

Experimental Study of Hemostasis with Local Injection of Fibrin Glue to Treat Hemorrhagic Ulcer in the Upper Digestive Tract in Comparison with Ethanol

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Since 2000, we have been conducting studies on endoscopic hemostasis with local injection of fibrin glue (FG). The procedure has produced satisfactory results in treating patients with hemorrhage of the upper digestive tract who were difficult to treat with more conventional method, such as clipping or ethanol injection. There have been no reports on the histopathological effects of FG. In this study, the effects and efficacy of locally injected FG were investigated by using rats and the result was compared with the effects of ethanol (E). Sprague-Dawley rats were subjected to a laparotomy under ether anesthesia, followed by mechanical creation of a hemorrhagic ulcer at the pyloric vestibulum. In group E, in comparison with the control group, a hemostatic effect was recognized; but tissue damage was intensified and infiltration by inflammatory cells increased during the several days following the procedure. In group FG, the hemostatic effect was almost comparable to that of group E and there were only limited signs of tissue damage, which were much milder than in group E. In the latter group, the development of a postoperative perforation was observed, whereas no animals in the sham or group FG experienced similar conditions. Compared with E, local injection of FG may be a practical procedure for endoscopic hemostasis.

Key words: hemorrhagic ulcer, gastroduodenal, hemostasis with local injection, fibrin glue, ethanol

Introduction

For endoscopic hemostasis to treat hemorrhage of the upper digestive tract and after endo-

scopic mucosal resection (EMR) and polypectomy, procedures such as local injection of ethanol^{1)~3)}, hypertonic saline-epinephrine (HSE)^{4)~6)}

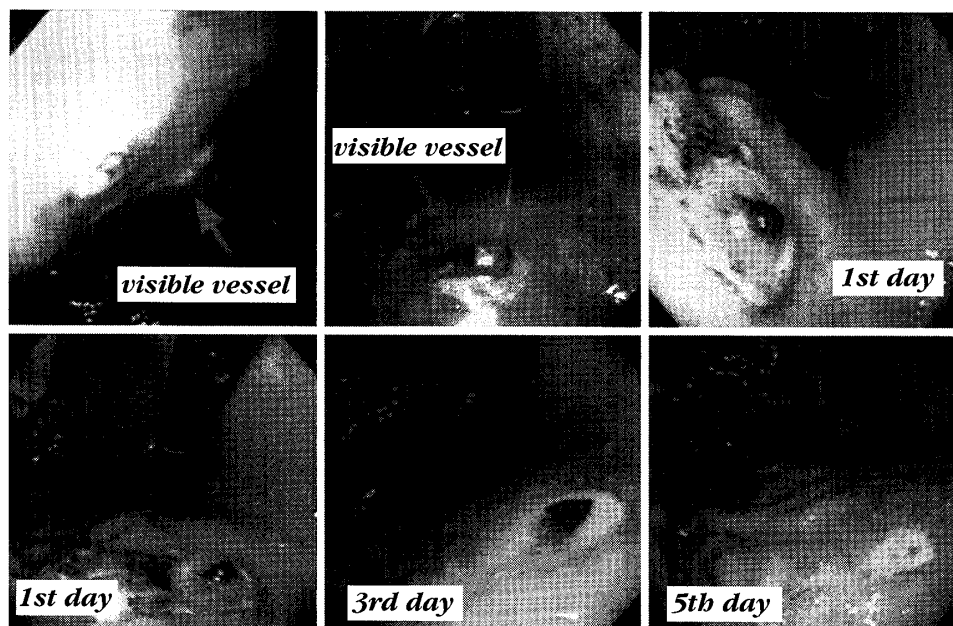


Fig. 1 Hemostasis of bleeding gastric ulcer with fibrin glue

This ulcer is located in the upper front wall of the gastric body. Because this region is not readily accessible for endoscopic clipping, fibrin glue was locally injected instead.

and sclerosants^{7)~9)} and clipping^{10)~13)} have been employed. However, ulcers located in certain areas cannot be observed from the front, thus presenting a problem in clipping^{10)~13)} (Fig. 1). Local injection of ethanol (E) may cause tissue damage and is therefore associated with problems such as hemorrhage from multiple sites involved in an extensive ulceration³⁾¹⁴⁾ and the optimum quantity to be injected to treat the original diseases¹⁵⁾. It was reported that, compared to E, HSE takes longer for effectuation and outcome is less reliable³⁾, and the effect of polidocanol is inferior to fibrin glue sealing¹⁶⁾.

