Duodenal injuries, though rare, carry high rates of morbidity and mortality. The aim of the study was to evaluate the healing of the duodenal wall with the use of a Small Intestinal Submucosa (SIS) patch. Material and methods. We studied 40 Whistar-Albino rats divided into two groups. In group A, we created a small defect in the duodenal wall, which was immediately covered with a SIS patch. In group B, the SIS patch was sutured over the defect after 6-8 hours, in order to induce peritonitis. The animals of both groups were sacrificed after 2, 6, 12 and 16 weeks respectively. In addition, we studied the immunohistochemical expression of TGF-β, which is a major constituent of SIS, and plays a central role in the healing process. Results. Histology showed progressive development of the layers of the duodenal wall over the patch as early as the 2nd week in some of the animals of group A. Mucosa developed later on in the animals of group B, presumably due to the more intense inflammation elicited by peritonitis. Expression of TGF-β was initially more pronounced in the epithelial cells of the regenerating mucosa of animals of group A, but it was maintained in higher levels in the animals of group B, which showed delayed mucosa degeneration. Conclusions. SIS appears to be both efficient and safe for the management of duodenal trauma. TGF-β seems to play an important role in the healing process, inducing regeneration of the stroma, and controlling epithelial growth. Key words: duodenal defect, healing, immunohistochemistry, regeneration, small intestinal submucosa, TGF-β

Duodenal defects are rare, but present high morbidity and mortality rates (1, 2, 3). Surgical repair is always needed for these severe injuries. Several methods have been proposed in the past, including pyloric exclusion and pancreatoduodenectomy (4, 5). Covering the duodenal defect with patches of small intestinal serosa, first proposed by Kobolt and Thal in 1963, is an alternative approach (6), which is easier and faster, and bears a minimum risk of stenosis and subsequent obstruction of the duodenum. Variations in the method of applying the patches and the different materials used have frequently brought controversial results in the literature. Among them, De Ugarte in 2004 reported successful repair of a duodenal defect in a rat model with the use of a single layer Small Intestinal Submucosa (SIS) patch (7). Surgical repair was performed immediately after the duodenal defect was created in the former study. In the present study, we report the successful delayed surgical repair of an iatrogenic duodenal defect with the use of a 4-ply SIS patch in a rodent model.
We also studied the immunohistochemical expression of Transforming Growth Factor-β (TGF-β) in the duodenal tissue during the healing process, which is a major constituent of SIS, and is known to be an important molecule that regulates tissue repair.

**MATERIAL AND METHODS**

Forty adult Whistar Albino rats were stratified into two study groups of twenty animals each. Group A constituted the control group representing immediate surgical repair of the duodenal injury, while group B was the study group of delayed duodenal injury repair. Small Intestinal Submucosa (4-ply SurgiSIS, ES Soft Tissue Graft 2x3, Cook Biotech Inc, Indiana, IN, USA) was used for repair of the duodenal defect. The research protocol was approved by both the Animal Research Committee of the Aristotle University of Thessaloniki and the Veterinary Department of the Prefecture of Thessaloniki (Decision no. 13/4626/27.05.2005). Anaesthesia was initially induced by diethyl ether, followed by 30 mg/kg Midazolame intraperitoneal infusion to maintain it. After a median laparotomy, the antimesenteric surface of the duodenum was exposed and a defect was created on it with surgical scissors. We made every effort in order to achieve a standard duodenal defect of 7 mm in diameter. We succeeded this goal in 33/40 (82.5%) of the animals. Only three animals of group A, and four animals of group B deviated by a few millimeters from this standard (range 5-8 mm).

All animals were treated using a SIS patch, and no injury was left to heal by itself. The defect in animals of group A was immediately repaired with an 8mm-long SIS patch, secured to the edges of the wound with the use of a 6/0 Vicryl full-thickness running suture (Ethicon, Cincinnati, OH, USA). Animals of group B were initially left with the duodenal defect open, while the abdominal wall was closed. The animals returned to their cages and remained there for six hours without anaesthesia. Then they were re-operated, and the SIS patch was sutured over the defect in the same manner as in animals of group A. Povidone-iodine 10% was used for prevention of wound infection, while the surgical field and all instruments and materials used were sterile. A single dose of 50 mg/kg Cefuroxime was infused in both groups after the operation.

