasm. It is clear that the response has been tested only in a situation in which the vessel was irradiated before application of blood. It is not clear whether subsequent irradiation of the vessel (in a model in which blood could be washed out temporarily to allow irradiation) will preserve the therapeutic response.

The study is less useful regarding the potential therapeutic use of this modality. The authors speculate that UV irradiation may be delivered directly at the time of surgical exposure of basal vessels, through the tip of an endovascular catheter. However, subarachnoid vessels may react differently to this type of injury than femoral arteries in an experimental animal. UV light may cause profound damage that could later result in vascular aneurysmal failure, dissection, or occlusion. Otherwise, it may be completely ineffective in the prevention of human vasospasm after subarachnoid hemorrhage (SAH).

Nevertheless, I encourage the authors to pursue this type of research. The next experiments should aim to better define the dose-response curve of this injury response and to apply it in a different model system, such as SAH in a canine or primate model. For the present, I hope that no one will be tempted to introduce this therapy in humans. Much more work must be performed to ensure that this approach will do more good than harm to the vessel wall.

Issam A. Awad
Denver, Colorado

It has been demonstrated that cerebral artery stretch injury, performed in vivo with an angioplasty balloon before experimental SAH, prevents development of vasospasm (1); the most likely mechanism seems to be some type of temporary derangement of the vessel wall contractile mechanisms (2). This observation has resulted in attempts to prevent clinical vasospasm by “prophylactic” balloon angioplasty soon after SAH and aneurysm repair (3). In this experimental study, creating an injury to rat femoral arteries with UV irradiation similarly seemed to prevent vasospasm in response to periarterial blood application, impair pharmacological contractile responses, and cause smooth muscle cell death. Contrary to some of the authors’ implications, it is not at all clear that this is related to inflammation, either in its prevention or suppression. Although UV irradiation may be anti-inflammatory when inflammation exists, the arteries were normal when they were irradiated. The observations are interesting, and although clinical application may seem distant, it seems no less reasonable to irradiate susceptible arteries intraluminally by an endovascular route than to subject patients to the risk of mechanical dilation on a prophylactic basis.

J. Max Findlay
Edmonton, Alberta, Canada


The authors have examined the effect of UV light irradiation on the prevention of experimental vasospasm in the rat femoral artery model. The current study confirmed the findings of their previous work with rabbit carotid arteries and demonstrated that UV light administered before the exposure to blood can inhibit the development of arterial narrowing in these models.

The clinical relevance of these findings is suggested in the Discussion section; however, significant barriers to implementation are raised by the investigators’ findings. The exposed arteries demonstrate functional changes long after vasospasm is clinically relevant in SAH patients. The current study suggests that this is because UV light acts on arteries by promoting apoptosis. It seems reasonable to think that in the cerebral vasculature, this might affect autoregulation and produce unwelcome side-effects. The ability to shield surrounding tissue from the effects of UV irradiation also may be a limiting factor in the central nervous system. Given the long-term morphological and functional changes described in this study, it seems that this therapy would not be appropriate for prophylactic use.

Warren R. Selman
Cleveland, Ohio

The authors have described the induction of arterial dilation in rat femoral arteries after treatment with UV irradiation and before the placement of an experimental perivascular blood clot. They used 108 rats to determine the effects of experimental perivascular blood on histological appearance, calculated lumen area, smooth muscle cell counts, induction of apoptosis, and pharmacological properties of the arteries with and without treatment by UV irradiation. They found an increase in vessel caliber after treatment with UV light as well as a decrease in smooth muscle cell counts, a reduction in pharmacological reactivity, and an increase in markers of cell death. It seemed that the UV-induced cell death occurred through nonapoptotic processes. On the basis of their results, the authors suggest a possible role for UV irradiation during surgery or embolization in the prevention of human chronic cerebral vasospasm, after validation of their results in an intracranial model.

A weakness of this article is that no baseline femoral artery lumen cross sectional area measurements were obtained at time zero in the VS or VS + UV groups. The first measurements were taken 12 hours after the various treatments. By this time, the VS and VS + UV groups already demonstrated decreased area compared with the control or UV-only arteries. Because vasospasm does not occur immediately, it would
be beneficial to demonstrate that the arteries have a normal diameter at the time the perivascular blood clot is placed. Nonetheless, if it is assumed that baseline femoral arterial areas were comparable among all groups, and that the model used induces acute and chronic vasospasm reproducibly, the results are intriguing. It is interesting that UV irradiation prevents delayed experimental vasospasm, although it does not alter acute vasospasm. The pathobiological process that underlies this protective effect against delayed vasospasm is not entirely clear. Although the authors have convincingly demonstrated that it is correlated with loss of vascular smooth muscle cells, presumably as a result of the death of the cells, there was no evidence of classic necrotic or apoptotic death. The proapoptosis-associated protein Bax appeared prominently within vascular cells in UV-treated arteries at Days 5 and 7, and the antiapoptotic protein Bcl-2 was found in UV-treated arteries at Days 3, 5, and 7. Because only semiquantitative data were obtained without statistical analysis, it is not known whether these changes in Bax and Bcl-2 were statistically significant. No differences in the caspase CPP-32 or terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling staining (both markers for apoptosis) were observed. Thus, we are left with the provocative suggestion that UV irradiation causes vascular smooth muscle death through caspase-independent (nonnecrotic, nonapoptotic) processes.

UV irradiation in the femoral artery rat model seems to cause little inflammatory reaction of the vessels. Whether this therapy will ultimately prove useful for the prevention of clinical chronic vasospasm depends on results in future experimental intracranial models of chronic vasospasm. The authors should be encouraged to pursue this novel therapy for vasospasm, which may have advantages compared with angioplasty.

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From, J. (Jean) Cruveilhier’s Anatomie pathologique du corps humain, ou Descriptions, avec figures lithographiées et coloriées, des diverses altérations morbides dont le corps humain est susceptible. Paris, Bailliére, vol. 2, 1829–1842. (Courtesy, Rare Book Room, Norris Medical Library, Keck School of Medicine, University of Southern California, Los Angeles, California.)