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Quantitation and Control of Pulpal Bleeding *

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Abstract

The purpose of this investigation was to use a quantitative technique to evaluate the hemostatic properties of gelfoam and thrombin. To accomplish this, blood was drawn from dogs, labeled in vitro *with* ^{51}Cr *and then reinjected back into the donor dogs. When pulp exposures were made, all blood shed from the site was collected in 30 second intervals to allow calculation of the rate of bleeding. The results indicate the method is convenient and quite sensitive. Repeated, direct contact of cotton balls or gelfoam to exposure sites prolonged bleeding compared to the indirect absorption of shed blood. Hemostasis was obtained more rapidly following packing of the exposure site with gelfoam when left in place, compared to untreated controls. Thrombin application with either cotton balls or gelfoam provided rapid, complete hemostasis even after removal of the packing.*

Introduction

Occasionally it is difficult to obtain hemostasis following removal of the coronal pulp during a pulpotomy procedure. It has been suggested that such pulps are "hyperemic," and that continued bleeding indicates inflammatory changes present in the radicular pulp.[1] Complete hemostasis is desirable before application of medicaments to pulpal stumps to lessen the possibility of these substances gaining access to the systemic circulation via open venules and/or capillaries. Various techniques of controlling pulpal hemorrhage have been suggested including constant irrigation with saline rinse,[2] application of a calcium hydroxide suspension,[3] epinephrine,[4] hydrogen peroxide,[1] and Chloramin T.[5] However, the method most commonly advocated is direct pressure with a sterile cotton pellet.

Surprisingly little attention has been given to research designed to quantitate bleeding rate from pulpal sites[6] or to compare methods of obtaining hemostasis. The present report describes a simple method for quantitating the rate of pulpal bleeding in experimental animals, and provides data comparing several different methods of obtaining hemostasis.

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Materials and Methods

1. Labeling of blood with $^{51}CrO_4^{-2}$.
   Mongrel dogs of either sex weighing 20-25 kg were anesthetized with pentobarbital, 30 mg/kg i.v. Immediately a 5 ml blood sample was obtained in a heparinized syringe and labeled with radioactive chromate according to the method of Gray and Sterling.[7] The blood sample was spun down in a clinical centrifuge, the plasma discarded and the red blood cells resuspended in 5 ml of phosphate buffered saline (Grand Islands Biological). After re-centrifuging the red blood cells and discarding the saline rinse, a new, 5 ml volume of phosphate buffered saline (PBS) containing 2mCi of $^{51}CrO_4^{-2}$ (200 Ci/gm Cr from New England Nuclear) was added to the red cells, the tube capped, and the contents tumbled (16 rpm) at room temperature for 1 hour. At the end of 1 hour, the red cells were spun down, the supernatant containing unreacted $^{51}CrO_4^{-2}$ discarded, and the red cells resuspended in fresh PBS to rinse away any free $^{51}CrO_4^{-2}$ trapped between the cells. The resuspension and recentrifugation was repeated two more times to give a total of three rinses. The red cells were then resuspended in 5 ml of PBS and injected intravenously into the donor dog.
2. Experimental Design
   After allowing 20-30 minutes for the ^{51}Cr-labeled red blood cells to mix with the circulating blood volume, 5 ml blood samples were obtained from the saphenous vein at 30 minute intervals to insure that the counts per minute of radioactive red cells per ml of blood were constant.

   Maxillary and mandibular anterior teeth were prepared as follows: Using a #558 carbide bur in a high-speed handpiece with copious air-isotonic saline spray, the tooth was sectioned at the junction of the middle and gingival thirds of the clinical crown creating an exposure site of approximately 1 mm. The

dentinal area surrounding the exposure was deepened 1 mm and widened 2 mm to provide a depression to facilitate hemorrhage collection. A stopwatch was begun, and all radioactive blood coming from the exposure site was collected on cotton balls for 30 second intervals until the bleeding stopped. All cotton balls used in a 30 second interval were combined into a test tube for quantitation of the amount of radioactive ^{51}Cr present in the cotton balls. The activity of ^{51}Cr was counted to at least 10,000 in a Packard Auto-gamma counter, and corrected for background radioactivity.

