Journal of Pediatric Surgery (2005) **40**, 1881–1886

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Graft serosectomy in adult small bowel transplantation without vascular reconstruction in rats improves graft survival by induction of vascular endothelial growth factor

Kyeong Deok Lee[a], Atsuyuki Yamataka[a,*], Yoshifumi Kato[a], Hiroyuki Kobayashi[a], Geoffrey J. Lane[a], Keiko Maeda[b], Yuko Kojima[c], Noriyoshi Sueyoshi[c], Takeshi Miyano[a]

[a]*Department of Pediatric General and Urogenital Surgery, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b]*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[c]*Division of Biomedical Imaging Research, Biomedical Research Center, Juntendo University School of Medicine, Tokyo 113-8421, Japan*

Index words:
Small bowel transplantation;
Rats;
Serosectomy;
Vascular endothelial growth factor

Abstract

Purpose: The aim of this study was to assess whether adult small bowel grafts (ASBGs) can survive transplantation without vascular reconstruction if graft serosectomy (SS) is performed.

Methods: Syngeneic ASBG transplants were performed in 85 Lewis rats. The entire serosa was removed just before transplantation in the SS group (n = 50) and left intact in the nonserosectomy group (n = 35). Transplanted ASBG was harvested 1, 3, 5, 7, 14, 21, or 28 days after transplantation and studied using staining with hematoxylin-eosin, immunohistochemistry for protein gene product 9.5, S-100, CD34 and vascular endothelial growth factor (VEGF), and quantification of VEGF messenger RNA (mRNA). Adult small bowel graft viability was assessed blindly using a mucosal surface expansion score (0, no mucosa; 1, mucosa on one fourth of graft; 2, mucosa on one half of graft; 3, mucosa on three fourths of graft; and 4, circumferential mucosa on graft).

Results: No rejection was identified in any ASBG. Average mucosal surface expansion score and VEGF mRNA expression were significantly higher in the SS group (both $P < .01$). Vascular endothelial growth factor protein was detected in enterocytes from day 3 posttransplant in the SS group. Distribution of protein gene product 9.5 and S-100 was normal in SS-group ASBG.

Conclusions: Our results suggest that SS allows VEGF mRNA and, subsequently, VEGF protein in ASBG to be induced very soon after transplantation, which may contribute to the survival of ASBG transplanted without vascular reconstruction.

© 2005 Elsevier Inc. All rights reserved.

When small bowel grafts harvested from fetal or neonatal rats aged up to 3 days old are transplanted into a pouch created in the omentum of a recipient, they revascularize and grow even without vascular reconstruction and can be anastomosed to the recipient's native bowel [1-4]. However,

Presented at the 38th Annual Meeting of the Pacific Association of Pediatric Surgeons, May 22-26, 2005, Vancouver, Canada.

* Corresponding author. Tel.: +81 3 3813 3111; fax: +81 3 5802 2033.
E-mail address: yama@med.juntendo.ac.jp (A. Yamataka).

0022-3468/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2005.08.030

grafts harvested from rats aged more than 6 days cannot survive without vascular reconstruction [1,2]. We hypothesized that the presence of serosa on the bowel graft may prevent revascularization between older grafts and the recipient omentum.

The aim of this study was to assess whether adult small bowel grafts (ASBGs) can survive transplantation without vascular reconstruction if its serosa is removed. Vascular endothelial growth factor (VEGF) is a potent angiogeneic glycoprotein known to be one of the important factors for angiogenesis [5]. We also studied whether VEGF plays a role in revascularization of ASBG.

1. Materials and methods

1.1. Animals

Twelve-week-old (adult) and five-week-old Lewis rats were purchased from SLC Japan (Shizuoka, Japan). All experimental protocols were approved by the Institutional Animal Care and Use Committee at Juntendo University School of Medicine (Experimental Protocol No. 160079) in accordance with guidelines set forth in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Before surgery, rats were deprived of food for 1 day and given double-agent antibiotic therapy with cefmetazole sodium (100 mg/kg) by subcutaneous injection and tobramycin sulfate (3 mg/kg) by intramuscular injection on the day of surgery. All rats were anesthetized with inhalational isoflurane.