Postoperative analgesia was provided by bupivacaine (0.25%, 2-3 drops to the wound prior to closure) and acetaminophen-treated drinking water (3 mg/ml) for 48 hrs post surgically. Water administration commenced immediately after surgery and food was provided on the second postoperative day. Five animals from each group were sacrificed at the end of the 2nd, 6th, 12th and 16th postoperative week respectively. Euthanasia was achieved through intraperitoneal administration of sodium pentobarbital 250 mg/kg. The SIS implantation site of each animal was procured, fixed in 10% formalin and sent for pathology examination. Tissue sections were embedded in paraffin, cut and stained with eosin and hematoxylin. The repair process was evaluated for the presence of mucosa, muscularis popria and serosa. Inflammatory reaction was also evaluated and graded in a 0 to 3 scale (0 = no inflammation, 1 = mild, 2 = moderate, and 3 = severe inflammation). Immunohistochemical investigation was performed on paraffin sections, using an automated avidin-streptavidin method, and a polyclonal antibody against TGF-β (dilution 1:25, Spring Bioscience, Pleasanton, CA, USA).

TGF-β expression was evaluated separately in the epithelial cells and the stroma in a 0 to 3 scale (0 = no reaction, 1 = weak, 2 = moderate, and 3 = strong reaction). The histological slides were evaluated by two independent pathologists (D. M. and S. M.) blinded to the treatment received, and the mean value of the two scores provided for each case was used for further analysis. A 75% concordance rate was achieved between the two pathologists. Fisher’s exact test was used for statistics regarding the time needed for the healing process (presence or absence of mucosa over time for example), and Student’s t-test for statistics of quantitative characteristics such as intensity of inflammation and immunoreaction to TGF-β antibodies.

**RESULTS**

At the time of surgical repair (6-8 hours after the defect was created), animals of group B presented a blood clot at the site of the trauma, and signs of peritonitis: indolence, food refusal, and a small quantity of perito-
neal fluid. Two animals (one of group A, and one of group B) died within the first 48 postoperative hours due to pancreatic injury during the operation. The early post-op feeding may have contributed to the death of these two animals. The remaining animals tolerated the procedure and the feeding schedule well, and returned to normal activity within 3 to 5 days, while they gained weight (preoperative mean weight: 355.97 g; mean weight by the end of the 2nd week: 372.18 g; p<0.005). At the time we sacrificed the animals, no leakage was observed around the duodenal trauma and the patch. Mucosal regeneration was observed only in 2 out of 4 animals of group A at the end of the 2nd week (tab. 1). No muscular or serosal regeneration was observed in either group at this time (fig. 1 and 2). Mucosal regeneration was delayed in group B, and first appeared in 3 out of 5 animals (60%) at the end of 6th postoperative week. Mucosal regeneration included the formation of normal crypts and villi.

Developed muscular layer and serosa was observed in a minority of the animals of both groups at 12th week. Most animals of both groups presented complete duodenal wall regeneration at the end of the 16th postoperative week (fig. 3). None of the differences observed between the two groups and regarded the time needed for the healing process reached statistical significance. Inflammation was more intense in group B of animals during the first six weeks, and subsided considerably in both groups later in the course (1+ to 0 at 12th and 16th week). Intensity of inflammation did not differ significantly between the two groups. A limited foreign body type giant cell reaction

![Fig. 1. An animal of group A with immediate surgical repair of the duodenal defect showing mucosal regeneration at the upper middle portion of the micrograph (single arrow – end of 2nd week). A giant cell reaction is seen around stitches at the lower left part of the picture (double arrows – H&E, X100)](image)

![Fig. 2. An animal of group B with delayed surgical repair of the duodenal defect showing no mucosal regeneration (arrows) at the end of the 2nd week of the experiment (H&E, X25)](image)

### Table 1. Healing process, inflammation intensity, giant cell reaction, and TGF-β immunohistochemical expression in duodenal tissues during repair

