Evaluation of Ethyl Violet as an Alternative Dye to Crystal Violet to Visualize the Vessel Wall during Vascular Anastomosis

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Abstract

Crystal violet (CV) ink has been used as a skin marker worldwide. It has been reported to be useful for vessel wall visualization of microvascular anastomoses. Contrastingly, it has been found to be carcinogenic and inhibit migration and proliferation of venous cells. In some countries, its use in the medical field has been restricted. Therefore, it is necessary to consider alternatives to CV. In this present study, we compared the time required for the anastomosis of a 0.8-1 mm diameter vessel in the chicken wrist artery using CV and a CV-free dye (ethyl violet; EV). The surgeon, microscope, and anastomosis microsurgical tools were standardized for comparison. CV and EV were changed for each anastomosis. The same surgeon performed 30 anastomoses using each dye. No visually obvious differences were noted in the vascular transections with CV and EV. As per the results, no statistically significant difference was observed in the time required for anastomosis using CV and EV. EV conforming to California Proposition 65 may be an effective alternative to CV for vascular visualization of microvascular anastomoses. However, further studies on the effectiveness of the EV in clinical cases are needed.

Keywords: anastomosis, dye, crystal violet, microsurgery, neurosurgery

Introduction

Crystal violet (CV) (tris(4-(dimethylamino)phenyl)methyl chloride, Gentian violet),1,2 a triphenylmethane dye, has been widely used in the human body and veterinary medicine as a biological dye.3,4 It is also used as a dye in the textile processing industry to produce a dark purple color.5 CV is useful in the field of neurosurgery, as it is often utilized for visualization of the dissected vessel walls with a diameter of approximately 0.8 mm during vascular anastomosis in pediatric Moyamoya disease.1,6-8 Moreover, CV is known to have strong bactericidal and antifungal activities against Staphylococcus aureus, including MRSA, and Pseudomonas aeruginosa, although it is reported to be ineffective against Gram-negative bacteria and Mycobacterium tuberculosis.9,10 An advantage of using CV is that it has bactericidal and growth-inhibitory activities even at low concentrations and has been observed to be effective against MRSA at concentrations as low as 0.1%.10 In contrast, CV is reported to act as a carcinogen, and its use in the medical field has been restricted in Japan.5 However, no reports of its carcinogenesis have been confirmed when used for medical purposes.11

Considering these points, there is a need to identify an alternative to CV. In this study, we report on the use of ethyl violet (EV) (C31H42ClN3, C.I. Basic Violet 4)11 for microvascular anastomosis in response to the California Proposition 65 and its potential as a substitute for CV. EV was obtained from a surgical marking pen (Sandel 4-in-1 Marker, Nipro, Osaka Japan). Details of the cytotoxicity data were not disclosed by the manufacturer; however, they were verified by the manufacturer. The solvent of the ink used was isopropyl alcohol.

Materials and Methods

We used EV from an ink pen with California Proposition 65-compliant ink (Sandel 4-in-1 Marker, Nipro, Osaka Ja-
EV Use for Vascular Anastomosis Vessel Visualization

Fig. 1
(Left) Image of the vessel before (upper left) and after (lower left) anastomosis using crystal violet.
(Right) Image of the vessel before (upper right) and after (lower right) anastomosis using ethyl violet.
White arrows: Stained vascular transected surface before the start of anastomosis.
White arrowhead: After completion of anastomosis.

pan). The cytotoxicity data were undisclosed; however, it was verified by the manufacturer and is available in the public domain. In addition, isopropyl alcohol was used as a solvent. A stereomicroscope was used for the vascular anastomosis technique. A surgeon with an experience of performing more than 200 clinical cases of microvascular anastomosis was in charge of all the anastomoses. The procedure consisted of harvesting a chicken wing superficial ulnar artery or ventral metacarpal artery \(^{12,13}\) with a diameter of 0.7-1 mm, cutting it, visualizing the vessel wall with CV and EV, performing end-to-end anastomosis, and measuring the time required for six stitches of the anastomosis. The vessel diameter was measured in the collapsed vessels. The anastomosis method was then integrated into a 6-needle suture. After ligating the supporting suture with two stitches at 180 degrees, the anterior wall between the two supporting sutures was sutured with two stitches. After reversing the vessel and microvessel approximator clamp, the posterior wall was anastomosed with two stitches. To mark the blood vessels, EV was squeezed out of a skin pen and prepared into a liquid. CV and EV were thereafter applied to the tip of the micro-tweezers and used to mark the blood vessels. The number of anastomoses was 30 for each stain, adding up to a total of 60 anastomoses. A 10-0 nylon suture was used for the anastomosis (Fig. 1). For the vascular anastomosis, CV and EV were changed each time to avoid interfering with the learning curve. The tools for the microsurgical procedures were standardized with those that the surgeon was familiar with. Anastomosis time was defined as the time from the preparation of the vessel’s transected end and the start of the first needle application to the end of the six stitches and cutting of the thread. The Wilcoxon rank-sum test was used for statistical analysis, with a significance level set at 5%. The statistical analysis was carried out using the JMP Pro statistical software ver. 14 (SAS Institute, Inc., Cary, NC).

All statistical results are presented as mean, standard error of mean or median, and standard deviation. This study was conducted with the approval of the Ethical Review
Committee of our hospital.