Using all maxillary (6) and mandibular incisors (6) and canines (4) for convenience, it is possible in the dog to make sixteen separate exposures on individual teeth to simulate pulpotomies. Five dogs were used in this manner.

A. Effect of *direct* cotton ball application with and without thrombin.

In these experiments, 24 teeth were studied. All teeth were treated as follows: Cotton balls were placed directly on the bleeding exposure site for 30 seconds and then replaced by a fresh cotton ball.[1] This procedure was continued (with the exception of a cotton ball moistened with either saline or thrombin in saline and applied during the period between 240 and 270 seconds) until bleeding stopped spontaneously or until samples had been collected from each tooth for ten minutes. Alternate teeth received a cotton ball moistened with thrombin* (1000 μ/ml) after the 240 second blood collection. Thus, thrombin in saline, or a saline-moistened control cotton ball were in contact with the exposure site for only 30 seconds. In some preliminary experiments, gelfoam was used in place of cotton balls both to absorb shed blood and to apply thrombin.

B. Effect of *indirect* absorption of shed blood.

These experiments were done by carefully placing dry cotton balls adjacent to, but never directly on, the exposure site for 30 second intervals to determine if physical contact of the cotton ball promoted bleeding and if avoidance of direct contact would shorten bleeding time.

A second method of indirect absorption of shed blood was accomplished by gently packing a small piece of gelatin foam** onto the exposure site followed by absorbing blood coming around or through the foam with dry cotton balls in the conventional manner.

3. Calculations:

Since the blood level of ^{51}Cr labeled red blood cells was constant, dividing the amount of ^{51}Cr labeled red cells collected on cotton balls for 30 second intervals (cpm) by the concentration in blood (cpm/μl) yields the volume of blood (μl) which hemorrhaged over that time interval, assuming the hematocrit is constant. Bleeding times can be estimated then to the closest 30 second interval.

The assumption is that the hematocrit of the shed blood is identical to that of circulating blood. No attempt was made to measure the hematocrit of the microliter volumes of shed blood.

All data points are the mean of the number of values indicated within the parentheses. The brackets signify ± one standard error of the mean. Levels of significance were calculated using Student's t test.

4. Hemostatic agents

Thrombin was made fresh each day from a dry powder injected with 1 ml of isotonic saline resulting in a final concentration of 1000 μl/ml. Cotton balls or small pieces of gelatin foam were dipped into the solution and lightly blotted before application to the exposure site.

Gelatin foam was used directly from sterile packets by simply trimming a piece to a convenient size.

Results

A. Effect of direct cotton ball application with and without thrombin.

The data shown in Figure 1 indicate that the rate of bleeding from an exposure site, plotted on the ordinate, fell rapidly from a value of 39 μl/30 seconds during the first 30 seconds to a steady rate of about 5 μl/30 seconds after 120-150 seconds. Several teeth stopped bleeding spontaneously at 120-150 seconds. At 240 seconds, half the teeth were treated for 30 seconds with a cotton ball moistened with thrombin (experimental teeth) while the other half were treated with a cotton ball moistened with saline (control teeth). Thrombin treatment led to a rapid fall in bleeding rate to about 1.5 μl/30 seconds within the next 30 seconds and to virtually zero flow within the next 30 seconds (Figure 1 dash line). Similar results were obtained in preliminary experiments using gelfoam moistened with thrombin. The control teeth continued to bleed at a relatively constant rate of about 5 μl/30 seconds. Two of the 10 control teeth spontaneously stopped bleeding at 360 seconds and 510 seconds. The remaining 8 teeth continued to bleed for the entire 10 minutes.

The insert in Figure 1 demonstrates the constancy of ^{51}Cr activity in the blood of five dogs.

*Bovine thrombin, Parke-Davis, Detroit, Michigan.

