

Evaluation of the novel anti-inflammatory agent tetrandrine as a pulpotomy medicament in a canine model

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Abstract

Tetrandrine, a bisbenzylisoquinoline alkaloid with unique broad-spectrum anti-inflammatory properties, was evaluated as a pulpotomy medicament in a canine model. Histological sections were evaluated after three days (acute inflammation) and six weeks (chronic inflammation) by two criteria: 1) intensity and degree of inflammation, and 2) extent of pulp involvement. The results of the three-day dressings revealed significant neutrophil infiltration in only 30% of teeth treated with tetrandrine, compared with 81%, 84%, and 100% of teeth treated with Ledermix,[®] (Lederle Pharmaceuticals, Wolfrathausen, Germany), formocresol[®] (Creighton Pharmaceuticals, Sydney, Australia) and saline (controls) respectively ($P < 0.01$). After six weeks, there was significant lymphocyte infiltration in only 30% of teeth treated with tetrandrine, compared with 66%, 90%, and 100% on teeth treated with Ledermix, formocresol, and saline controls respectively. ($P < 0.01$). In both three-day and six-week specimens in tetrandrine-treated teeth the extent of inflammation was limited to less than one-third of the coronal section of the pulp, whereas teeth treated with Ledermix or formocresol showed cellular infiltration extending to greater than two-thirds of the pulp ($P < 0.01$). Comparative studies with berbamine, a natural analog of tetrandrine, showed that it was less potent than tetrandrine, but significantly better than Ledermix and formocresol on both acute and chronic pulp inflammation ($P < 0.05$ and $P < 0.01$ respectively). These results suggest that tetrandrine may have value as a pulpotomy medicament. (Pediatr Dent 15:259–66, 1993)

Introduction

Despite the well-documented decline in dental caries in the permanent dentition, extensive dental decay in the primary dentition that progresses to the dental pulp remains a common problem in pediatric dental practice.¹ An endodontic technique commonly used to manage early pulp infection in primary teeth is amputation of the coronal pulp or pulpotomy. The aim of this procedure is to maintain healthy radicular pulps for the normal life span of the primary tooth. In spite of its putative clinical success, the pulpotomy technique has been questioned for safety and effectiveness of currently available medicaments.

Pulpotomy agents remain unsatisfactory for several reasons. The most commonly used medicament, formocresol, is associated with systemic toxicity and carcinogenic potential, so that its safety—especially in children—is questioned.^{2–6} Pulp dressing with most medicaments, such as formocresol and zinc oxide eugenol, often leads to chronic pulpal necrosis because of the medicaments' inflammatory properties. Calcium hydroxide, while successful for dressings on healthy pulp tissue,⁷ is associated with severe internal root resorption after pulpotomies of primary teeth, and is not used extensively as a pulpotomy agent for this reason.^{8,9} Preparations containing corticosteroids such as Ledermix[®] (Lederle Pharmaceuticals, Wolfrathausen, Germany) also have been tried as pulp-capping medicaments, but these, too, are associated with histological evidence of persistent pulpal inflammation and necrosis.^{10–12}

In recent years, electrosurgery,^{13–16} and lasers¹⁷ have been studied as alternatives to formocresol pulpotomy with varying degrees of clinical success. Chemicals used for homeostatic control such as ferric sulphate¹⁸ also have been tested as experimental agents, but long-term clinical success is still unclear.

The ideal medicament for pulp dressing after pulpotomy should be nontoxic and possess antimicrobial activity and an anti-inflammatory potential to control pre-existing inflammatory states and surgically induced inflammation. The utility of an anti-inflammatory agent in pulp dressing is recognized, but the inclusion of an effective agent for endodontic use has been impeded because currently available anti-inflammatory agents are not very effective and are far too toxic for dental use. To date, only the corticosteroids have been tried, and although short-term animal experiments and clinical reports^{19–20} have indicated putative control of inflammation, histological studies of long-term pulp treatment with corticosteroids have generally shown unfavorable results.²¹