Results

Images of the vascular transection with CV and EV and post-anastomosis with CV and EV are presented in Fig. 1. No visually obvious differences were noted in the vascular transections with CV and EV. The results of the anastomosis of 60 cases are shown in Table 1. The longest and shortest anastomotic times are also summarized in Table 1. The average anastomosis time (second) (mean (range) ± SD/SE) was 448.9 (362−616) ± 53.19/9.71 for anastomosis with CV and 450.1 (377-536) ± 43.27/7.89 for EV. Time taken for anastomosis with CV and EV was found to be not significantly different as per the result of the Wilcoxon rank-sum test.

Discussion

As per the results of this study, it was found that EV may be as useful as CV for visualization of vessel walls smaller than 1 mm. CV has used as a colorant and disinfectant. As a medical material, it is used in the surgical field as a pen to mark the skin. Furthermore, various other uses have been reported in which it may remain in the body. It is often used to clarify anastomotic vessel walls in microvascular anastomosis. Further, it is also used as a marker to easily identify the vessel wall torsion, for spraying the subarachnoid space, etc. However, there are concerns as regards the risk of allowing these chemicals to remain in the body. Moreover, the World Health Organization has issued a position statement via the Codex Alimentarius Commission that states the following: “In view of the FAO/WHO Joint Expert Committee on Food Additives conclusions on the available scientific information, there is no safe level of residues of gentian violet or its metabolites in food that represents an acceptable risk to consumers. For this reason, competent authorities should prevent residues of gentian violet in food. This can be accomplished by not using gentian violet in food producing animals.” In response to this recommendation, in June 2019, Health Canada announced the withdrawal of approval for nonprescription drugs containing gentian violet after conducting a risk assessment, and the company voluntarily suspended sales of the product. Meanwhile, in dermatology, studies have investigated CV’s antibacterial, antiangiogenic, anti-neoplastic, and wound healing properties. CV is known to be a potentially low-cost, well-tolerated topical agent that has been examined for a variety of potential applications in dermatology.

In Japan, the Food Safety Commission conducted a food health effect assessment of CV in veterinary drugs in 2022. As a result, genotoxicity could not be ruled out, and carcinogenicity was suggested. Subsequently, Japan has been instructed to discontinue the sale of CV ink in the medical field from 2023, and the recommendations state that the use CV ink in the medical field, “whether as an active ingredient or as an additive, shall not be permitted.” However, if there is no alternative and the benefits outweigh the risks, the risks (genotoxicity and carcinogenicity) should be explained to the patient, and CV ink should be administered after obtaining consent. However, when used for medical purposes, no reports of carcinogenesis have been confirmed. The molecular formula of EV is C31 H42CIN3, and it has a molecular weight of 492.14 and CAS registration number: 2390-59-2. It is soluble in cold water, appears violet blue in hot water, and is easily soluble and appears violet in ethanol. The European Commission has requested EFSA to evaluate each dye covered by the “Guidance on methodological principles and scientific methods to be considered when establishing reference points of action (RPAs) for pharmacologically active substances in foods of animal origin” and has reported the results of its review. In it, EV has been reported as follows: “Based on the chemical structure of ethyl violet and the absence of safety concerns upon oral exposure with respect to allergenicity or blood dyscrasias, the guidance document can be applied to establish an RPA for ethyl violet.”

In the field of neurosurgery, vascular anastomoses of less than 1 mm in diameter are required to be performed. Furthermore, the vessel wall in patients with pediatric Moyamoya disease is extremely thin; therefore, improved visibility is useful for successful surgery. CV ink is used to improve the visibility of the thin vessel walls. To improve the visibility of the blood vessel walls, blue is the most visible color because blood is red in the surgical field; however, there is no specific requirement for the ink to be CV. To improve the visibility of the thin vessel walls, ink of a color similar to that of CV is also effective. In addition, CV-free ink must not be prohibited in order to be used in a form that remains in the body during surgery. No carcinogenicity has been indicated with the EV used in this study.

| Table 1 Results of measurement of anastomosis time with stained materials |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   | Median(s)         | Quartile range    | Mean(s)           | SD             | SE             | Longest(s)         |
| CV                | 439.5             | 72.25             | 448.9             | 53.19          | 9.71           | 616               |
| EV                | 446               | 73.25             | 450.1             | 43.27          | 7.89           | 536               |

EV, ethyl violet; CV, crystal violet; s, second; SD, standard deviation; SE, standard error

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Limitations

Future challenges include reproducing this experience in the microvessels of the human body in a clinical setting. In addition, the thickness of the vessel wall of chicken wing vessels used in this study, which was about 0.8-1 mm, was thicker than the vessels on the brain surface in Moyamoya disease. Therefore, it is necessary to examine the usefulness of EV for extremely thin vessels, such as those on the surface of the brain in pediatric Moyamoya disease.

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This study did not receive any external funding.

Ethics Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional Research Committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The procedures followed in the experiments on human subjects were conducted in accordance with "Ethical Guidelines for Medical and Health Research Involving Human Subjects ( Provisional Translation as of March 2015)" and its later amendments.

Abbreviations

CV, crystal violet; EV, ethyl violet

Conflicts of Interest Disclosure

The authors declare no conflicting or competing interests. All authors who are members of the Japan Neurosurgical Society have registered online and supplied the self-reported COI Disclosure Statement Forms through the website.

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