

# Failure of Cyanoacrylate Tissue Glue (Flucrylate, MBR4197) to Stop Bleeding from Experimental Canine Gastric Ulcers

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*A plastic tissue adhesive, trifluoroisopropyl 2-cyanoacrylate (Flucrylate™, MBR4197), was tested for hemostatic efficacy in acute laparotomy experiments using a canine model of acute bleeding gastric ulcer. An improved delivery system suitable for endoscopic use was developed. Hemostatic efficacy of the adhesive was tested in both briskly bleeding ulcers and in oozing ulcers after partial treatment with a heater probe. In pilot studies at laparotomy, primary and adjunctive cyanoacrylate therapy of 81 bleeding ulcers were evaluated in seven unheparinized foxhounds. Hemostasis was produced in 11% of ulcers treated with cyanoacrylate alone and in 31% of ulcers treated with cyanoacrylate as an adjunctive after partial heater-probe treatment; no sham-treated control ulcers stopped bleeding under the conditions of the experiment. To evaluate Flucrylate™ using our standard heparinized ulcer model, a randomized study was performed in six heparinized foxhounds at laparotomy. Ulcers were randomized to treatment with cyanoacrylate alone, adjunctive cyanoacrylate, heater probe alone or untreated control. Sham-treated control ulcers or ulcers treated with cyanoacrylate alone did not stop bleeding; 42% of ulcers treated with cyanoacrylate as an adjunctive stopped bleeding; all ulcers treated with a heater probe stopped bleeding. In this experimental model of acute bleeding gastric ulcer, trifluoroisopropyl 2-cyanoacrylate (Flucrylate™, MBR4197) did not stop severe bleeding and was unpredictable as an adjunctive treatment.*

The ideal endoscopic treatment for acute bleeding upper gastrointestinal lesions would be: (1) convenient and easy to use; (2) 100% effective in stopping bleeding; and (3) nontoxic and minimally injurious to the underlying tissue. Electrocoagulative techniques (1, 2), lasers (3–6), heater probes (7), hemostatic clips (8), and injection techniques (9) have all been considered for treatment of upper-gastrointestinal hemorrhage. Although some are better than

others, none of these techniques fulfills all three requirements for the ideal agent.

Cyanoacrylate tissue glues adhere strongly to tissue and to virtually all other substances except Teflon or polyethylene. They can be applied from a distance as an aerosol spray. Although cyanoacrylates are relatively nontoxic, the shorter-chain analogs cause local tissue necrosis (10–12), and some analogs occasionally cause fibrosarcomas and other tumors when embedded in rodent tissue (11, 13, 14). Application of cyanoacrylates to the surface of bleeding gastrointestinal lesions is technically possible and may prove to be permissible clinically if they can be shown to safely stop brisk bleeding. Layers of cyanoacrylate sprayed on bleeding lesions may fall off in time, but toxicity of degradation products

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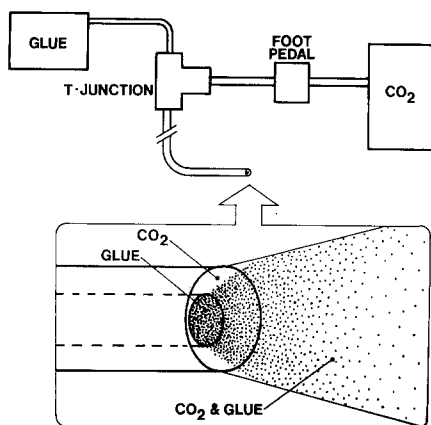


Fig 1. Schematic drawing of glue delivery system used in these studies.

absorbed from the gastrointestinal tract seems minimal (16). Trifluoroisopropyl 2-cyanoacrylate (Flucrylate™, MBR4197) aerosolizes easily and polymerizes uniformly in less than 10 sec; it has shown minimal animal toxicity (15). In this study, we utilized a previously described canine model of acute bleeding gastric ulcer (16) to test the efficacy of this cyanoacrylate tissue adhesive in stopping severe bleeding.

## MATERIALS AND METHODS

**Instrument Design.** Trifluoroisopropyl 2-cyanoacrylate (Flucrylate™, MBR4197) was supplied by the 3M Corporation (3M Center, Minneapolis, Minnesota) in 10-ml aerosol push-button bottles as a 5% solution with 95% freon propellant.