Since 1988, local injection of fibrin glue (FG) to treat hemorrhagic ulcers began to be reported^{17)~19)}. However, all were clinical reports and none examined its histological effects.

Since October 2000, we have applied FG for hemorrhagic control and found it effective in endoscopic hemostasis in 37 patients (Table) those who did not respond favorably to E administration or clipping²⁰⁾; who suffered from hemor-

Table Cases of endoscopic hemostasis with local injection of fibrin glue

Cases	No. of cases	Rebleeding after therapy (cases)
Endoscopic hemostasis cases		
Gastric ulcer	13	0
Gastric cancer	3	0
EMR or polypectomy cases		
Gastric polyp	11	0
Colon polyp	10	0
Total	37	0

From October 2000 to April 2002

rhagic ulcers while under anticoagulant therapy following a condition such as a cerebral infarction; or those who required care following a polypectomy and EMR (Figs. 2, 3).

In the present study, rats were used to compare the histological changes, such as hemostatic effects and efficacy in tissue regeneration of fibrin glue with those of ethanol that has been used for hemostatic purpose.

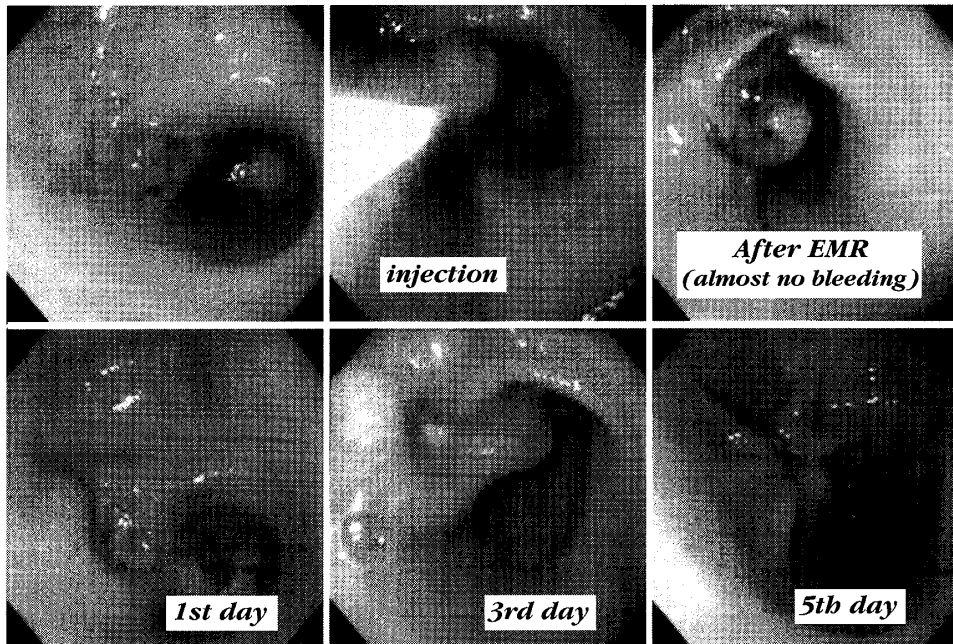


Fig. 2 EMR of the stomach

Injecting fibrin glue into a polyp (3 cm in diameter) in the pyloric vestibulum. EMR was conducted, after which hemorrhage was hardly noted and the succeeding healing process was satisfactory.