In the present study, we examined the pulpal response to a novel anti-inflammatory agent, tetrandrine, as dressing after pulpotomy in canine teeth. We then compared the results with berbamine (a natural analogue of tetrandrine) and two pulpotomy medicaments currently in use. Tetrandrine, a bisbenzylisoquinoline alkaloid, has been found to inhibit both cellular^{22–24} and molecular^{25–29} mediators of inflammation, and is largely devoid of immunosuppressive^{30–31} and toxic side effects.^{32–33}

Methods and materials

Animals

Nine mongrel dogs (five males and four females) approximately one year old were used for the experiments. The dogs had complete permanent dentitions free of dental caries, severe gingivitis, or evidence of dental trauma. The animals were kept in an air-conditioned animal house and maintained on a normal diet for the duration of the experiments, either three days or six weeks.

Pulpotomies

The dogs were anesthetized with IV pentobarbital sodium at doses of 30 mg/kg, and intubated with a cuffed endotracheal tube. Pulpotomies were performed on the incisor and premolar teeth. In each dog, approximately equal numbers of teeth were assigned randomly to receive each medicament and normal saline controls.

The teeth were isolated with rubber dam and the operating field and teeth were cleaned with 70% alcohol and 0.2% chlorhexidine gluconate. Sterility was maintained throughout the operating procedures. A sterile, round diamond bur rotating at high speed and cooled with a fine stream of sterile normal saline was used to create the endodontic access cavities. Full coronal pulpotomies were performed using a gentle technique⁷ with the same bur rotating at high speed and cooled with sterile saline. Hemostasis was achieved using pressure applied with sterile cotton pellets. After applying the pulpal medicaments, the access cavities were filled with a double seal of Cavit® (Espe, Seefeld/Oberbay, Germany) and Kalzinol® (Dentsply Ltd, Waybridge, Surrey, England).

Medicaments

Tetrandrine (Fig 1), with a molecular weight 622.73 daltons and empirical formula $C_{38}H_{40}O_6N_2$, was obtained from the People's Republic of China. The powder, of > 98% purity, was dissolved in phosphate-buffered saline and 20% 0.1 N hydrochloric acid. The pH was adjusted to 7.2 using 0.1 N sodium hydroxide to obtain a stock con-

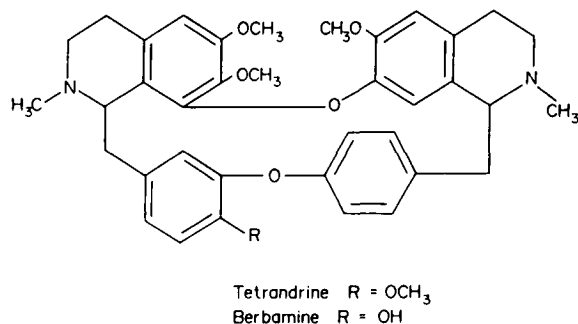


Fig 1. Chemical structure of tetrandrine and berbamine. The molecular weight of tetrandrine is 622.7 daltons and of berbamine is 608.7 daltons.

centration of 10 mg/ml. Tetrandrine solutions were mixed with sterile zinc oxide powder at liquid/powder ratios of 0.5 ml/300 mg.

Berbamine, a water-soluble analog of tetrandrine (Fig 1) was dissolved in normal saline at a stock concentration of 20 mg/kg. Solutions of berbamine were mixed with sterile zinc oxide liquid/powder ratios of 0.5 ml/300 mg.

Ledermix cement was mixed according to the manufacturer's instructions and applied directly onto the pulpal surface.

Formocresol at Buckley's concentrations of 35% cresol and 19% formaldehyde was applied in the usual clinical manner. A pellet of formocresol was placed on the pulp surface for 5 min, after which a layer of zinc oxide eugenol dressing was applied.

Zinc oxide powder was mixed with normal saline at liquid/powder ratios of 0.5 ml/300 mg as dressings for control teeth.

The doses of tetrandrine and berbamine used for the experiments were derived from results of the authors' previous *in vitro* work.²⁵⁻³⁸ Also, dose-response experiments were performed first to determine the optimal concentrations of tetrandrine and berbamine for pulpotomy use. These optimal concentrations were used for comparison with other medicaments.