The delivery system which we devised for these studies consists of a 2.4-mm OD flexible polyethylene catheter containing an inner tube of 1.2 mm OD within its lumen (Figure 1). Pressing the button on the pressurized bottle allows the cyanoacrylate with freon to flow through the inner catheter. Dry CO<sub>2</sub> gas flows through the space between the inner and outer tubing. The CO<sub>2</sub> flow rate is adjustable and is activated by an electronic foot-pedal switch. The glue with its freon propellant mixes at the distal end of the catheter with the CO<sub>2</sub>. The Venturi effect caused by the rapid flow of CO<sub>2</sub> around the tip of the inner catheter hastens glue exit and atomizes the glue so that it is delivered in a fine spray. CO<sub>2</sub> alone is used to blow off obscuring blood from an ulcer prior to application of glue.

**Preliminary Studies.** The optimal method of applying Flucrylate™ evolved from preliminary laparotomy experiments on dogs under general anesthesia. The fundal gland mucosa was exposed via gastrotomy. To assess adhesiveness Flucrylate™ was applied to normal, abraded, or thermally coagulated mucosa. The glue was also applied to standardized experimental ulcers made with a modified suction biopsy instrument—the “ulcer maker” (16). The glue was used both as primary therapy

for briskly bleeding ulcers and as secondary adjunctive treatment after bleeding had been slowed to an ooze with a heater probe. We included the treatment category of cyanoacrylate glue after partial heater-probe therapy to test whether the glue could stop residual ooze after partial treatment with a modality known to be effective (7). Bleeding experimental ulcers were treated in heparinized and unheparinized animals to evaluate the effect of heparin on glue hemostasis. The CO<sub>2</sub> flow rate and the distance of the glue delivery catheter from the mucosa were varied to determine the most effective method of applying the glue.

**Final Study.** The ability of Flucrylate™ to stop bleeding from acute experimental gastric ulcers (16) was evaluated at laparotomy in heparinized foxhounds. Intravenous pentobarbital anesthesia and endotracheal intubation were used. After surgical hemostasis the dogs were given intravenous heparin, 200 units/kg initially and 100 units/kg one hour later. Core temperature, vital signs, and fluid status were monitored in a controlled-environment operating chamber. In each experiment a long anterior gastrotomy exposed the fundal gland mucosa.

Standard-sized acute gastric ulcers were made in fundal gland mucosa with the ulcer maker. After its creation, each ulcer's initial bleeding rate was quantified by collecting the blood lost in a preweighed glass beaker for the first 3 min. Ulcers were then categorized by their initial bleeding rate as mild (<1 cc/min), moderate (1–2 cc/min) or severe (>2 cc/min). Within each bleeding category, treatment was randomized to one of four groups: cyanoacrylate glue alone, heater probe alone, adjunctive cyanoacrylate treatment after partial heater-probe treatment, or sham-treated control. Experiments were terminated when splanchnic vasoconstriction was observed. This endpoint was determined clinically by gastric mucosal cyanosis or by diminished bleeding volume (two mildly bleeding ulcers in succession) after more than one hour's surgery.

In ulcers randomized to glue alone, a jet of CO<sub>2</sub> (flow ≈ 100 cc/sec; pressure ≈ 60 psi) was used to remove obscuring blood and to dry the ulcer. Flucrylate™ with freon was then sprayed on the ulcer in a 1-sec burst using a jet of CO<sub>2</sub>. If bleeding continued, the ulcers were treated immediately with an additional 1-sec application of glue. Ulcers randomized to heater probe alone were treated with multiple 1-sec applications of the 3.2-mm-diameter heater probe at 150° C until bleeding stopped (maximum number of applications = 25). Ulcer bleeding that persisted after the above two treatments was quantified for minutes 6–9 after the ulcer's creation.

Ulcers randomized to adjunctive treatment with Flucrylate™ were first partially treated with the heater probe until the blood flow from the ulcer had slowed to an ooze. Blood flow was then quantified for 1 min, following which cyanoacrylate therapy was applied as described above. A final 3-min blood collection was made from those lesions continuing to bleed after the second treatment interval.