**Gelfoam, Upjohn, Kalamazoo, Michigan.

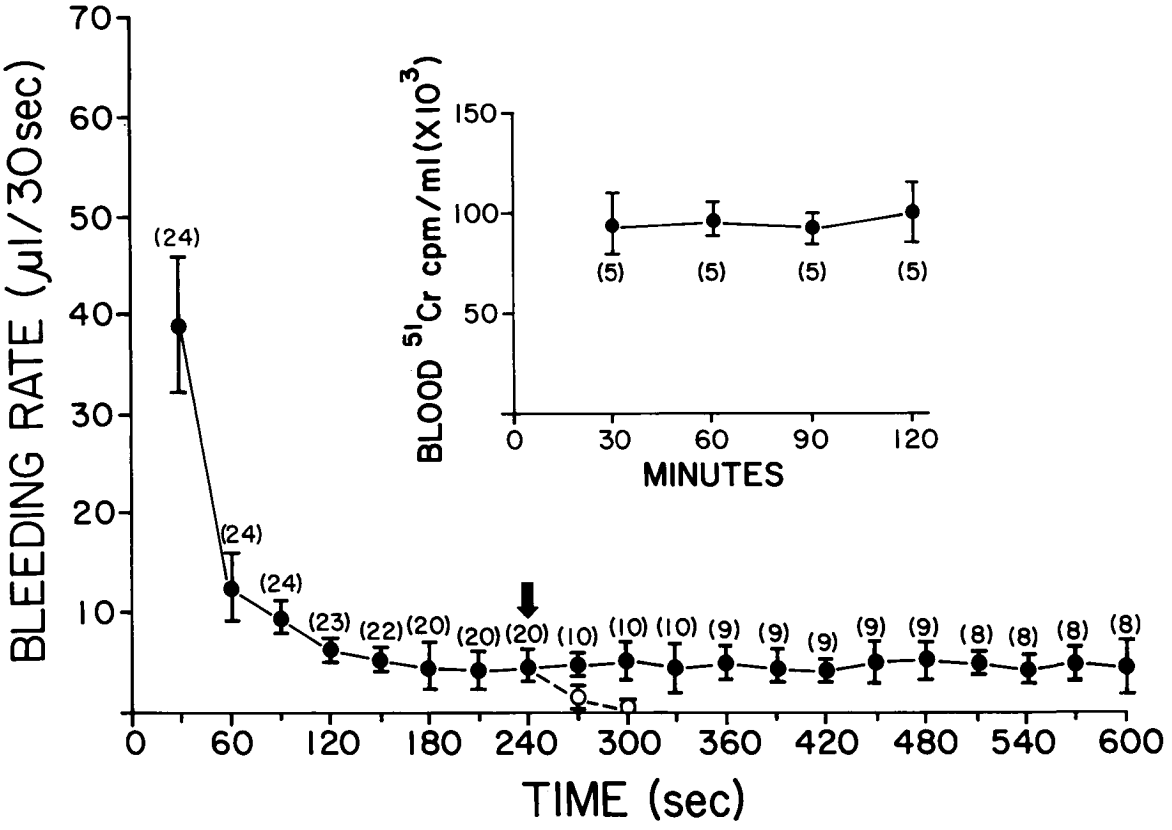

Fig. 1. The effects of direct cotton ball application to exposure sites as a function of time. At 240 seconds, half the sites were treated with topical thrombin. Numbers in parentheses indicate the number of teeth studied. Reductions in the numbers in parentheses indicate spontaneous cessation of bleeding. Solid circles represent the mean of the untreated teeth while the open circles indicate the mean of the thrombin-treated teeth (treatment instituted at 240 seconds at the arrow). Brackets indicate ±1 standard error about the mean.

Insert shows the constancy of blood ^{51}Cr concentration over a 2 hour period. The numbers in the parentheses indicate the number of dogs used in the experiments. The solid circles represent the mean and the brackets ±1 standard error of the mean blood ^{51}Cr concentration.

B. Effect of *indirect* absorption of shed blood.

The data in Figure 2 indicated by the solid circles and solid line plot the rate of bleeding on the ordinate as a function of time on the abscissa. Care was taken to avoid any direct contact of the cotton balls with the exposure site. It is clear that the rate of bleeding fell rapidly from approximately 45 µl/30 seconds measured at the end of the first 30 seconds, to between 5-7 µl/30 seconds after 120-150 seconds. This is very similar to what was observed in Figure 1 when the cotton balls were allowed to contact the exposure site. Subsequent data are different however. Over the time period of 120-210 seconds, the bleeding rates of these teeth fell spontaneously to zero. This contrasted with those teeth in Figure 1 in which the cotton balls were allowed to touch the exposure site. In those cases, most of the teeth continued to bleed for 600 seconds at which time the measurements were discontinued.

