Efficacy of platelet-rich plasma as a shielding technique after endoscopic mucosal resection in rat and porcine models

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Background and study aims: The aims were to assess the efficacy of endoscopic application of Platelet-rich plasma (PRP) to prevent delayed perforation and to induce mucosal healing after endoscopic resections.

Patients and methods: Colonic induced lesions were performed in rats (n=16) and pigs (n=4). Animals were randomized to receive onto the lesions saline (control) or PRP. Animals underwent endoscopic follow-up. Thermal injury was assessed with a 1–4 scale: (1) mucosal necrosis; (2) submucosal necrosis; (3) muscularis propria necrosis; and (4) serosal necrosis

Results: Saline treatment showed 50% of mortality in rats (P=0.02). Mean ulcerated area after 48 hours and 7 days was significantly smaller with PRP than with saline (0.27±0.02 cm² and 0.08±0.01 cm² vs. 0.56±0.1 cm² and 0.40±0.06 cm²; P<0.001). The incidence of thermal injury was significantly lower with PRP (1.25±0.46) than in controls (2.25±0.50); P=0.006. The porcine model showed a trend toward higher mucosal restoration in animals treated with PRP than with saline at weeks 1 and 2 (Median area in cm²: 0.55 and 0.40 vs. 1.32 and 0.79)

Conclusions: Application of PRP to colonic mucosal lesions showed strong healing properties in rat and porcine models.
was used. Syringes were centrifuged, obtaining 3 different layers; erythrocytes at the bottom, PRP and platelet-poor plasma (PPP) on the top (Fig. 1). PRP was activated with the addition of 20 mM CaCl$_2$ just before administration. Activated PRP becomes a viscous solution after 3 to 5 minutes, as a solid clotted jelly mass.

**Fig. 1** Four-step method to obtain platelet-rich plasma (PRP). a Peripheral blood was drawn from each animal. b PRP was obtained by centrifugation of blood at room temperature. c Three different layers were obtained: erythrocytes at bottom, PRP in the middle, and platelet-poor plasma on the top. d PRP was filled into a syringe.

**Shielding technique**
To perform endoscopic shielding technique (EST), healing coverage agents were applied over mucosal lesions with a catheter through the endoscopic working channel, covering as a shield the total surface of the ulcer. A volume of 1 mL in rats and 5 mL in pigs was applied to each animal. EST with PRP was performed positioning the tip of the catheter over the ulcer, then activated PRP was gently sprayed onto the lesion, and after 1 to 2 minutes the clot was firmly organized (Fig. 2).

**Colonic induced lesions in rats**
After a 24-hour fasting period with free access to drinking water, rats were anesthetized by isoflurane inhalation (1.5% with 98% O$_2$) and placed in a supine position. Remaining feces were flushed away by injecting water through the anus. A drop of lubricating jelly (Aquagel; Ecolab, Leeds, England) was applied on the anal sphincter to facilitate insertion of the endoscope. The endoscope was then gently passed through the anus and further introduced under endoscopic vision. Water was injected through the endoscope’s working channel to visualize the lumen of the colon.
Occasionally, the colon was inflated with air for better visualization of the lumen.

Colonic lesions were performed in the left colon, at 6 cm to the anal margin by Coagrasper Haemostatic Forceps (Olympus, Tokyo), with a power setting of 40W over 4 seconds [6,7] (Fig. 3). This technique produces deep thermal injury in the acute phase (48h), with development of peritonitis in the late phase, 7 days after the damage. Animals were randomly divided into 2 groups (n=8) for EST with PRP and saline. Animals were allowed to eat after the intervention. Rats underwent endoscopic follow-up at 48 hours and 7 days after EMR, and were euthanized by anesthetic overdose.

After sacrifice, the colon was opened longitudinally to examine colonic mucosa. Full-thickness samples of approximately 4 cm were taken from the proximal left colon surrounding endoscopic lesions.

**Colonic EMR-induced lesions in pigs**

In the porcine model, food was not allowed 12 hours prior to the procedure. Preparation for colonoscopy was done with saline irrigation. Colonoscopy was performed under sedation with propofol. Mucosal elevations were created at 25 cm to anal margin by submucosal injection of saline, then EMR with snare polypectomy and blended current was performed (Fig. 4). Animals were randomly allocated to EST with PRP or saline (n=2 each), and underwent endoscopic follow-up at 7 days and 14 days after EMR. Finally, pigs were euthanized and necropsied to obtain colonic samples as described above.

