Histologic Evaluation of Organ Preservation Injury and Correlation With Cold Ischemia Time in 13 Intestinal Grafts


ABSTRACT

Lesions produced in the graft mucosa due to harvesting, storage, and implantation must be graduated to assess the subsequent protocolized biopsy specimens. The aim is to identify type and intensity of graft mucosal lesions observed immediately after implantation. Congestion, hemorrhage, microthrombi, neutrophilic infiltrates, shortening of villi, epithelial detachment, erosion, and crypt loss were separately evaluated by two pathologists in mucosal biopsy specimens from 13 grafts. Each change was assessed as normal, mild, moderate, or severe and by splintering the summation of points a global score was designed. Cold ischemia time was registered. Correlation between the pathologists’ evaluations and between final preservation injury degree and cold ischemia time was determined using the “index of correlation rho (ρ)” (Spearman’s test). The same changes were assessed in 19 biopsy specimens from day 2 to day 6 (3.6 ± 1.1) to determine their evolution. Congestion was found in 7 biopsy specimens, microthrombi in 2, hemorrhage in 4, neutrophils in 6, villous atrophy in 8, epithelial detachment in 9, erosions in 2 and/or crypt loss in 2. The maximum degree of preservation injury was expressed as intense congestion and hemorrhage associated with epithelial detachment and villous atrophy. The global preservation score was grade 3 in 2 cases, grade 2 in 5, grade 1 in 2, and grade 0 in 4. There was positive correlation (ρ = 0.915) in the evaluation between pathologists (P < .01), total agreement in 9 biopsy specimens, and partial agreement (only 1 point disagreement) in 4. Mean cold ischemia time was 327 ± 101 min. (135–480). There was positive correlation (ρ = 0.694) between preservation score and cold ischemia time (P < .01). In the follow-up biopsy procedures, histological injury decreased by at least one grade in every case. Additionally, karyorrhexis was observed in 3 grafts and very occasional apoptosis in 2 others. This scale achieves good reproducibility and allows graduate preservation injury in intestinal transplantation.

INTESTINAL mucosa is very sensitive to ischemia [1,2] and it could determine a primary nonfunction or delayed graft function. Preservation changes could be related to cold ischemia time [3–6]. A tool that allows physicians to identify and graduate lesions produced in the graft’s mucosa due to harvesting, storage, and implantation to assess the subsequent docketed biopsy specimens and to separate it from the early immunologic damage is necessary.

The aims of this study were to identify and to assess intestinal mucosal lesions in grafts immediately after implantation; to create a simple scale for graduate preservation injury; to compare final preservation injury grades with cold ischemia times; and to determine the evolution of these histologic lesions in follow-up mucosal biopsy specimens.

METHODS

Two pathologists separately evaluated histologic changes in hematoxylin-eosin–stained, 3- to 5-μm sections from 13 immediately

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post-implantation ("time zero") mucosal biopsy specimens from isolated small bowel grafts. Congestion, hemorrhage, microthrombi, neutrophilic infiltration in lamina propria shortening of villi, epithelial detachment, erosions, epithelial karyorrhexis and apoptosis, and crypt loss were rated based on severity as normal, mild, moderate, or severe and a scale was designed with the total sum score. Cold ischemia time (period in minutes between the conservation of the graft and their implantation) for each graft was registered. Correlations between both pathologists' ranking and between final score and cold ischemia time was evaluated by using rho Spearman's index (r; measure from –1 to +1 indicating negative or positive associations respectively, and 0 indicating neither correlation nor independence and it is used in less than 20 samples for degree of association between variables). To know the evolution of the histologic lesions 19 intestinal biopsy specimens from day 2 to day 6 after transplantation were studied.

RESULTS

Preservation injury evaluations are listed in Table 1. The global preservation score was grade 3 in 2 cases, grade 2 in 5, grade 1 in 2, and grade 0 in 4 (Table 2). There was positive correlation (r = 0.941, P < .01) in the evaluation between pathologists. There was total agreement in 9 biopsy specimens and partial agreement (only 1 point disagreement) in 4. The mean cold ischemia time was 327 ± 101 minutes (range, 135 to 480 minutes). There was positive correlation (r = 0.681, P < .05) between preservation score and cold ischemia time. In the follow-up examination, the biopsy specimens' histologic injury decreased by at least one grade in every case. Karyorrhexis was observed in 3 grafts and just occasional apoptosis in other 2.