The second method of indirect absorption of blood involved absorbing blood that leaked through or around a piece of gelatin foam placed directly on the exposure. The data shown by the open circles and dashed line in Figure 2 are from teeth treated in this manner. There were no statistical differences between the bleeding rates of either group until 90 seconds after the bleeding had started. At that point, and at all subsequent points, the gelatin-sponge treated teeth bled at lower rates ($p<0.05$ indicated by asterisks), and stopped bleeding sooner than the other group in which shed blood was absorbed indirectly.

Discussion

The present report describes a very simply isotopic method for measuring bleeding rates that is clearly superior to previous methods.[6] The bleeding times obtained in this report are somewhat shorter than those reported for humans *in vivo* but much closer to those found for human blood *in vitro*.[8]

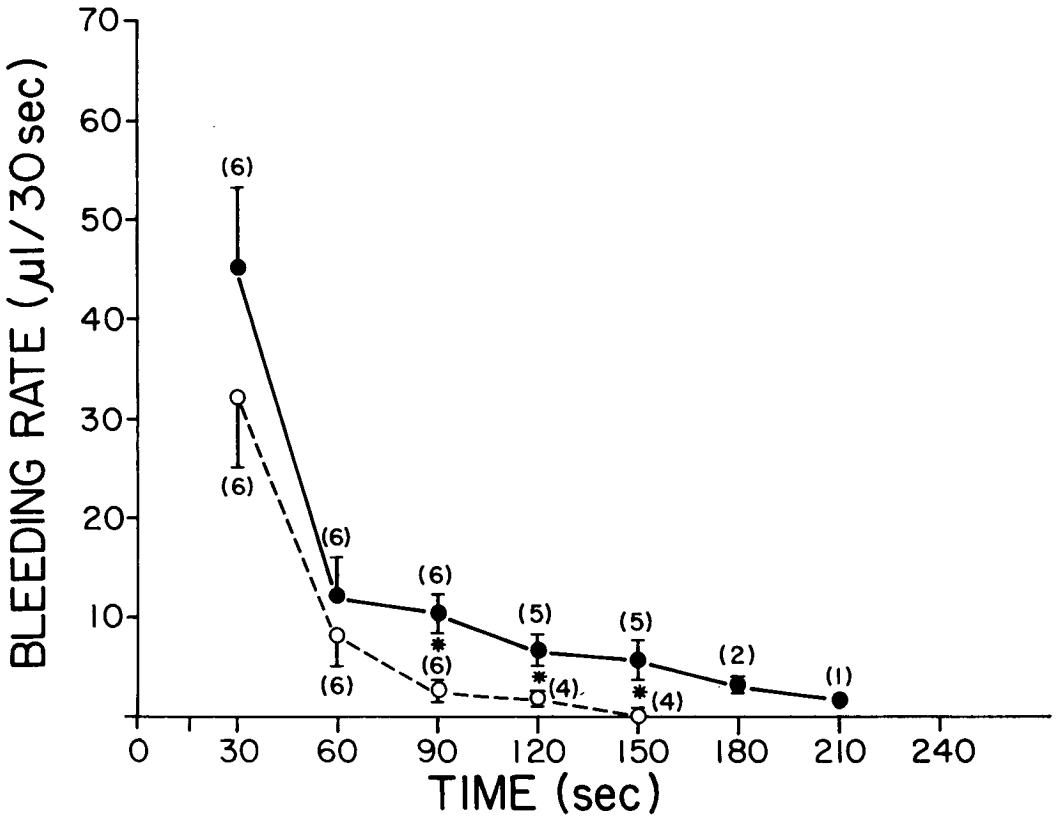

Fig. 2 The effect of indirect absorption of blood. The solid circles and line indicate data obtained using cotton balls with care being taken not to touch the exposure site. The open circles and dashed line were obtained by absorbing blood which had leaked around a piece of gelatin foam placed directly on the exposure site. Changes in the numbers in parentheses indicate spontaneous cessation of bleeding. Asterisks signify statistical significance at $p<0.05$.

