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Value of mucosal biopsies in the monitoring of acute small bowel rejection

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Abstract The value of mucosal biopsies in evaluating small bowel rejection is controversial. In this study, the value of mucosal biopsies was estimated in unmodified porcine small bowel rejection. Ten animals received the distal half of the small bowel as a heterotopic loop (Thiry-Vella loop). The allografts were followed by proximally and distally harvested full-thickness and mucosal biopsies every other day, starting from the 3rd day and continuing until the grafts became necrotic. The histological parameters in both types of biopsies were semiquantitatively scored from 0 to 3 and compared with each other. The difference in mean values on subsequent days was not remarkable, the results favoring slightly higher values in full-thickness than in mucosal biopsies. Our results suggest that multiple mucosal biopsies are adequate in monitoring morphological changes of small bowel grafts during rejection and that the proximal and distal ileum are similarly affected by acute rejection.

Key words Small bowel transplantation, mucosal biopsy, pig · Mucosal biopsy, small bowel transplantation, pig · Pig, small bowel transplantation, acute rejection

Introduction

The aim of this study was to evaluate whether the histological changes in superficial layers of the bowel wall during acute small bowel rejection can reliably be evaluated when mucosal biopsies are used. For this purpose we biopsied heterotopic porcine small bowel allografts by full-thickness and mucosal methods and compared the results with each other. Biopsies obtained from the proximal and distal ends of the grafts were compared separately.

Materials and methods

Animals

Outbred female piglets weighing 16–22 kg were used for the experiment. The animals were not fed for 12–18 h before the operation. Twelve animals were operated as non-littermate pairs, each animal serving as a donor and a recipient. Two animals were lost because of technical reasons on the 3rd day (one due to arterial thrombosis and one due to paraplegia) and were excluded from the study. The control group consisted of six animals that received an autotransplant using a surgical technique similar to that in the allotransplant group.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" formulated and prepared by the Institute of Laboratory Animal Resources and published

by the National Institutes of Health (NIH publication no. 86–23, revised 1985). The study was authorized and conducted in accordance with Finnish legislation.

Anesthesia

The piglets were operated on under general anesthesia with endotracheal intubation using ketamine 20–30 mg/kg (Ketalar; Parke-Davis, Barcelona, Spain), azaperon 6 mg/kg (Stresnil; Orion, Espoo, Finland), diazepam 2.5 mg (Stesolid Novum; Kabi Pharmacia, Stockholm, Sweden), and atropine 0.01 mg/kg (Atropin; Orion) in induction. The anesthesia was maintained with enfluran (Ephrane; Abbott, Campoverde, Italy), nitrous oxide, and pancurone 0.1–0.15 mg/kg (Pavulon; Organon, Oss, Holland). Ceftriaxone 500 mg (Rocephalin; F. Hoffmann-La Roche AG, Basel, Switzerland) was given i.m. as antibiotic prophylaxis and was continued at the same daily dosage for 2 days postoperatively.

Surgical technique

Following a midline incision, the distal half (ileum) of the small intestine was harvested and perfused via the artery with approximately 200 ml cold (+ 4°C) Ringer's solution containing heparin 5000 IU/1000 ml (Heparin; Leiras, Turku, Finland) until the venous effluent was clear and the transplant macroscopically bloodless. Thereafter, the harvested bowel (average length 6.7 m) was kept in cold isotonic saline solution. The continuity of the remaining small bowel was restored by end-to-end anastomosis in one layer with 5–0 polyglyconate monofilament (Maxon; Davis + Geck, Gosport, UK). After systemic heparinization of the recipient (Heparin, 3500 IU; Leiras), the graft vessels were anastomosed end-to-side to the recipient's aorta and vena cava below the renal artery with a running stitch of 6–0 polypropylene (Prolene; Ethicon, Norderstedt, Germany). The graft was placed heterotopically with both ends as enterostomies (Thiry-Vella loop). The piglets received liquids starting on the 1st day after the operation and a normal diet from the 3rd day onwards. In the control group, the distal half of the small bowel was harvested, perfused, anastomosed, and placed heterotopically in the same way. The total ischemic time ranged from 80 to 145 (124 ± 20) min in the allograft group and from 60 to 98 (81 ± 14) min in the control group.