Histological sections

At the end of the experiments, the animals were sacrificed using IV pentobarbital sodium. The teeth were examined macroscopically with a dental probe, and those with clinically evident inadequate seals of the endodontic access cavities were omitted from the study. Immediately after sacrifice, the endodontic access cavities were reopened using a diamond bur rotating at high speed and cooled by a stream of 10% buffered formalin. The jaws were placed in 10% buffered formalin and the teeth dissected out, immersed in decalcifying solutions containing 30% formic acid and 10% formalin for a week, embedded in paraffin blocks, and sectioned midlongitudinally. Several sections of the pulp were prepared, and three representative sections of each root stained with hematoxylin and eosin.

Assessment of inflammation

The histological sections were examined by one of the authors (WKS) under a light microscope at low and high power. The assessment of inflammation on each tooth was done blind—without reference as to the pulp treatment previously rendered. Inflammation was assessed by noting the infiltration of neutrophils in the three-day experiments, and lymphocytes in the six-week experiment. Degree of inflammation in each root was graded as to whether it was mild, moderate, or severe. Inflammation was considered to be mild if there were 1–10 inflammatory cells per microscopic field at 400x magnification, moderate if there were 11–50, and severe if there were >50. Extent of inflammation along the root was graded as to whether it affected less than a third, one-third, two-thirds, or the entire pulp.

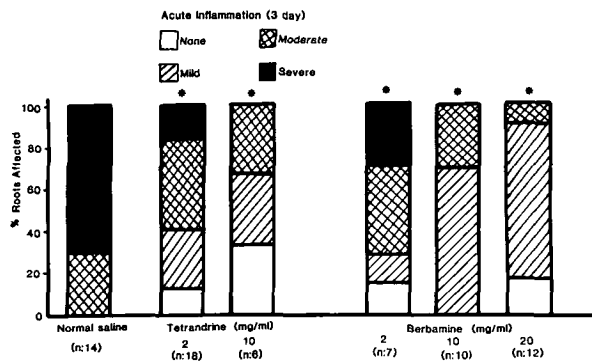


Fig 2. Degree of intensity of acute inflammation in dose-response effects of tetradrine and berbamine. The pulps were dressed with various concentrations of tetradrine and berbamine. After three days, the degree of inflammation was assessed in each case. A dose-response effect is observed with both compounds. The differences in results between normal saline and all concentrations of tetradrine and berbamine are statistically significant ($P < 0.01$). $N =$ number of roots assessed at each concentration of medicament.

Statistical analysis

The analysis of variance (ANOVA) tests were used to determine statistical differences in results among the groups.

Results

Dose response effects of tetradrine and berbamine

The three-day pulpal responses to tetradrine at doses of 2 and 10 mg/ml and berbamine at doses of 2, 10, and 20 mg/ml compared to control teeth receiving normal saline are shown in Fig 2. As indicated in the figure, in the control teeth, a total of 10 of 14 teeth (71%) showed severe inflammation, and the rest moderate inflammation. Furthermore, the inflammation extended to at least two-thirds of the root in the majority of these control teeth (Table 1). By contrast, 10 mg/ml of tetradrine resulted in approximately one-third of the total number of treated roots with no inflammation, another third with only mild inflammation, and the rest with moderate degrees of inflammation. A lower concentration of tetradrine of 2 mg/ml resulted in an increased proportion of the percentages of teeth with moderate inflammation (44%), and severe inflammation (17%). These differences were statistically significant ($P < 0.01$).

Similarly, in the case of 20 mg/ml berbamine, 17% of the roots showed no inflammation, 75% moderate inflammation, and 8% severe inflammation. Less remarkable, but still significant results were obtained with berbamine at concentrations of 10 mg/ml and 2 mg/ml ($P < 0.01$).