Several techniques could minimize the technical problems associated with hemostasis following pulpotomy. One could simply wait until hemostasis had been obtained, and then attempt to rinse away the accumulated blood with sterile saline—a technically difficult procedure which runs the risk of dislodging the clot and reinstituting bleeding. Alternatively, one could carefully absorb the blood without touching the bleeding site with the cotton absorbent (as in Figure 2). This requires ideal operating conditions, i.e., excellent access, visibility, and lack of patient movement. In the absence of ideal conditions, such attempts may result, inadvertently, in prolongation of bleeding if the cotton ball disturbs the clot (as in Figure 1). If one packs the site with cotton or gelfoam, allows hemostasis to occur and then removes the absorbent, the fresh clot is disturbed and bleeding resumes. Although some clinicians advocate gentle continuous irrigation of the pulp chamber while attempting to obtain hemostasis in an effort to remove blood that might promote pulpal inflammation, this technique is time consuming and is often difficult to perform.

The topical use of thrombin, either on cotton balls or gelfoam, offers several advantages. It promotes rapid hemostasis (Figure 1) and is easy to apply. Upon removal of the cotton ball or gelfoam, the pulpal site does not begin bleeding again, as it does in the absence of thrombin, but leaves a shiny, dry membrane-like surface. Rapid hemostasis and more consistent results are obtained if the thrombin-containing material is placed after the bleeding rates decline spontaneously to more manageable levels (Figures 1-2). The use of cotton balls placed directly on the exposure site tends to promote and prolong bleeding (Figure 1) presumably because, as shed blood soaks into the cotton balls and fibrin deposition begins to occur, the forming clot is torn away from the site when the cotton ball is removed.

Similar results were obtained with small pieces of gelatin sponge. When either cotton balls or pieces of gelatin foam moistened with thrombin were applied directly to the exposure site, removal of the cotton ball did not restart the hemorrhage. Visual inspection of the exposure site revealed a shiny, reflective membrane-like area over the site.

When untreated gelfoam was packed on the exposure sites and cotton balls then used to absorb the blood, the bleeding times were reduced (Figure 2) compared to controls where shed blood was carefully absorbed without allowing direct contact to the exposed tissue. The

former technique was easier to master than the latter since no particular care was required in absorbing the blood. If, after obtaining hemostasis, the gelfoam was removed, bleeding began again. When gelfoam or cotton was packed on exposure sites, hemostasis was obtained more consistently with gelfoam which presumably is more compressible and better adapted to the shape of the exposure site. Since cotton is less deformable, it did not adapt or pack well. Further, gelfoam, unlike cotton, is resorbable and could possibly be left in place under a permanent restoration. Cotton, if left in place, would act as a foreign body.

Thus, while gelfoam with thrombin was an effective hemostatic agent, gelfoam without thrombin was also effective if left in place. A possible disadvantage of such a technique is the fact that the gelfoam is saturated with blood and may produce a delay in pulpal healing by promoting inflammation while the gelfoam and blood cells are resorbed.[9]

The technique utilizing the thrombin-saturated gelfoam raises the question of possible adverse effects from systemic thrombin absorption. Since only 0.01-0.05 ml of thrombin solution (1000 μl/ml) was placed in a tooth, the objection seems exaggerated in light of a recent report[10] where surgeons have irrigated 5 ml of an identical thrombin concentration on large wound areas of patients on low-dose heparin therapy without any adverse effects.

The results warrant further investigation of the tissue response and long term effect of gelfoam with and without thrombin when placed in contact with vital pulp. Such a technique could be utilized for hemorrhage control during pulpotomy procedures in both primary and permanent teeth.

Conclusions

The use of radioactive red blood cells in dogs permitted the evaluation of the hemostatic properties of gelfoam and thrombin applied to pulpal exposure sites. Repeated direct contact of either cotton balls or gelfoam to the exposure sites prolonged bleeding compared to the indirect absorption of shed blood. Hemostasis was obtained more rapidly following gelfoam packing if left in place, compared to untreated controls. Thrombin application with either cotton balls or gelfoam produced rapid, permanent hemostasis.

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