**Assessments**

Mucosal healing was evaluated as mean ulcerated area after 48 hours and 7 days in rats, and 7 days and 14 days in pigs. The mucosal healing rate was defined as a percentage of mucosal restoration. Measurement of mucosal lesion was performed comparing to the open forceps (5 mm) or by direct measurement with the specimen.

Thermal injury was evaluated with a 1 to 4 scale [6]: 1) mucosal necrosis; 2) submucosal necrosis; 3) transmural necrosis; and 4) peritonitis, microperforation, in hematoxylin and eosin (H&E) histological sections using a conventional microscope (Olympus, Shinjuku-ku, Tokyo, Japan).
Statistical analysis
All data in rats are reported as the mean ±SD, whereas in pigs as median. Statistical analyses were performed via unpaired student’s t test with SPSS software version 14.0 (SPSS Inc, Chicago, Ill). A P value < .05 was considered statistically significant.

Results

Mucosal healing
Delayed stricture or bleeding was not noticed in any animal included in the study. In rats, saline treatment showed 50% of mortality, being 0% with PRP (P = 0.02). Mortality was secondary to severe thermal injury with appearance of colon perforation in all animals. Basal ulcers were similar in both groups (0.41 ± 0.03 cm² with PRP vs. 0.38 ± 0.05 cm² with saline), but a significantly higher percentage of mucosal restoration was observed with PRP than with saline (80.5% vs. 2.4%; P < 0.001). Mean ulcerated area after 48 hours and 7 days was significantly smaller with PRP than with saline (0.27 ± 0.02 cm² and 0.08 ± 0.01 cm² vs. 0.56 ± 0.1 cm² and 0.40 ± 0.06 cm²; P < 0.001) (Table 1). Rats treated with PRP showed a strong healing activity at 48 h with high mucosal restoration at day 7 (Fig. 5).

In pigs, PRP showed a trend toward higher mucosal restoration than did saline, at weeks 1 and 2 (median area in cm²: 0.55 and 0.40 vs. 1.32 and 0.79; P = 0.12, respectively (Table 2 and Fig. 6). These data confirmed a higher mucosal healing rate with PRP than with saline, respectively (75.3% vs. 43.9%).

Thermal injury
Acute basal histologic assessment was not performed to avoid sacrifice of animals. EST with PRP induced a marked trend to less deep thermal injury in both studies. In rats, the incidence of thermal injury was significantly lower with PRP (1.25 ± 0.46) than with saline (2.25 ± 0.50); P = 0.006 (Table 1). Saline group showed necrosis of the muscularis propria and serosa on day 7, whereas rats treated with PRP showed superficial necrosis without injury in muscularis propria (Fig. 7). In pigs, PRP was associated with a significant reduction in thermal injury (Table 2) and mucosal inflammation with partial restoration of the epithelium in Week 2 (Fig. 8).

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**Table 1** Mucosal healing and thermal injury in experimental model with rats treated with platelet-rich plasma (PRP) or saline.

<table>
<thead>
<tr>
<th></th>
<th>PRP group (n=8)</th>
<th>Saline group (n=8)</th>
<th>Test</th>
<th>P-value</th>
</tr>
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<tr>
<td>Mortality (%)</td>
<td>0</td>
<td>50</td>
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<td>Basal ulcer (cm²)</td>
<td>0.41 ± 0.03</td>
<td>0.38 ± 0.05</td>
<td>n.s.</td>
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<tr>
<td>Mean ulcerated area at 48 hours (cm²)</td>
<td>0.27 ± 0.02</td>
<td>0.56 ± 0.1</td>
<td>&lt; 0.001</td>
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<tr>
<td>Mean ulcerated area at 1 week (cm²)</td>
<td>0.08 ± 0.01</td>
<td>0.40 ± 0.06</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Mucosal restoration (%)</td>
<td>80.5</td>
<td>2.4</td>
<td>&lt; 0.001</td>
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<tr>
<td>Thermal injury</td>
<td>1.25 ± 0.46</td>
<td>2.25 ± 0.50</td>
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</table>

Values are given as mean ±SD.

