Evaluation of the vascularity of an isolated bowel segment using fluorescein angiography in rats

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ABSTRACT

Aims: An isolated bowel segment (IBS), a viable loop of bowel completely free of its mesenteric attachments, has potential clinical use in bowel lengthening and other techniques. The end point of successful creation of an IBS is the development of adequate collateral circulation. In this experimental study, we report the use of fluorescein test to demonstrate collateral vascularity of the IBS in rats. A simple technique for the visualization and photography of fluorescein in the tissues is also described.

Materials and Methods: In nine Wistar rats, 5 cm of distal ileum was anastomosed side-to-side to 5 cm of proximal colon using 6/0 PDS. In one rat (control), the colon patch was isolated from the rest of the large bowel immediately and fluorescein test was performed. The remaining eight (study group) rats were operated after 8 weeks and the procedure followed as in the control rat. In the last rat, the mesentery of the colon patch was severed; and 2 weeks later, the fluorescein test was performed. Results: The colon patch in the control rat showed complete lack of fluorescence; in the seven rats tested after 8 weeks, patchy fluorescence was demonstrated in the colon patch; and in the last rat, bright fluorescence of the whole patch could be demonstrated within a few seconds following fluorescein injection. Conclusion: This study describes a simple technique for the fluorescein test. The observation that the vascular collaterals were more abundant after mesocolic division was obviously due to the opening up of collaterals over a period of time. The fluorescein test is a rapid and easy test and can be easily reproduced.

KEY WORDS: Collateral circulation, colon patch, fluorescein test, isolated bowel segment

INTRODUCTION

An isolated bowel segment (IBS) is a viable loop of bowel that is completely free of its mesenteric attachments. The basic idea of the procedure to create an IBS was derived from the colon patch graft (CPG) procedure described in 1981.[1] The CPG is itself a model of an IBS. The uses of an IBS are many; these include functional and absorption studies on a completely denervated bowel segment and its potential clinical use as a bowel lengthening technique. The end point of successful creation of an IBS is the development of adequate collateral circulation to the bowel segment. The exact time required to create an IBS in the animal model is not clear. In the rat, the time required to create an IBS was 7 weeks after myoenteropexy, 5 weeks after hepatoenteropexy and 4 weeks after omentoenteropexy.[2,3] Thus it seems that the time required to create an IBS may vary with the technique employed. In these experimental studies, the authors used histological studies on the IBS to establish viability of the IBS and demonstrate the collaterals, while others demonstrated the developing collaterals of the IBS by the injection of radioopaque microfil silicone compound.[4] However, none of these methods is probably easily applicable in routine clinical practice. Due to this existing uncertainty regarding the exact time required to create an IBS, it becomes important in clinical practice to establish the presence of adequate collateral vascularity to the IBS. If its original mesentery can be divided. To our knowledge, a rapid, simple, easily applicable and reproducible technique to this effect has not been previously described. In this experimental study, we report the use of fluorescein test to demonstrate collateral vascularity of the colon patch in a CPG model of IBS in rats. A simplified technique for the visualization and photography of fluorescein in the tissues is also described.
MATERIALS AND METHODS

Nine Wistar albino rats, weighting 200-250 gm, were anesthetized using intraperitoneal ketamine hydrochloride (50 mg/kg). Under aseptic conditions, the abdomen was entered through a midline incision. A side-to-side anastomosis was performed between 5 cm of proximal large bowel (colon patch) and 5 cm of distal small bowel using continuous 6/0 PDS stitch [Figure 1]. In one animal (control), soon after the anastomosis, the colon patch was isolated from the rest of the large bowel, its mesentery was severed and fluorescein test was performed [Figure 2]. In the remaining eight (study) animals, the abdomen was closed with 4/0 Vicryl after the anastomosis. After 24 h of fasting, liquid feed was allowed and a standard rat diet was started 48 h later. All eight animals were reoperated after 8 weeks. In seven animals, the colon patch was then isolated from the large bowel, its mesentery severed and fluorescein test performed [Figure 3]. In the last animal, the mesentery of the colon patch was severed and the abdomen closed. After 2 weeks, the animal was reoperated, the colon patch isolated from the rest of the large bowel and fluorescein test performed.

The fluorescein test was performed in a dark room. The light from a cold light source was allowed to fall on the animal. Fluorescein (0.5 ml) was injected into the tail vein of the animal. A blue filter was placed in the path of the light so that the tissue fluorescence could be visualized in blue light. The result was simultaneously photographed using a 400 ASA Kodak film mounted on a camera fitted with a close-up lens.

RESULTS

The colon patch in the control rat showed complete lack of fluorescence [Figure 4]. In the seven rats tested after 8 weeks, patchy fluorescence was demonstrated in the colon patch, which gradually deepened [Figure 5]. In the last rat, 2 weeks after severing the mesentery, bright fluorescence of almost the whole of the patch could be demonstrated within a few seconds following the fluorescein injection [Figure 6]. The result was readily visualized with the naked eye and photographed by the method described.

DISCUSSION

In the present study, the CPG has been employed as a model of IBS. When IBS is created in the clinical setting, it is important to confirm the development of adequate collateral circulation before the original mesentery of the IBS is severed. The present study describes a simple technique for this purpose. In contrast to the earlier reported techniques like histology and injection of radioopaque compounds, the fluorescein technique described in the present study is simple, rapid and inexpensive and easily reproducible. When the mesentery of the colon patch was severed and fluorescein test was performed 8 weeks after the anastomosis, patchy fluorescence appeared in the
performed 2 weeks after the mesentery was severed, the patch demonstrated much brighter and rapid fluorescence. This observation that the vascular collaterals were more abundant after mesocolic division was made by other authors also.[4] We presume that this is due to the opening up of collaterals due to the relative ischemia induced by dividing the original blood supply to the colon patch. The fluorescein test has earlier been described for use in plastic surgery.[5,6] It has also been used to assess the viability of ischemic bowel in experimental models and in clinical practice.[7] The standard technique describes the use of a Wood’s lamp to visualize the tissue fluorescence. The result is then photographed using two color filters – an excitation filter fitted to electronic flash unit and a barrier filter fitted to the camera.[8] In the present study, we employed a single blue filter fitted to the light source. The advantage of this technique is that results could be easily visualized with the naked eye and simultaneously photographed. To our knowledge, such a simple technique that allows simultaneous visualization and photography of fluorescein has not been previously described. The use of a high-speed film for photography such as the one employed by us has been previously described.[9] Because we did not employ the barrier filter, the background appeared blue, but the contrast with fluorescein was so good that we did not find the use of a barrier filter mandatory. However, it may be employed if it is felt necessary to do so.

REFERENCES


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