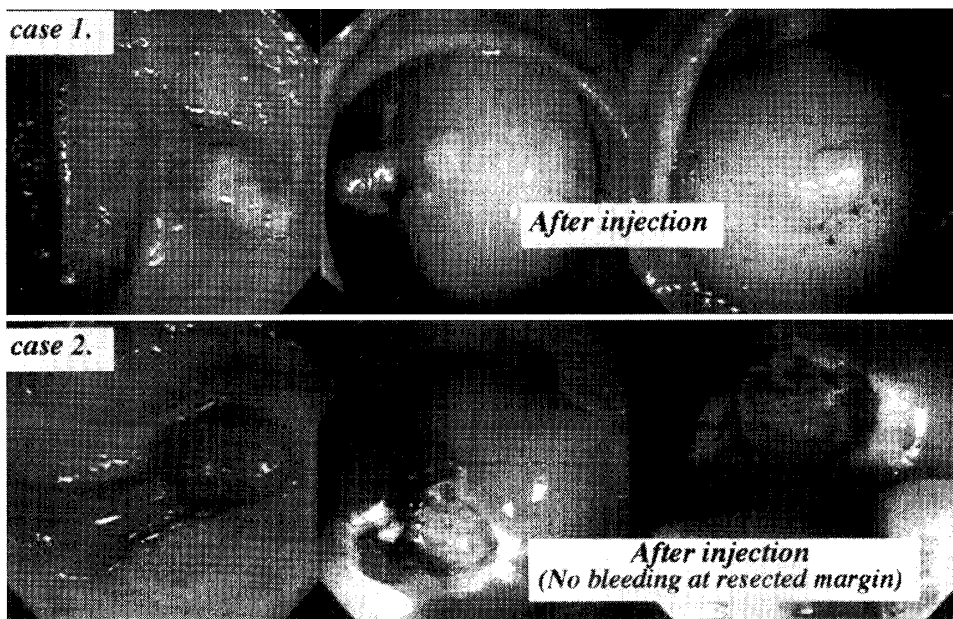


Fig. 3 EMR of the colon

EMR was conducted in 2 examples of colonic polyps by injecting 1 ml of fibrin glue into the root of the polyp. After the procedure, hemorrhage from the resected margin was barely noted.

Materials and Methods

Sprague-Dawley rats (aged animals, Sankyo

Labo Inc, Shizuoka, Japan) were subjected to a laparotomy under ether anesthesia. Having made

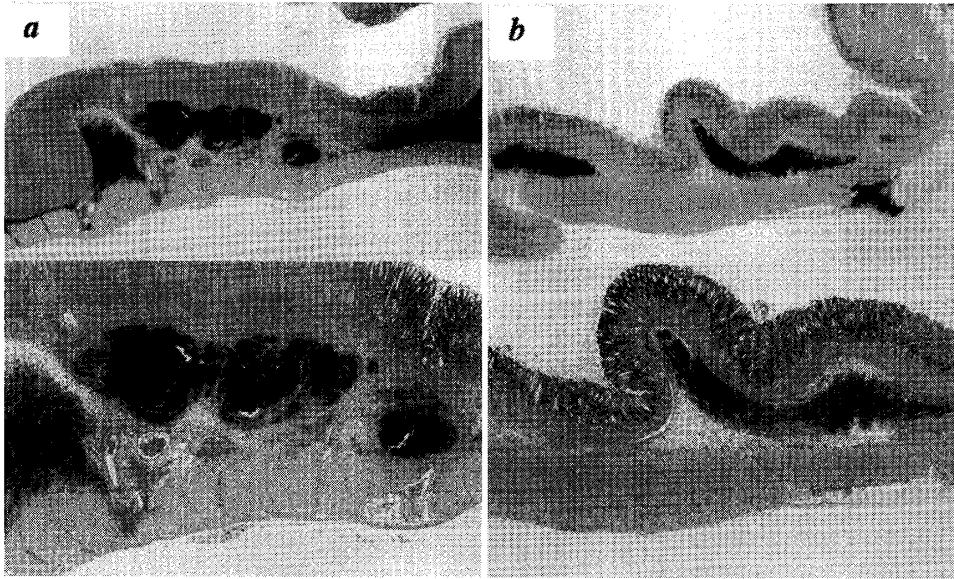


Fig. 4 Local injection of saline (sham)

Light microscopy (HE staining, upper row $\times 5$, lower row $\times 10$). a: immediately after injection. b: on the 5th day.