At all concentrations of tetradrine and berbamine, the extent of inflammation observed in treated teeth was limited mainly to the occlusal one-third of the pulp, with the

Table. The extent of acute inflammatory response in the pulp after 3-day dressing with various concentrations of tetradrine and berbamine

Medicament (mg/ml)	Number of Roots (Per cent) Extent of Pulp Affected (Thirds)				
	0	< 1	1	2	3
Saline (control)					
0 (N = 14)	0	0	2 (14)	10 (72)	2 (14)
Tetradrine					
2 (N = 18)	8 (44)	0	7 (39)	3 (17)	0
10 (N = 6)	2 (33)	0	1 (17)	3 (50)	0
Berberamine					
2 (N = 7)	1 (14)	0	4 (57)	2 (29)	0
10 (N = 10)	0	2 (20)	6 (60)	2 (20)	0
20 (N = 12)	2 (17)	6 (50)	4 (33)	0	0

The differences in results were statistically significant between the saline controls (no medicament) and all concentrations of tetradrine and berbamine ($P < 0.05$).

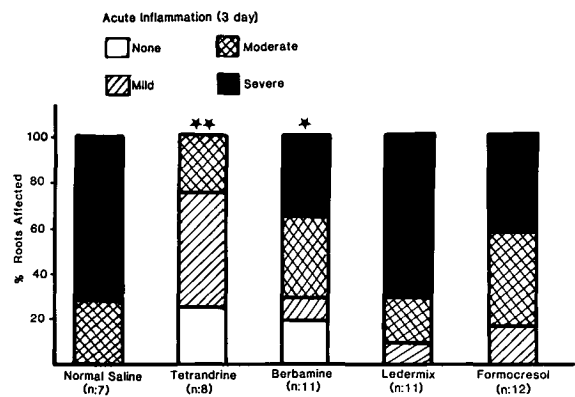


Fig 3. Degree and intensity of acute inflammation after three-day pulp dressing with various medicaments. The difference between normal saline and tetradrine (10 mg/ml) and berbamine (20 mg/ml) is statistically significant at $P < 0.01$ and $P < 0.05$, respectively, indicating that both compounds show significant anti-inflammatory activity on the dental pulp. No significant differences are observed between normal saline controls and Ledermix and formocresol. $N =$ number of roots assessed using each medicament.

least amount of inflammation observed in the teeth with the highest concentrations of tetradrine and berbamine (Table 1).

For these experiments, pulpotomies were performed on a total of 72 roots. Five (7%) roots were broken or had inadequate cavity seals, leaving a total of 67 roots for final analysis.

Comparative effects of tetradrine, berbamine, formocresol, and Ledermix after three days

In the next set of experiments, the concentrations of tetradrine and berbamine selected were 10 mg/ml and

20 mg/ml respectively. Fig 3 shows the degree of inflammation observed in the pulp after dressing with the various medicaments for three days. As can be observed from Fig 3, in the pulps dressed with tetrandrine, only two of eight teeth (25%) showed moderate inflammation and the remainder mild or no inflammation. In those dressed with berbamine, moderate or severe inflammation occurred in eight of 11 (73%) and no inflammation in 27%. Furthermore, in the tetrandrine- and berbamine-treated teeth in which inflammation was present, this was confined mainly to the coronal third of the pulp (Fig 4). By contrast, severe or moderate inflammation occurred in all seven control roots dressed with normal saline, nine of 11 (91%) of Ledermix-treated, and 10 of 12 (83.4%) of formocresol-treated teeth. Furthermore, in most of these teeth, inflammation extended to at least two-thirds of the pulp (Fig 4).

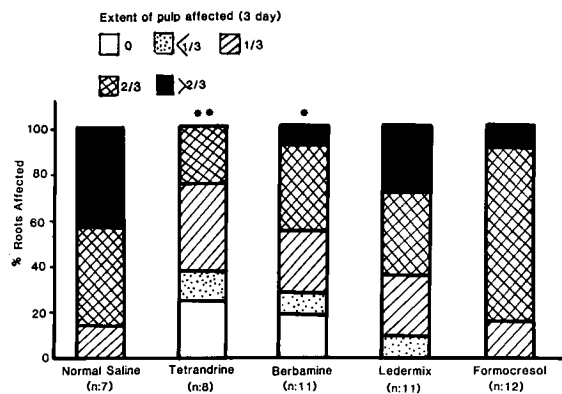


Fig 4. The extent of pulp involved after three-day dressing with various medicaments compared to normal saline controls. The results show that in the roots dressed with tetrandrine and berbamine, respectively, inflammation—if present—tended to remain localized to the coronal one-third of the pulp. By contrast, in the roots dressed with normal saline, Ledermix or formocresol, respectively, inflammation tended to extend to greater than two-thirds of the pulp. N = number of roots assessed using each medicament.