1.2. Small bowel transplantation

Laparotomy was performed on a 12-week-old adult Lewis rat (n = 85) weighing 195 to 230 g. Adult small bowel graft (jejunum, 10 mm in length) was obtained with a small amount of mesentery intact to ensure serosal integrity, and stocked in cold saline. The lumen of the ASBG was flushed with cold saline to remove any contents. The entire serosa was removed circumferentially from the ASBG in the serosectomy (SS) group (n = 50) and was left intact in the nonserosectomy (NSS) group (n = 35). Serosectomy was performed under an operating microscope with 3.5× magnification. All ASBGs were then transplanted as free grafts, each into a pouch created in the omentum of a 5-week-old Lewis rat weighing 110 to 145 g. The omental pouch was closed with 7-0 Prolene sutures to surround the ASBG and the wound closed in 2 layers with continuous 4-0 Dexon sutures (Cyanamid Medical Device Co, Inc, Anyang, Korea). Transplantation in this study was syngeneic (intraspecies transplantation), and no immunosuppressant was required.

1.3. Graft harvesting

Recipients were killed 1, 3, 5, 7, 14, 21, or 28 days after transplantation, and ASBG, harvested. All grafts were washed in physiological saline and divided vertically into 2 pieces: one was fixed in 15% neutral buffered formalin and embedded in paraffin wax for hematoxylin-eosin (H-E) staining and immunohistochemistry to examine graft viability, mucosal surface expansion score (MSES), enteric nervous system (ENS), and vasculogenesis; the other was snap-frozen in liquid nitrogen and stored at −80°C for quantification of VEGF messenger RNA (mRNA). Hematoxylin-eosin staining for MSES and immunohistochemistry with protein gene product (PGP) 9.5 and S-100 for ENS were performed on ASBG harvested 1, 2, 3, and 4 weeks after Syngeneic transplantation (SyTx) (n = 55). Immunohistochemistry with CD34 (a marker of vascular endothelial progenitor cells [6]) and VEGF, and quantification of VEGF mRNA for vasculogenesis were performed on ASBG harvested 1, 3, 5, and 7 days after transplantation (n = 30).

Nontransplanted age-matched normal jejunum (n = 15) was used as control tissue for H-E staining, immunohistochemistry, and mRNA quantification.

1.4. Hematoxylin-eosin staining and immunohistochemistry

Each ASBG was cut into 5-μm-thick serial sections and treated alternately with H-E or immunohistochemistry. Sections were flooded with 10% blocking serum for 30 minutes, followed by incubation with primary antibodies for 60 minutes. Sections were then incubated with biotinylated second antibodies at room temperature for 30 minutes, then placed in a freshly prepared methanol–0.3% H_2O_2 solution for 15 minutes, and then incubated with avidin-biotin peroxidase complex at room temperature for 30 minutes. Bound antibodies were visualized with 3,3′-diaminobenzine containing 0.003% H_2O_2. Between each incubation step, sections were washed with Phosphate-buffered saline (pH 7.4) 3 times, except before the addition of primary antibodies. Negative staining was obtained by incubation without primary or secondary antibody.

1.5. Mucosal surface expansion score

Graft viability was evaluated blindly using a MSES on a random selection of ASBG (n = 55: 20 from the NSS group, 35 from the SS group). Mucosal surface expansion score was 0 if there was no mucosa, 1 if mucosa was present on up to one fourth of the graft, 2 if mucosa was present on up to one fourth but less than one half of the graft, 3 if mucosa was present on more than one half but less than three fourths of the graft, and 4 if mucosa was present on at least three fourths to all of the graft. Four MSES were randomly selected from H-E–stained ASBG sections and averaged to give the mean MSES for each ASBG. Graft death was defined as MSES of 1 or less.

1.6. Quantification of VEGF mRNA

1.6.1. Ribonucleic acid isolation and reverse transcription

Ribonucleic acid isolation and reverse transcription were performed according to the modified methods described

Download English Version:

https://daneshyari.com/en/article/4160870

Download Persian Version:

https://daneshyari.com/article/4160870

Daneshyari.com