**Table 2** Mucosal healing and thermal injury in porcine model treated with platelet-rich plasma (PRP) or saline.

<table>
<thead>
<tr>
<th></th>
<th>PRP group (n=2)</th>
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<th>Test</th>
<th>P-value</th>
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<td>Basal ulcer (cm²)</td>
<td>1.62</td>
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<td>Mean ulcerated area at day 7 (cm²)</td>
<td>0.55</td>
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<tr>
<td>Mean ulcerated area at day 14 (cm²)</td>
<td>0.40</td>
<td>0.79</td>
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<td>Mucosal restoration (%)</td>
<td>75.3</td>
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<tr>
<td>Thermal injury</td>
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<td>2.5</td>
<td></td>
<td></td>
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</table>

Values are given as median.

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Fig. 5 Follow-up in both group of rats at 48 hours and 7 days. Macroscopic images (left) in saline group. Endoscopic follow-up (right) in animals treated with platelet-rich plasma.
Fig. 6  Endoscopic follow-up in porcine model in both treated animals, saline (left) and platelet-rich plasma (right), at baseline, Day 7, and Week 2 of follow-up.

Fig. 7  Histologic study of colon sections with thermal injury in both groups of rats on Day 7. Saline group (left) shows necrosis of the muscularis propria and serosa. Rats treated with platelet-rich plasma (right) show superficial necrosis without injury in muscularis propria.
Discussion

This study showed that in both animal models, use of EST with application of PRP to endoscopic-induced mucosal lesions in the colon induced strong healing properties and reduced deep thermal injury. We need a way to prevent delayed perforation after large mucosal resections. One option is clip closure of the mucosal defect, but its use is limited to lesions smaller than 40 mm and the techniques can sometimes be complex [3]. The tissue-shielding method with polyglycolic acid sheets is promising approach, but it takes at least 35 minutes [2,8].

Endoscopic and tomographic study of colon rats has been assessed by our group [7], as well as the proper method for preparing the colon before colonoscopy [9]. We have previously described an experimental model in rats that reproduces deep thermal damage in the colon, allowing application of endoscopic treatments, which showed that the muscular layer was thinner in the proximal left colon [6]. Our group has recently performed performing EST with newly developed hydrogels that congeal over mucosal lesions after therapeutic endoscopy as a quick and safe technique for preventing delayed perforations after therapeutic endoscopy [10,11].

PRP gel contains high levels of platelets, which release high quantities of key growth factors and recruits cells to the site of tissue damage for repair [4,12], which is essential for physiologic mucosal healing. Recently, it has been reported that topical use of PRP gel promotes wound healing in primary colonic anastomosis [13]. Anabolic effects are directly correlated to platelet number [5], so we selected the PRP fraction of plasma to obtain better results. PRP preparation was carried out with a standardized protocol [14], where mean PRP platelet amount is about 4 to 5 times greater than that observed within peripheral blood samples. Our study revealed that PRP has 2 beneficial effects: prevention of delayed perforation after therapeutic endoscopy and induction of mucosal healing after deep thermal injury. We initiated the experimental study with rats because those animals are widely used in research. A porcine model was selected to reproduce daily practice. EST with PRP has proven to avoid mortality in rats, because platelet clot acts as a shield with a mechanical defense associated with a thicker layer. We have also found, in both rat and porcine experimental models, that treating ulcers with PRP results in significantly faster and stronger healing of mucosa, with restoration of areas of deep thermal injury. Furthermore, PRP, as an inexpensive and easy-to-obtain gel, shows triple biological activity, hence it can be called a true “tri-bio” shield because it is bioadhesive, biodegradable, and bioactive. Despite these promising results, our study has limitations because it is preclinical and preliminary. One of the most important criticisms is that we do not have data on delayed bleeding or stricture, so we cannot evaluate the efficacy of PRP in prevention of these complications. Neither have we performed an acute histologic assessment of thermal injury and or evaluated the concentration of PRP in this protocol but data in those areas are available from previous studies.

In conclusion, EST with application of PRP to EMR-colonic mucosal lesions showed strong healing properties in rat and porcine models. These data suggest that EST may be a new and easy way to treat and manage mucosal lesions resulting from therapeutic endoscopy.

Competing interests: None

References