Submucosal hemorrhage was exaggerated, in contrast to the effect seen after injecting fibrin glue. On the 5th day, fairly acute bleeding was still apparent.

an incision at the anterior mid-portion of the gastric wall, a hemorrhagic ulcer was mechanically induced by cutting into the mucosal layer at the pyloric vestibulum. Fibrin glue (FG, Bolheal, Teijin, Tokyo), E, or saline was locally injected at this ulcerous site and the surgical wound was closed, after that the animals fasted and were maintained on a glucose solution. On the 1st, 3rd, and 5th day after this treatment, the stomachs were excised and histopathologically examined to find the hemostatic and regenerative effects of each agent and any associated tissue damage.

Comparisons were made among the following three groups: one that received a local injection of FG, the second that had a local injection of absolute ethanol E, and the third that received a local injection of physiological saline (sham). The amount to be injected was set at 0.05~0.1 ml for each agent. A 27G injection needle was used for hemostasis. Each group was composed of 7 rats. For histopathological observation, the tissue samples were stained with hematoxylin-eosin (HE) and

phosphotungstic acid hematoxylin (PTAH) and the histological changes were compared.

Handling of the animals and the details of the present experiment were approved by the Committee for Experimental Animal Facilities of this university.

Results

Although the local injection did not cause any tissue damage in the sham group, hemorrhage into the mucosa and submucosal region was noted in the histopathological specimens of this group on the 5th day (Fig. 4). Although no hemorrhage was noted in the group E, tissue damage was intensified following the injection. Infiltration by inflammatory cells was also evident in this group (Fig. 5). Group FG, on the other hand, indicated some signs of tissue damage, which was much milder than that of group E. The fibrin that had been injected into the submucosal region persisted until the 5th day, suggesting a sustained hemostatic effect in this group (Fig. 6). The sham group and group FG were free of per-

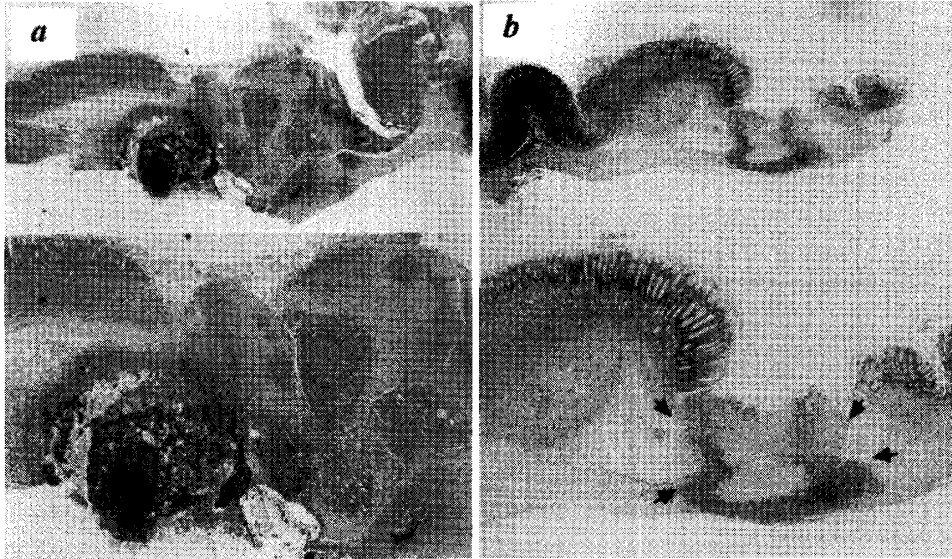


Fig. 5 Hemostasis with a local injection of ethanol

Light microscopy (HE staining, upper row $\times 5$, lower row $\times 10$). a: immediately after injection. b: on the 5th day.