These differences were statistically significant ($P < 0.01$), indicating that tetrandrine has strong anti-inflammatory actions on the pulp.

In this group of experiments, pulpotomies were performed on 54 roots. Of these, 49 were available for final analysis, as five (9%) were found to have inadequate seals of the endodontic access cavities.

Comparative effects of tetrandrine, berbamine, formocresol, and Ledermix after six weeks

Fig 5 shows the effects of the various pulp dressings after six weeks. As can be observed from the figure, in the tetrandrine-treated group, 11 of 16 (69%) and in the berbamine-treated group, five of 11 (45%) teeth showed little or no inflammation. Furthermore, in the case of the tetrandrine- and berbamine-treated groups, inflammation, where present, tended to be localized to the coronal third of the

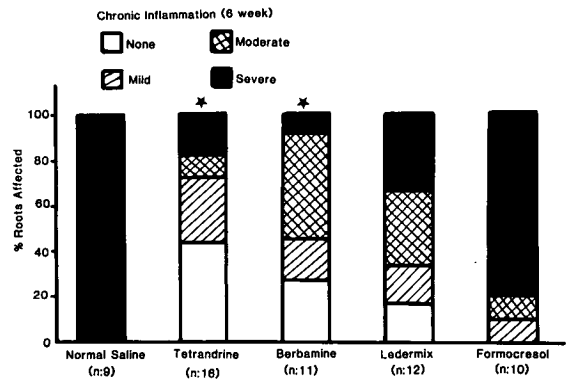


Fig 5. The degree and intensity of chronic inflammation after six-week dressing with various medicaments compared to normal saline controls. The differences in results between tetrandrine and berbamine respectively and normal saline were statistically significant ($P < 0.01$), indicating that these compounds have significant long-term anti-inflammatory activity on the dental pulp. There were no statistical differences in results between normal saline and Ledermix and formocresol respectively. N = number of roots assessed using each medicament.

pulp (Fig 6). By contrast, only a small percentage—four of 12 (33%) in the Ledermix-treated group, and one of 10 (10%) in the formocresol-treated group—showed little or no inflammation and the remainder of the teeth showed severe or moderate inflammation. In the group treated with normal saline, all nine teeth showed severe inflammation. The differences in pulp responses were statistically significant, ($P < 0.01$). In addition, in the control teeth

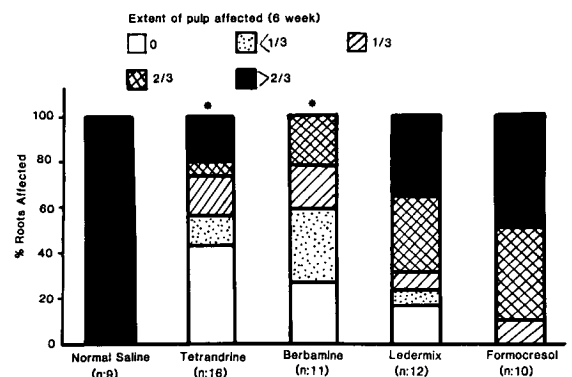


Fig 6. The extent of pulp affected after six-week dressing with various medicaments compared to normal saline controls. The results show that in the roots dressed with tetrandrine and berbamine, inflammation—if present—tended to remain localized to the coronal one-third of the pulpal surface. In contrast, in the roots dressed with normal saline, Ledermix, or formocresol respectively, the inflammation tended to extend to at least two-third of the pulp. N = number of roots assessed using each medicament.

treated with normal saline—as well as those treated with Ledermix and formocresol—inflammation extended to > 2/3 of the pulp. These differences were statistically significant ($P < 0.01$).