<table>
<thead>
<tr>
<th>Week</th>
<th>Patch application</th>
<th>n</th>
<th>Repair</th>
<th>Inflammation</th>
<th>Giant cells</th>
<th>Epithelial TGF-β</th>
<th>Stromal TGF-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>immediate</td>
<td>4’d</td>
<td>2 M</td>
<td>2.50 (±0.5)</td>
<td>3</td>
<td>2.25 (±0.29)</td>
<td>1.75 (±0.5)</td>
</tr>
<tr>
<td></td>
<td>delayed</td>
<td>4’d</td>
<td>0</td>
<td>3.00 (±0)</td>
<td>4</td>
<td>1.33 (±0.29)</td>
<td>1.33 (±0.58)</td>
</tr>
<tr>
<td>6th</td>
<td>immediate</td>
<td>5</td>
<td>4 M, 1 MP, 1 S</td>
<td>2.50 (±0.55)</td>
<td>4</td>
<td>1.40 (±0.65)</td>
<td>1.30 (±0.45)</td>
</tr>
<tr>
<td></td>
<td>delayed</td>
<td>5</td>
<td>3 M</td>
<td>2.60 (±0.55)</td>
<td>3</td>
<td>1.13 (±0.48)</td>
<td>1.13 (±0.42)</td>
</tr>
<tr>
<td>12th</td>
<td>immediate</td>
<td>5</td>
<td>5 M, 2 MP, 1 S</td>
<td>0.75 (±0)</td>
<td>0</td>
<td>0.75 (±0.35)</td>
<td>0.25 (±0)</td>
</tr>
<tr>
<td></td>
<td>delayed</td>
<td>5</td>
<td>5 M, 1 MP, 1 S</td>
<td>0.80 (±0.45)</td>
<td>0</td>
<td>1.20 (±0.57)</td>
<td>0.50 (±0)</td>
</tr>
<tr>
<td>16th</td>
<td>immediate</td>
<td>5</td>
<td>5 M, 5 MP, 5 S</td>
<td>0.40 (±0.2)</td>
<td>0</td>
<td>0.50 (±0)</td>
<td>0.50 (±0)</td>
</tr>
<tr>
<td></td>
<td>delayed</td>
<td>5</td>
<td>5 M, 4 MP, 3 S</td>
<td>0.40 (±0.2)</td>
<td>0</td>
<td>0.90 (±0.35)</td>
<td>1.20 (±0.35)</td>
</tr>
</tbody>
</table>

n – number of animals, M – mucosa, MP – muscularis propria, S – serosa
a – mean inflammation intensity in 0 to 3 scale; b – number of cases showing giant cells; c – mean expression in 0 to 3 scale; d – one animal of each group died within 48 hours after the surgical procedure.
Immediate and delayed surgical repair of duodenal defects in rats with small intestinal submucosa patch

Immediate and delayed surgical repair of duodenal defects in rats with small intestinal submucosa patch was observed mainly around stitches in most of the animals of both groups at 6th and 12th week, but in no animal in the subsequent weeks. Immunohistochemical expression of TGF-β was much higher in both the epithelial cells and the stroma of the animals of group A than the animals of group B at 2nd and 6th week (tab. 1; fig. 4). TGF-β expression was generally lower in the subsequent weeks of surveillance (12th and 16th week), but it was found to be maintained higher in both the epithelial cells and the stroma of the animals of group B than the animals of group A.

Normal stroma was immuhistochemically negative for TGF-β, while normal villi generally presented a light staining for TGF-β. No difference between the two groups regarding TGF-β expression reached statistical significance, except of the much higher expression of TGF-β in the epithelial cells of group A than in Group B at the second week after operation (p < 0.0044).

DISCUSSION

Our study is the first to evaluate the delayed surgical repair of a duodenal injury using SIS patch as the only material. The purpose of this study was to prove that inflammation caused by peritonitis due to delayed surgical repair, does not affect the healing properties of SIS, making it a safe and efficient material for delayed management of duodenal trauma. In the group of animals with immediate surgical repair (group A), mucosal regeneration was observed in some of the lesions at the end of the 2nd postoperative week. In the group of animals with delayed surgical repair (group B), mucosal regeneration was observed in 60% of the animals at the end of the 6th postoperative week. Earlier regeneration of the mucosa in the animals of group A is attributed to the earlier application of the patch to the trauma, and the smaller degree of inflammation.