Control ulcers were sham treated with ten 1-sec applications of the cold heater probe (~23° C) for the first treatment period. Bleeding was then quantified for 1 min. For the second treatment period two 1-sec bursts of CO<sub>2</sub> gas (flow ≈ 100 cc/sec; force ≈ 60 psi) plus freon propellant without Flucrylate™ were applied. Bleeding was

## CYANOACRYLATE ULCER TREATMENT

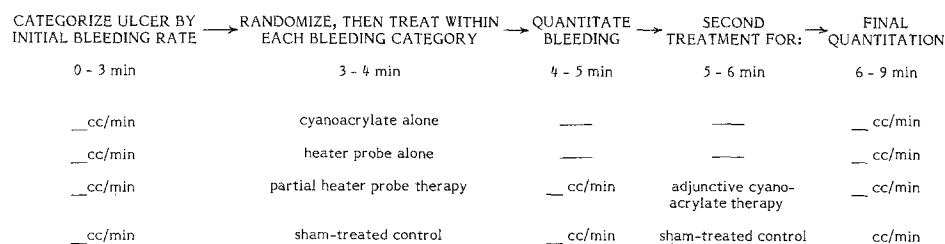


Fig 2. Summary of experimental design.

quantified for a final 3 min after the second treatment period.

Following the total observation period of nine minutes, any ulcers still bleeding were stopped with the heater probe in order to minimize total blood loss. The experimental design is summarized in Figure 2.

While using Flucrylate™ the investigators and their assistants wore goggles and industrial-grade face masks supplied by the 3M Company. Working without these protective devices resulted in subjective ocular and/or respiratory irritation.

At the conclusion of each experiment, the animal was sacrificed and the stomach removed. The stomach was pinned out on a paraffin slab and fixed in Hollande's solution for 24–48 hr. The ulcers were then removed, embedded in a paraffin–piccolyte mixture, sectioned at 4–6  $\mu$ m, and stained with hematoxylin–eosin–Alcian blue for histological examination.

## RESULTS

### Preliminary Studies

Flucrylate™ did not adhere to normal mucosa, but it did adhere to mucosa injured by abrasion or the heater probe. When sprayed on acutely bleeding ulcers, the glue stuck to the base of the ulcer better than to its rim.

The glue delivery system was evaluated in acutely bleeding ulcers in both heparinized and unheparinized dogs. The duration of glue application was varied from 1 to 13 sec and the pressure of CO<sub>2</sub> from 25 to 80 psi. A CO<sub>2</sub> jet of 60 psi ( $\approx$ 100 cc/sec) with 1-sec bursts of Flucrylate™ was chosen because it produced a consistently uniform glue layer without causing separation of the gastric wall. At a CO<sub>2</sub> pressure of 80 psi, Flucrylate™ was sprayed in a thin uniform layer, but the force of the CO<sub>2</sub> gas dissected the gastric mucosa from the submucosa, depositing the glue in the deep layers of the gastric wall. Longer Flucrylate™ applications resulted in thick layers that cracked after polymerization. Shorter applications did not deposit an adequate amount of glue for adherence. Low-pressure CO<sub>2</sub> (<60 psi) produced a nonuniform layer of glue.

Flucrylate™ was sprayed varying distances from acute ulcers. A 2- to 3-cm circle of glue could be reproducibly applied when the catheter tip was held 1–2 cm from the surface of the mucosal defect.

Pilot studies of the hemostatic effect of Flucrylate™ on bleeding experimental gastric ulcers in four heparinized mongrel dogs showed continued oozing of blood from beneath the layer of glue. To assess the effect of heparinization on Flucrylate™ hemostasis, 81 ulcers were made at laparotomy in seven unheparinized foxhounds (20–27 kg). After an initial 1-min bleeding quantification, treatment was randomized to Flucrylate™ alone, adjunctive Flucrylate™ therapy after partial heater-probe coagulation, or untreated control. Five of 44 ulcers treated with glue alone stopped bleeding for the observation period of up to 5 min; 5 of 16 adjunctively treated ulcers stopped bleeding; 0 of 21 untreated controls stopped bleeding. The difference in ulcer hemostasis between either treatment group and untreated control was not statistically significant ( $P > 0.05$ , Fisher's exact test). Selected lesions were studied histologically as described above.

### Final Study

Forty-eight ulcers were made in six heparinized foxhounds (23.6–26.5 kg). Initial bleeding rate was mild in 23, moderate in 19, and severe in six.

The 7 sham-treated control ulcers continued to bleed with a spontaneous decrease in bleeding rate of  $66 \pm 17\%$  (mean  $\pm$  SD) from the first 3 min to the final 3 min. All 17 ulcers randomized to treatment with the heater probe stopped bleeding completely after a mean of 12 (range 6–21) 1-sec applications at 150° C.

None of the 12 ulcers treated with cyanoacrylate glue alone completely stopped bleeding despite a reduction in bleeding rate of  $82 \pm 20\%$  (mean  $\pm$  SD) (Table 1). After glue application blood collections were often incomplete because there was oozing from multiple sites along the perimeter of the glue circle.