Postoperative monitoring

Full-thickness and mucosal biopsies of the graft were taken immediately after harvesting; thereafter, the graft was followed by biopsies every other day starting on the 3rd day after transplantation until the graft was macroscopically necrotic. For biopsy specimens, both ends of the bowel loop were loosened, about 10 cm of the gut resected, the antistomal part of which was used for biopsy specimens, and new stomas were created. A segment was cut off as a full-thickness biopsy and four mucosal biopsies were taken with a forceps from the adjacent segment. The specimens were fixed in buffered formalin, processed routinely, and embedded in paraffin. The sections were stained with hematoxylin-eosin, Masson's trichrome, periodic acid-Schiff, and methyl green-pyronin. Ten animals were followed for 7 days, seven animals for 9 days and three animals for 11 days. All six animals in the control group were followed for 11 days.

Some of the biopsies in the acute rejection group were totally necrotic and therefore not readable. They were mostly biopsies from the 9th and 11th days. Of the four mucosal biopsies harvested in each session, an average of 3.3 were adequate for evaluation after processing. If none of the four mucosal biopsies in one sample was readable, it and its corresponding full-thickness pair were excluded from the analysis. Because only three animals could be followed until the 11th day and as these biopsies were mostly necrotic, the 11th day results were not included in the analysis. After this exclusion, there were ten proximal and ten distal biopsy pairs obtained on the 3rd and 5th days, nine on the 7th day, and seven on the 9th day. In the control group, the number of biopsy pairs was six on each day.

Histological scoring

Five variables for the lamina propria, epithelium, and mucosa (infiltration of inflammatory cells in lamina propria, epithelial swelling, crypt abscesses, blunting of villi, and sloughing of epithelium) were scored blindly. Every parameter was scored separately. The scale used was from 0 to 3, with 0 indicating no pathological alterations and 3 indicating extreme changes. The quantitative criteria for scoring the key histological parameters have been described elsewhere [10]. For every specimen, the average of the five parameters, called the "acute rejection index", was calculated.

Statistics

The values are presented as mean ± one standard deviation. The comparison of full-thickness with mucosal biopsies, and of proximal with distal biopsies, was made statistically using the Wilcoxon signed rank test. The acute rejection group was compared with the control group using the Mann-Whitney U-test. A probability of below 0.05 was accepted as statistically significant. The individual values of full-thickness and mucosal biopsies are shown as scattergrams and the correlation coefficients counted.

Results

In the acute rejection group, the mean acute rejection index of the full-thickness biopsies was higher than that of the mucosal biopsies from the 3rd to the 7th day in the proximal segments. This difference, although not great, was statistically significant ($P < 0.05$; Fig. 1). In distal segments, both types of biopsies received, on the average, equal values until the 9th day, the difference was not statistically significant. When the proximal biopsies were compared with the distal ones, full-thickness and mucosal biopsies separately, the difference was not statistically significant either.

The difference between the acute rejection and the control group was significant on the 7th and 9th days in proximal biopsies, and on the 5th, 7th, and 9th days in distal biopsies, regardless of the type of biopsy (Fig. 1).

Because the mean values of the proximal and distal biopsies did not show much difference, the value of each biopsy pair in the acute rejection group, both proximal and distal, is presented in a combined scattergram. The values on the operation day 0, mostly values near zero, were excluded from the scattergram. In Fig. 2, the x axis represents the full-thickness biopsy and the y