In this group of experiments, pulpotomies were performed on 64 roots. Of these, 58 were available for final analysis, as six (9%) were found to have inadequate seals of the endodontic access cavities.

Fig 7a shows the typical histological appearance of the coronal half of a root pulp dressed with tetrandrine after six weeks. The pulp appears essentially normal and free of inflammation, throughout the entire root (Fig 7b). Figs 8a,b show the typical appearance of a pulp dressed with berbamine. While there is mild inflammation in the coronal third of the root as shown in Fig 8a, the rest of the tooth remains essentially normal (Fig 8b). By contrast, Fig 9 depicts the typical appearance of teeth dressed with normal saline, formocresol, or Ledermix respectively. The histological expression of these teeth usually shows severe inflammation extending throughout the entire root lengths. For comparison purposes, histological analysis of several negative control roots, (teeth without treatment) also were included. These teeth all showed normal pulp histology (Fig 10).

Discussion

Our laboratory researchers are engaged in the discovery and development of novel compounds suitable for treating chronic inflammatory and autoimmune diseases. In particular, we are looking for a broad-spectrum anti-inflammatory agent without toxic or immunosuppressive side effects; one compound with this immunopharmacological profile is tetrandrine, a bisbenzylisoquinoline alkaloid extracted from the tuberous root of the rainforest creeper *Stephania tetrandra*. Tetrandrine has been used in China since antiquity to treat rheumatic diseases. In particular, its efficacy in treating silicosis,^{34,35} a disease unresponsive to treatment with conventional immunosuppressive drugs, led us to hypothesize that tetrandrine may have unique anti-inflammatory properties. In vitro experiments in our laboratory have shown that it has inhibitory effects on neutrophils, monocytes, natural killer cells, mast cells,

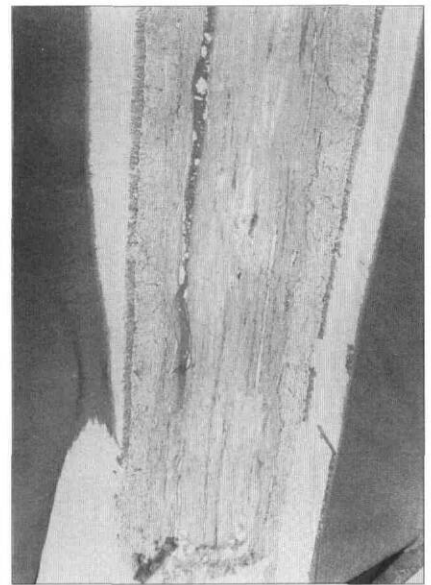


Fig 7a. (left) Section of root pulp after six-week dressing with 10 mg/ml tetrandrine. A few dentin chips (arrows) are observed at the amputation site where the dressing material had been removed during histological processing. Below the amputation site, the pulp appeared free of inflammation (Hematoxylin and eosin stain. Magnification 40 x). **Fig 7b.** (right) Section of the apical half of the root in Fig 7a, showing normal appearance of the pulp (Original magnification 40x).



Fig 8a. (left) Section of root pulp after six-week dressing with 20 mg/ml berbamine. A mild degree of infiltration of lymphocytes is observed extending from the site of amputation to about one-third of the length of the root. (H & E original magnification 40x). **Fig 8b.** (right) Section of the apical half of the root in 8a showing a normal pulp, free of inflammation (Original magnification 40x)

platelets, as well as inflammatory mediators and cytokines such as histamine, prostaglandins, leukotrienes, platelet activating factor (PAF), interleukin-1 (IL-1), and tumor necrosis factor (TNF).^{22-26,36-39} In vivo studies have shown that it lacks immunosuppressive properties, with no suppression of antibody production,^{24,31} no increased susceptibility to infection in the form of experimental brucellosis,³¹

and only a slight prolongation of cardiac transplant rejection,³⁰ but definite suppression of the actions of IL-1, TNF, and PAF in the air-pouch model of inflammation.²⁹ It also has been shown to be effective in animal models of autoimmune diseases such as experimental allergic encephalitis³² and insulin-dependent diabetes mellitus.³³ Its exact molecular mode of action is unclear, but may be related to its capacity to interfere with transmembrane signaling,⁴⁰ block calcium channels,⁴¹ or induce apoptosis.³⁹

Tetrandrine differs from most currently available anti-inflammatory agents in that it appears devoid of toxic side effects at therapeutic doses. Systemic safety of tetrandrine has been proven in previous clinical trials in humans³⁴ as well as in our own animal experiments^{31, 33} in which analyses of appearance, behavior, weight, blood chemistry, hematology, and organ histology were essentially normal.