TABLE 1. MEAN RATE OF BLEEDING (ML/MIN)

	<i>Initial</i>	<i>Final*</i>	<i>Decrease (%)</i>
Sham-treated control	1.12	0.38	66
Range	0.36–1.65	0.14–0.59	
Heater probe alone	1.20	0	100
Range	0.24–3.34		
Cyanoacrylate alone	1.14	0.20	82
Range	0.37–1.95	0.01–1.06	

\*Minutes 6–9 after the ulcer's creation.

In the 12 ulcers randomized to adjunctive Flucrylate™ treatment, partial heater-probe coagulation slowed the rate of hemorrhage from a mean of 1.26 cc/min (range 0.9 to 3.16 cc/min) to a mean of 0.19 cc/min (range 0.10–0.73 cc/min). When these partially treated ulcers were treated with Flucrylate™, five of 12 stopped bleeding. The rate of bleeding after partial heater-probe treatment did not predict which ulcers would stop bleeding when sprayed with glue.

Heater-probe treatment was superior to either sham treatment ( $P < 0.02$ , Fisher's exact test) or to cyanoacrylate alone ( $P = 0.001$ , Fisher's exact test), but not to cyanoacrylate following partial heater probe coagulation ( $P > 0.05$ , Fisher's exact test) (Table 2).

### Pathology

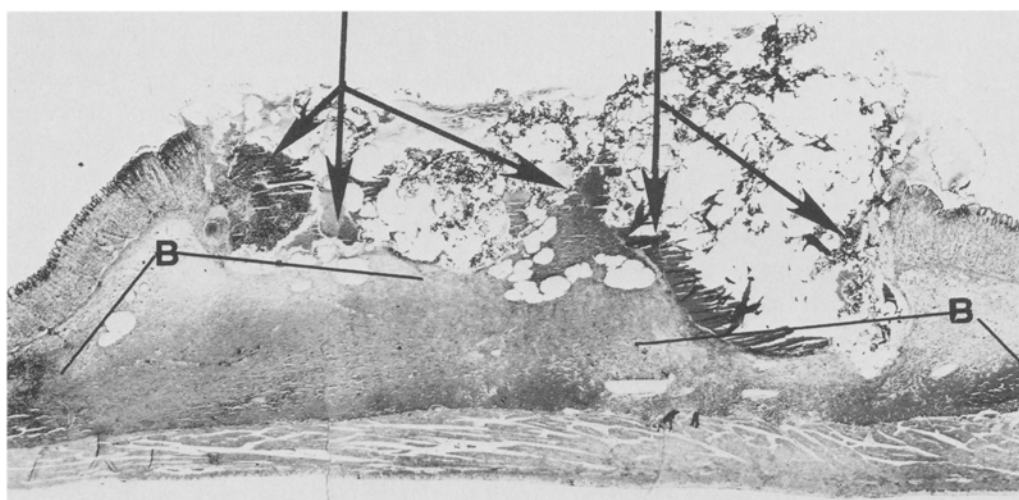
Gross examination of all glue-treated lesions showed a white crust of polymerized cyanoacrylate covering the ulcer; adherence to the ulcer rim was

irregular in lesions treated with the tissue glue alone. Adherence of glue to the rim of ulcers partially treated with the heater probe was more uniform. Flucrylate™ adhered to part of the ulcer base, but persistent bleeding beneath the glue layer often produced a bubble of blood which raised the layer of glue above the ulcer base.

Microscopically, four of the five ulcers that stopped bleeding in heparinized dogs after adjunctive treatment with cyanoacrylate tissue glue showed evidence of continued bleeding beneath the glue layer: hemorrhage could be seen under intact elevated mucosa, suggesting continued ulcer bleeding with dissection of blood along the path of least resistance (Figure 3). There was no microscopic evidence of Flucrylate™ below the submucosa and no acute damage to the muscularis externa in glue-treated lesions. Glue-treated ulcers from unheparinized and heparinized animals were identical in appearance. Sham-treated ulcers and heater-probe-treated lesions were indistinguishable from those reported elsewhere (7, 16).

### DISCUSSION

Alkyl 2-cyanoacrylate plastic tissue adhesives were developed in the 1950s for use in battlefield trauma. The structure of the parent molecule is shown in Figure 4. The first cyanoacrylates used, the methyl and *N*-butyl derivatives, provided adequate hemostasis for wounds of the liver (17), blood



**Fig 3.** Acute experimental ulcer after cyanoacrylate treatment. A dark irregular glue layer (arrows) covers a pool of blood (B) accumulating beneath it and dissecting under the surrounding mucosa (H & E Alcian blue,  $\times 9.2$ ).