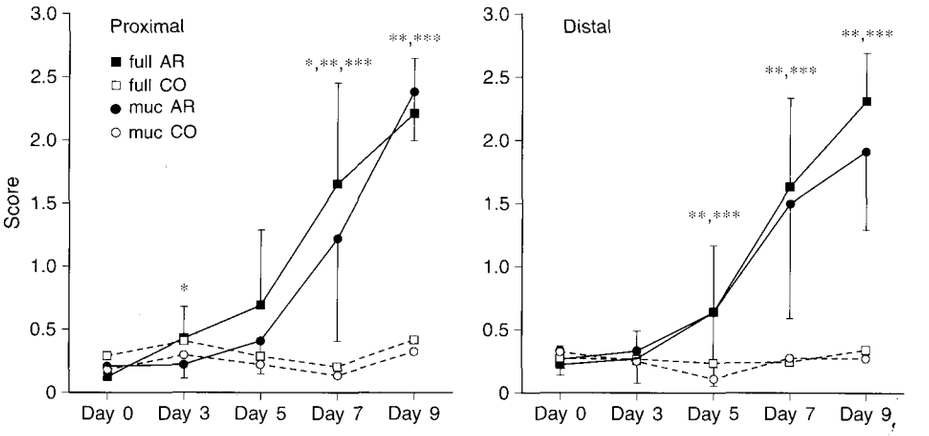

Fig. 1 Acute rejection index (mean value ± SD) in the proximal and distal mucosal biopsies on subsequent days. The parameters were scored from 0 to 3 according to the severity of the change (—●— —■— acute rejection group, —○— —□— control group) $*P < 0.05$ full-thickness biopsies vs mucosal biopsies in the acute rejection group; $**P < 0.05$ full-thickness biopsies in the acute rejection group vs the control group; $***P < 0.05$ mucosal biopsies in the acute rejection group vs the control group

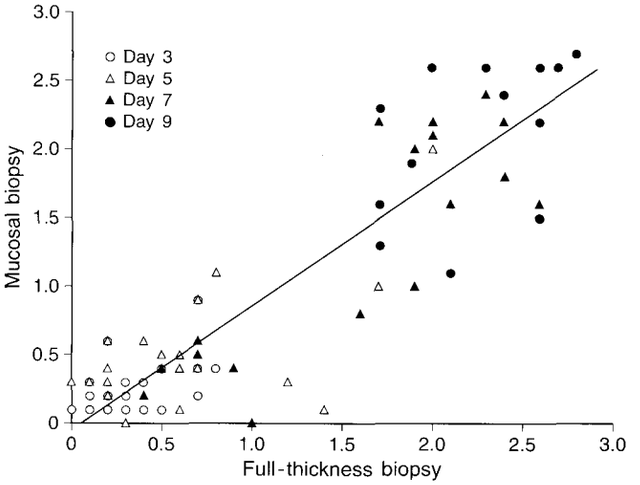

Fig. 2 Each point shows the acute rejection index evaluated in the full-thickness biopsy (*x axis*) and in the mucosal biopsy (*y axis*). The biopsies on subsequent days marked with different symbols ($r = 0.89$, $y = -0.03 + 0.90*x$)

axis the corresponding mucosal biopsy. The biopsies taken on subsequent days are marked with different symbols. The scattering is rather wide; the change is, in many cases, higher in the full-thickness than in the mucosal biopsy; in other cases, the opposite is true. A slight tendency towards higher values in full-thickness biopsies is seen; the correlation coefficent (r) is 0.89.

Discussion

The diagnosis of small bowel rejection is based on mucosal biopsies, clinical symptoms, and endoscopic findings [1]. Histopathological analysis is the gold standard for diagnosing small bowel rejection. Yet, none of the many functional tests and serum parameters investigated has been proven sensitive enough to detect early rejection.

The usefulness of mucosal biopsies in the monitoring of intestinal grafts is controversial. Mucosal biopsies are said not to give enough or relevant information because of the patchy nature of rejection or because the early morphological changes may not be evident in superficial layers [3–5, 8, 13, 14, 16, 17]. Yet, mucosal biopsies have been successfully used [2, 11] and, in clinical settings, morphological estimation is dependent on mucosal biopsies [9].

The morphological changes in the small bowel during acute rejection have been amply reported [8, 12, 13, 16, 17]. Many of the early changes are seen in superficial layers, i.e., in the mucosa and lamina propria.