A score was defined: grade 0, no changes or minimal; grade 1, mild congestion and villus atrophy; grade 2, focal congestion, hemorrhage, neutrophil infiltrate, villus atrophy, mild-moderate epithelial detachment, and focal erosion; and grade 3, congestion, severe hemorrhage, intense neutrophilic infiltrate with cryptitis, epithelial detachment, erosion, and loss of crypts.

DISCUSSION

Intestinal mucosa is very sensitive to interruption of the blood flow for periods longer than 20 minutes. Ischemia may produce changes in capillary permeability and histologic morphology and also reperfusion can cause tissue damage [1,2]. Histologic study of the intestinal mucosa in bowel transplantation had been performed on animal models with different cold ischemia times. The changes described follow from normal mucosa until transmural necrosis. The first morphologic mucosal change seems to be a subepithelial gap on the villus tip. With more severity, this gap could increase to complete epithelial denudation and finally a loss of the total villus, damage of the crypts, and total necrosis of the mucosa. Nevertheless, intestinal mucosa has a great capacity for regeneration and superficial lesions recuperate in 6 to 18 hours. This regeneration could be by migration of cells next to the injured area [1,2,7]. Ischemia-reperfusion injury causes loss of the intestinal barrier allowing bacterial translocation and septic consequences. Also, it is believed to be related to the development of rejection and graft dysfunction. Current hypotheses propose that the proinflammatory ambient could determine an antigenic expression and activation of the immune system which facilitate the rejection. Interleukin 6 could be involved in the pathophysiology of the damage [8].

Recognition of lesions produced in the mucosa of the graft due to harvesting, storage, and implantation is necessary to assess the subsequent docketed biopsy specimens. Pathologists must identify these specific lesions separating them from early immunologic damage. In 1994, Nakamura et al described the histologic characteristics of preservation injury and the effectiveness of this evaluation for the clinical course in 42 intestinal grafts. In their observations, graft injury at the end of preservation was characterized by edema on lamina propria, detachment of villus epithelium, and a decrease in villus height. Post-reperfusion injury showed epithelial denudation, congestion in lamina propria, capillary neutrophilic margination, and loss of crypts. These changes could be related to cold ischemia time [3]. These injuries are no different among the topographical regions of the gut [9].

One of the problems is the lack of consensus regarding the graduation of these injuries. Quaedackers et al [10]...
analyzed the multiple ways that were published and concluded that the ideal system is one that combined morphologic findings with the intensity of the damage from mild to severe; and they choose a combination of Park and Chiu’s classification because it had the major index of correlation [1,10,11]. With the same purpose, we have selected the principal findings on “time 0” mucosal biopsy specimens from intestinal transplantation in adult patients and we have elaborated a simple scale to graduate the injury in four degrees. Each grade of the scale was defined as follows: grade 0 (no changes or minimal), grade 1 (mild congestion and villus atrophy), grade 2 (focal congestion, hemorrhage, neutrophil infiltrate, villus atrophy, mild-moderate epithelial detachment, and local erosion), and grade 3 (congestion, severe hemorrhage, intense neutrophilic infiltrate and cryptitis, epithelial detachment, erosion, and loss of crypts).

Afterward, we tested the reproducibility of it comparing the evaluations made by two different pathologists with a positive correlation ($r = 0.941, P < .01$). The next step is to find drugs that allow us to reduce the injury and stimulate a better evolution of the grafts. In two studies remifentanil and guanylhydrazone CPSI-2364 have been used in experimental small bowel transplantation; in the future they could become a complementary treatment for prevention of secondary complications to ischemic reperfusion injury [12,13].

CONCLUSIONS

In this study maximum degree of preservation injury was expressed as intense congestion and hemorrhage associated with epithelial detachment and villus atrophy. There was positive correlation between cold ischemia time and preservation injury score. This new score achieves good reproducibility. Preservation injuries decrease notably or disappear in subsequent first-week biopsies. There was no association between preservation injury grade and development of rejection in this series. Observations have been on a limited number of samples but we have designed a tool that can be used in further studies.

REFERENCES