## CYANOACRYLATE ULCER TREATMENT

TABLE 2. ULCER HEMOSTASIS

Treatment group	# Hemostasis/ # treated
Heater probe	17/17
Cyanoacrylate alone	0/12
Adjunctive cyanoacrylate	5/12
Sham-treated control	0/7

vessels (18, 20), hollow viscera (19), kidney (20), and skin (21). Unfortunately, the methyl derivatives broke down quickly to irritating compounds which produced unacceptable tissue inflammation and necrosis (10–12). The longer alkyl 2-cyanoacrylate side-chain derivatives are easier to apply, less irritating, and more effective in stopping bleeding (10, 17, 22–24). However, before considering widespread clinical use of tissue glues, it must be determined whether glue residues remain embedded chronically in the gastric wall and whether they are carcinogenic.

Flucrylate™ (MBR4197) is the trifluoroisopropyl homolog of the alkyl 2-cyanoacrylates with a branched fluorinated side chain. In preliminary studies this substance has been shown to stop bleeding following dental extractions (13). The mechanism of action of the cyanoacrylates is not fully understood, but hemostasis may occur by mechanical tamponade of bleeding lesions. Because of this characteristic, we hoped that cyanoacrylate tissue adhesives might stop hemorrhage when applied endoscopically to bleeding ulcers. The theoretical advantages of tissue glues are that they do not thermally injure tissue beneath the ulcer as do other hemostatic methods, and they can be applied as a spray without touching the lesion and without precise targeting.

In our canine model of acute gastric ulcer bleeding, Flucrylate™ did not reliably stop bleeding in either briskly bleeding or slowly oozing ulcers. This was true whether or not the animals were heparinized (Figure 5). In fact, despite unimpaired coagulation in unheparinized animals, there was no statistical difference in ulcer hemostasis between Flucrylate™ treatment and no treatment. Additionally, the histology suggests that even when external bleeding could not be seen, the glue had only capped the surface while bleeding continued in the deeper tissue layers.

Martin et al used Flucrylate™ to chronically treat experimental gastric ulcers in dogs (25). In that study canine gastric ulcers were made endoscopically by lifting the mucosa and excising a plug of

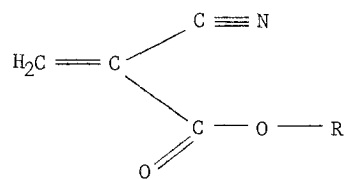


Fig 4. Cyanoacrylate monomer.

tissue. Animals were heparinized on days 4–8 following surgery. On days 4, 6, 8, and 11, the dogs were endoscoped, and Flucrylate™ (MBR4197) was sprayed on the healing ulcers. Blood loss was compared in control animals and cyanoacrylate-treated animals by measuring serial hematocrits. The results of that study showed a significantly smaller hematocrit drop in the glue-treated dogs compared to the untreated controls. However, because the experimental design and objectives differed from the present study, it is impossible to make a meaningful comparison.

Flucrylate™ (MBR4197) also has been used in a pilot clinical trial to control a variety of severely bleeding upper-gastrointestinal lesions in patients (26). Bleeding stopped in three of 6 patients successfully treated, and markedly slowed in another two treated patients. However, it is well established that over 80% of patients with upper-gastrointestinal bleeding stop spontaneously (27). Therefore, properly designed, prospective, randomized, controlled clinical trials are necessary to prove the efficacy of any hemostatic technique, but only after the technique is proven safe and effective in animal studies.

In conclusion, our controlled experimental studies indicate that Flucrylate™ is not an effective hemostatic agent for the treatment of briskly bleeding or slowly oozing experimental ulcers in heparinized or nonheparinized dogs.

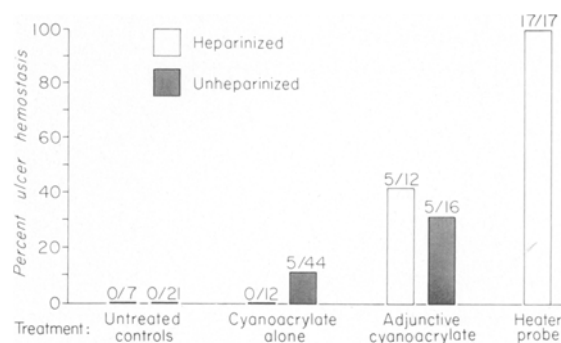


Fig 5. Effectiveness of cyanoacrylate tissue glue hemostasis.

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