Holmes et al. [8] studied canine jejunal allografts and used suction biopsy in the monitoring of the grafts. They saw great histological variability in different areas of the same section, as well as in sequential biopsy specimens from the same animal, and concluded that single slides are insufficient and unreliable for evaluating the degree of rejection. In a study with accessory small bowel transplants in rats, Rosemurgy and Schraut [16] found it very difficult at times to ascertain the actual phase of rejection when microscopic examinations were limited to an area of the mucosa approximating a mucosal suction biopsy in size, especially when the grafts were in the intermediate stage of rejection. Dennison et al. [3] studied allografts of terminal ileum as Thiry-Vella segment in dogs and concluded that the variability in histological appearance of mucosal biopsies does not allow mucosal morphology to be used as the sole index of graft rejection.

The number of biopsies required in the monitoring of heart and lung transplants has been established [7, 19–21]. Several biopsies might very well lead to better results in the monitoring of small bowel grafts as well [8, 9, 15, 16]. Based on the findings from these studies, we decided to do this comparison using four mucosal biop-

sies. It has recently been reported that endoscopically directed biopsies from macroscopically suspicious areas might lead to earlier recognition of rejection [6]. In this study, we did not want to take a stand on this question but rather to establish the value of mucosal random biopsies. All adequate specimens of the four mucosal biopsies were used in evaluating histological changes in one sample, and every parameter was assigned a value presenting the average view of these biopsies. The other possibility would have been to use the value of the biopsy in which the change was the strongest. This would have biased the results of the mucosal biopsies in the direction of higher values.

There are no generally accepted histological criteria for grading rejection in small bowel transplantation as there are for kidney, heart, and lung transplantation [18, 20, 21]. Due to the lack of a grading system, we chose five parameters that have been found to progress during rejection [2, 3, 6, 8, 10, 12, 16]. All parameters were scored separately and their mean value (acute rejection index) was used for analysis. Every parameter had an equal impact in the calculations.

When the proximal and distal biopsies were compared with each other, full-thickness and mucosal biopsies separately, the mean values were equal, and the small difference observed was not statistically significant. This suggests that the rejection process proceeds simultaneously along the longitudinal axis of the graft. This is in agreement with previous observations. In the study by Cohen et al. [2], where isolated proximal and distal pouches were used to monitor canine intestinal grafts, morphological changes seen in proximal biopsies closely followed those seen in distal biopsies or appeared 1 day later in the proximal pouch. Rosemurgy and Schraut [16] did not find any differences between jejunum and ileum during acute rejection in their study with rats.

In the evaluation of the correlation between full-thickness and mucosal biopsies, the proximal and distal pairs were analyzed as one group. Looking at individual pairs, the scored values were often different. In many cases, the change in the full-thickness biopsy was estimated as being greater than in the mucosal biopsy; in other cases, the opposite was true. The scattering can be partly explained by the subjective evaluation of parameters on the semiquantitative scale. The main reason seems to be the uneven distribution of morphological changes during rejection, especially when the changes are not yet strong. Full-thickness and mucosal biopsies were taken from adjacent segments, which might cause differences in individual cases. This interpretation is supported by the observation that a similar scattering of values was also noticed when proximal full-thickness biopsies were compared with corresponding distal full-thickness biopsies, or proximal mucosal with distal mucosal biopsies.

Although there was variation within pairs, the overall correlation was good. The values were not constantly higher in either type of biopsy. Only a slight tendency towards lower values in mucosal biopsies was seen proximally.

To conclude, our results confirm the observations that multiple mucosal biopsies can be satisfactorily used when monitoring morphological changes during bowel rejection. The proximal and distal parts of ileal allografts are similarly affected.

It is well recognized that the heterotopic position of the graft poses limitations to the interpretation of the results. However, it is expected that exclusion of the chyme affects the control group similarly, and so the validity of this study is maintained. Direct extrapolation to clinics must be done with caution when dealing with immunosuppressed patients and chronic rejection.

The precise value of mucosal biopsies can be evaluated only after uniform criteria have been established for the classification of acute and chronic rejection and an analysis has been done on how many mucosal biopsies are required to verify a certain grade of rejection with accepted probability. The situation differs from endocardial biopsies, as intestinal mucosal biopsies can be taken under visual control via an endoscope and directed to a suspected area. This raises an additional question when interpreting the results: what is the importance of a local change if the rest of the bowel is normal?

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