In both groups mucosal regeneration was complete by the end of the 12th week, while almost complete intestinal wall formation was seen at the end of the 16th week, which was the end point of our study. These findings are consistent with the results of previous studies on duodenal or small intestinal defects, where complete mucosal regeneration over the SIS patch was found (7, 8, 9). However, it should be mentioned that in studies where a tubular one-ply or four-ply SIS was interposed between isolated jejunal segments in dogs or rats, no mucosal regeneration was noted (8, 10). In addition, other determinants such as blunt versus penetrating trauma, size of the lesion, presence of necrosis or contamination could apparently affect the healing process and the end result in real life in humans.

The molecular structure of SIS acts as a biodegradable scaffold for cellular migration and consequent tissue repair. SIS contains important growth factors such as FGF-2 (Fibroblast Growth Factor-2) and TGF-β (Transforming Growth Factor-β), which elicit cellular migration and angiogenesis at the site of tissue defect, these being the principal actions in the process of tissue regeneration (8, 11). TGF-β

Fig. 3. An animal of group B at the end of 16th week showing complete mucosal regeneration at the left and middle portion of the picture (double arrows), and muscle wall and serosa formation at the right part (arrow – H&E, X100)

Fig. 4. Strong immunoreaction of TGF-β in regenerating villi at the upper right part of the micrograph (black arrows). The stroma also shows a moderate positive immunoreaction of TGF-β (white arrows – DAB, Hematoxylin, X100)
is a potent fibrogenic agent that stimulates fibroblast chemotaxis, and enhances the production of collagen, fibronectin, and proteoglycans (12). It decreases collagen degradation by decreasing matrix proteases, and increasing protease inhibitor activities. TGF-β has a strong anti-inflammatory effect, but may enhance some immune reactions (13). In vivo studies have confirmed that TGF-β increases granulation tissue, collagen formation, and wound tensile strength when applied locally or given systemically (14). TGF-β may act either as a positive or negative regulator of cell division (15). In general, TGF-β stimulates proliferation of mesenchymal cells, but inhibits growth of epithelial cells (16). It is noteworthy that the enterocytes normally express TGF-β, especially at the tips of the villi (17). TGF-β was more highly expressed in the enterocytes during the first weeks of our experiment in the group of animals with immediate surgical repair (group A) of the duodenal defect. This may be due to the earlier regeneration of the epithelium, highlighting an end point of the process, ie. inhibition of further growth of the epithelial cells.

TGF-β expression was lowered significantly in the subsequent weeks in the epithelial cells of group A of animals, while its expression was maintained comparatively higher in the epithelial cells of group B of animals, where delayed tissue repair was observed. In addition, TGF-β expression was found in the stroma of both groups of animals, and maintained its levels of expression during the experiment, following the evolution of the healing process and in keeping with the role of TGF-β in the stroma as described above.

CONCLUSIONS

SIS has demonstrated resistance to deliberate bacterial contamination in animal studies. SIS induces an integrative response with neovascularization through transmural capillary ingrowth, allowing the host to respond to the presence of bacteria and prevent colonization (18). This type of response is much different from an encapsulating foreign-body reaction elicited by synthetic materials, which restricts neovascularization of the graft, allowing bacteria to reside in an environment protected from the host’s immune response. It seems that SIS offers a substrate that allows the migration of new cells, promotes vascularization, and prevents infection, even if delayed surgical repair is applied to the duodenal trauma. New mucosa was functional as it was proved by the formation of villi and crypts, and the gain of body weight. Even though the discovery of the ideal material for such grafts is elusive, SIS seems to be a good choice for surgical restoration of duodenal defects as it appears to be effective for this purpose in other hollow organs and membranes of the body (19-23). More importantly, delayed surgical repair of the duodenal defect (as it most probably would happen in clinical practice), apart for some delay in the first few weeks, does not seem to affect significantly the end result, which most of the times was successful. In this regard, TGF-β seems to play a central role. Still, it is known that rodents respond to acute inflammatory stress through different genomic responses from humans (24), and any successful application of SIS in mice cannot guarantee an exactly similar result in men.

REFERENCES


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