On the 5th day, bleeding was under control but infiltration of inflammatory cells (an arrow) was very noticeable.

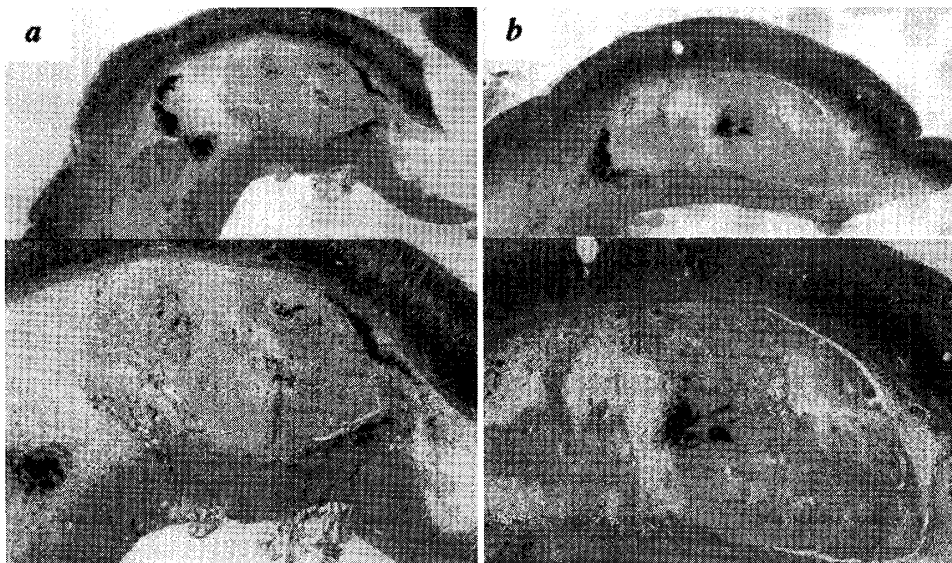


Fig. 6 Hemostasis with a local injection of fibrin glue

Light microscopy (HE staining, upper row $\times 5$, lower row $\times 10$). a: immediately after injection. b: on the 5th day.

Submucosally injected fibrin remained until the 5th day. Infiltration of inflammatory cells was slight and hemorrhage was minimal.

forations, which were recognized in group E.

Discussion

Recent progress in endoscopic therapeutic

technology has been truly remarkable, utilizing diverse chemical agents and materials. Since 1988, the application of a local injection of fibrin

glue to hemorrhagic ulcers has been reported occasionally^{17)~19)}. Starting in October 2000, we have been using fibrin glue and found it to be effective in 37 patients, those in whom E injections or clipping were found to be inadequate as a hemostatic measure²⁰⁾; those undergoing endoscopic hemostasis to be treated for hemorrhagic ulcers while being under anticoagulant therapy for a condition such as a cerebral infarction; or those requiring hemorrhagic control during procedures such as polypectomy and EMR.

The major features of hemostasis using fibrin glue are minimal tissue injury and the absence of adverse effects such as expansion of the ulcerous lesion due to a drug injection^{15)17)~19)}. There is a report stating that the presence of a sufficient quantity of fibrin is associated with a wound healing effect¹⁵⁾¹⁸⁾. It has been reported that at the wound site, the fibrin fuses with the neighboring tissue to form a fibrin mesh; and subsequently it aids in generating fibroblasts and collagen, thus promoting wound healing¹⁵⁾¹⁸⁾. These reports have described local injections of FG that were applied to clinical cases of ulcers: none delved into the histopathological outcome of the procedure. In the present study, rats were used to observe the effects of a locally injected FG on tissue, in comparison with E, the current choice for hemostasis in cases of hemorrhagic ulcer.