The results of our study with tetrandrine, have shown that it has significant therapeutic effects on the dental pulp. In both short- and long-term experiments, pulps dressed with tetrandrine had significantly less inflammation compared with control teeth without the medicament as well as those dressed with the currently used pulpotomy medicaments, formocresol and Ledermix. A dose response effect was observed; at 10 mg/ml of tetrandrine the majority of treated teeth showed little or no inflammation; at 2 mg the effects were less positive, but still significantly better than controls.

Of interest are the comparative effects using berbamine, a natural analog of tetrandrine, in which one of the methyl groups is replaced by a hydroxyl group (Fig 1). Berbamine's anti-inflammatory effects on the pulp are less positive compared to tetrandrine, although the results are still significantly better compared to the other medicaments. In this regard, in our previous investigations, it was noted that berbamine shows greater immunosuppressive effects,^{24, 31} whereas tetrandrine generally has more potent anti-inflammatory actions.²⁸ Compared to berbamine, tetrandrine suppressed the production of leukotriene B4 from human neutrophils²⁶ as well as interleukin-1 and tumor necrosis factor from human monocytes (to a greater extent).²⁸ However, berbamine reduced the incidence of experimental allergic encephalitis (EAE) in BB rats to a greater extent compared to tetrandrine.³²

The poor results of severe inflammation with the use of formocresol observed in this study, are consistent with those of previous long-term clinical studies,^{42, 43} confirming that biological success after pulpotomy with this me-



Fig 9. Section of a control root pulp after six-week dressing with normal saline. The section shows the extension of severe inflammation to the apical end of the pulp. A dense infiltration of lymphocytes and macrophages is evident. This histological picture also is typical of the results obtained with Ledermix and formocresol. (H&E original magnification 100x).

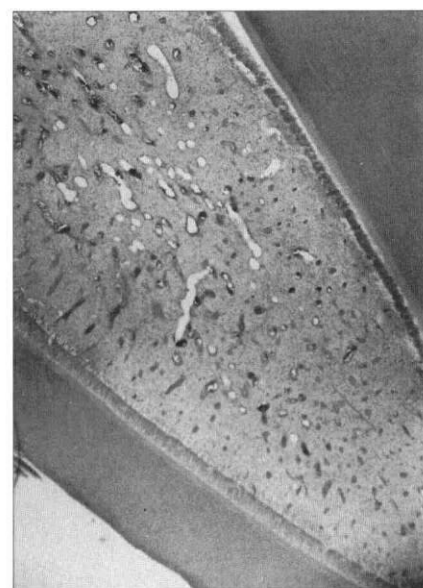


Fig 10. Section of untreated root pulp (negative control). Note normal appearance of pulp. (H&E original magnification 100x).

dicament usually is not achieved. Furthermore, formocresol, which is one of the most widely used medicaments,⁴⁴ remains controversial because of its high tissue toxicity^{2, 4, 45} as well as

inflammatory,³⁶ carcinogenic, and mutagenic potential.^{6, 45} Glutaraldehyde was suggested as a putatively better alternative to formocresol for pulpotomy in primary teeth but the potential for systemic toxicity is also present with this medicament.³

Thus, the demonstration of efficacy of tetrandrine in canine teeth underlines the potential value of this novel compound as a pulpotomy medicament, and indicates that clinical trials may be warranted.

Conclusions

Pulpotomies dressed with tetrandrine show significantly less inflammation compared with those performed with formocresol and Ledermix cement respectively. Its naturally occurring analog, berbamine, while not as potent as tetrandrine, is also significantly better than formocresol and Ledermix cement.

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