It has been proven in clinical practice that E showed a potent hemostatic effect^{1)~3)}. As frequently experienced in clinical practice, it caused spreading of the ulcerous lesion and accentuated tissue damage³⁾¹⁴⁾. Although there were no signs of hemorrhage, infiltration by inflammatory cells continued to intensify in group E. Among the three groups examined in the current study, this was also the only group that included animals that displayed postoperative perforation wounds, which, it was suspected, was due to excessive tissue damage. In the sham group and group FG, in-

filtration by inflammatory cells was slight with no further progression of the pathological process. Hemorrhage was noted in the sham group. In group FG, not only was hemorrhage totally absent: a sufficient quantity of fibrin persisted until the 5th day. In the normal process of recovery from ulceration, precipitation of fibrin at the wound site and subsequent fibrin mesh formation are essential¹⁵⁾¹⁷⁾, which explains the presence of fibrin in the sham group. In group FG, a larger number of fibrin layers were formed, showing that fibrin mesh formation had been augmented by external means during the natural process of woundhealing. In an examination on the 5th day following treatment in the present experiment, the process of spontaneous healing had not yet started but the treatment had already proved to be effective. The persistence of the fibrin layers indicated that the hemostatic effect of the treatment by a local injection had been preserved.

To examine wound healing, a longer experimental observation period is needed. However, the results of this study provided proof that a local injection of FG can be an effective hemostatic means in difficult cases that would otherwise present a problem in hemostatic control, e.g. prevention of minute bleeding in cases with disease conditions such as coagulation disorders and resisting conventional hemostatic procedures; and treating hemorrhage at sites such as the esophagus that require special care in the quantity of E or other hemostatics due to the possibility of tissue damage¹⁵⁾.

Conclusion

- 1) The effect of a local injection of FG on a mechanically induced hemorrhagic ulcer in the gastric wall was investigated in comparison with E.
- 2) The hemostatic effect of a local injection of FG was comparable to that of E.
- 3) Hemostatic treatment with a local injection of FG produced minimal tissue damage, and is as-

sociated with a sustained hemostatic effect due to the persistent presence of fibrin at the site, and caused only slight inflammatory cell infiltration. On the contrary, a local injection of E produced intensified tissue damage and evident inflammatory cell infiltration.

4) Although a longer experimental observation period is needed to examine wound healing, the results of this study provided proof that a local injection of fibrin glue produces safe hemostatic effects. It is believed that this procedure is an safe and effective hemostatic procedure.

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上部消化管の潰瘍性出血に対するフィブリン接着剤局注による
止血の実験的研究—エタノールとの比較—

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2000年以來、フィブリン接着剤(FG)の局注による内視鏡的止血を検討してきたが、クリッピング、エタノール(E)局注の実施し難い上部消化管の止血に対して満足しうる結果が得られた。これまで上部消化管の潰瘍性出血に対するFG局注による内視鏡的止血についてはかなりの報告がみられるが、多くは臨床例についての報告であり、その病理組織学的検討についての報告は殆ど見当たらない。ここではラットを用いて、実験的に上部消化管の潰瘍性出血を作製し、これに対するFG局注の効果と有効性について病理組織学的にE局注と比較しながら検討した。S-Dラットをエーテル麻酔下に開腹し、胃切開の後、胃幽門前庭部に機械的に出血性潰瘍を作製した。FGあるいはEを潰瘍部に局注した後、外科創を縫合した。その1, 3, あるいは5日後に胃を摘出し病理組織学的に検討した。E群ではcontrol群(生理食塩水局注)と比較して止血効果を確認したが、組織傷害は増強し、炎症細胞の浸潤が局注後数日間増加する像がみられた。FG群の止血効果はE群とほぼ同等であったが、組織傷害は限られた徴候がみられたのみで、E群よりも軽度であった。E群では胃壁の穿孔例がみられたが、FG群およびcontrol群では穿孔例はなかった。E群と比較してFG群は組織刺激性ないし傷害が少なく、潰瘍を増悪することもなく、組織に長時間停留して作用が持続的であった。以上よりFG局注は有効な止血法であり、上記利点をもつ内視鏡的止血法として加えうるものと考えられる。