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Measurement of Blood Flow in Reflected Muco-Gingival Tissue Flaps in Cats : Using the Radiolabeled Microsphere Method

Gary T. Wuchenrich

ABSTRACT

MEASUREMENT OF BLOOD FLOW IN REFLECTED MUCO-GINGIVAL TISSUE FLAPS IN CATS USING THE RADIOLABELED MICROSPHERE METHOD

by

Gary T. Wuchenich

Blood flow to the maxilla and mandible may vary due to differences in vascular supply and in physiologic, metabolic and pathologic conditions. In general it is accepted that oral tissues have an abundant blood supply; however, there is little information quantifying blood flow to these regions. In this study, the maxillary tissues were chosen to measure the blood flow in attached and reflected gingival tissue in adult cats using the radiolabeled microsphere method.

Sixteen cats with permanent dentition, clean mouths, and without inflamed gingiva upon visual inspection were used and divided into two groups. Group 1 consisted of six cats whose muco-gingival tissues were reflected on either the left (n=3) or the right (n=3) maxillary quadrant; their contralateral gingival tissues were left attached (n=6) and used as controls. Group 2 consisted of ten cats with maxillary muco-gingival flaps reflected on both maxillary left and right quadrants. Immediately prior to flap reflection, the gingival tissue of fifteen quadrants was injected with .6 ml of a solution of sterile saline,

2% plain lidocaine, or 2% lidocaine with 1:50,000 epinephrine. The gingival tissue of five quadrants received no injection of solution and were used as controls.

In both groups, all flaps were reflected for a minimum of 90 minutes before injection of ^{153}Gadolinium microspheres. The left femoral artery was cannulated for the collection of a reference blood sample. A catheter was placed in the left ventricle, via the right common carotid artery, to allow intraventricular injection and proper mixing of the microspheres. Two and a half million microspheres of ^{153}Gadolinium were injected over a 20 second interval. The cats were then euthanized and tissue flaps collected. The sample flaps were placed in a gamma counter and their blood flow was determined based on their radioactivity.

The attached gingival samples in group 1 had the lowest blood flow while those in group 2 injected with 2% plain lidocaine had the highest blood flow. In group 1, there was significant difference between the attached (control) and reflected gingival samples ($p=.027$). In group 2, there was significant difference between muco-gingival tissues injected with 2% plain lidocaine when compared with sterile saline or no treatment ($p<.05$). However, there was no significant difference ($p>.05$) in blood flow of gingival tissues injected with 2% plain lidocaine and 2% lidocaine with 1:50,000 epinephrine

117 minutes post injection, nor was there significant difference between the sterile saline and no treatment groups. The finding concludes that the increased blood flow is a function of the vasodilation properties of lidocaine and that the vasoconstrictor, epinephrine, has a restraining effect on increasing blood flow post operatively in reflected muco-gingival tissues.

LOMA LINDA UNIVERSITY

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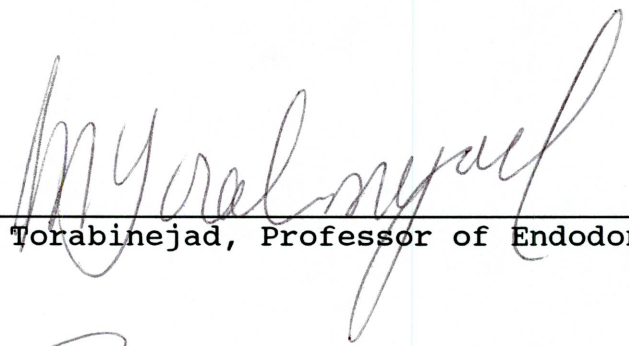

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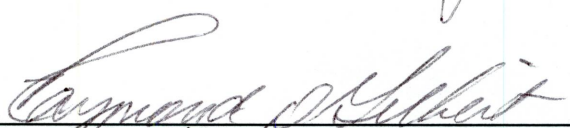

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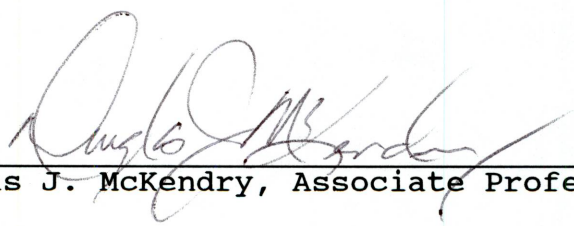

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CHAPTER 1

INTRODUCTION

Blood flow to the maxillary and mandibular tissues may vary from other body tissues due to differences in vascular supply and physiologic requirements. In general, it is accepted that oral tissues have abundant blood flow. However there is relatively little information regarding quantification of blood flow in healthy, diseased, or surgically traumatized oral gingival tissue. This study is undertaken specifically to examine the effects of injectable agents on blood flow in reflected gingival tissue.

Review of previous blood flow studies has indicated that blood flow to head and neck structures is not homogenous. Using isotope fractionation and particle distribution methods, Meyer (1) quantified blood flow in the oral mucosa, alveolar bone, tongue, and dental pulp in dogs. His findings suggest that blood flow in maxillary bone may be 30% higher than mandibular bone. Johnson et al (2) examined the relationship between epithelial thickness and blood flow in the mucosal regions of the cat. When epithelial thickness was correlated with blood flow, there was a significant positive correlation indicating that a thicker epithelium is associated with a higher blood

flow. The tissues of the palate and maxillary buccal mucosa had the greatest amount of blood flow. Kaplan and co-workers (3) used radiolabeled microspheres to measure gingival and alveolar bone blood flow in beagle dogs with periodontal disease and showed that increase blood flow in the periodontium is associated with increased severity of advanced periodontal disease. They also reported that gingival and alveolar bone blood flows were lowest in dogs with minimal periodontal disease (<10% bone loss) while dogs with moderate to severe bone loss (>20%) had significantly higher (250-400%) periodontal blood flows. Dorman and Bishop (4) induced periodontitis in dogs by removing 2mm of alveolar crest bone in the mandibular ramus. Vascular changes in the involved quadrant were studied 10-42 days postinduction by perfusing blood into each mandibular artery and recording the perfusion pressure. Their study reported increase mandibular arterial flow during chronic periodontitis in dogs. Actual flow to the damaged tissue was not determined.

Studies on pulpal blood flow measured in dogs have produced inconsistent values. Tonder and Aukland (5) measured blood flow in the dental pulp of canine teeth. Using a local hydrogen gas desaturation technique, they reported 17 ml/min/100g of tissue in pulpal blood flow in canine teeth of dogs. Meyer and Path (6) determined the

blood flow in dog pulps by hydrogen polarography and radioactive microsphere methods. They found flow discrepancies between these two methods, suggesting that the differences were due to venous-arterial shunting. Blood flow values in dental pulps of dogs ranged from 20-67 ml/min/100g of tissue.

Another method to determine blood flow is the ^{133}Xenon washout which was used by Kim and associates (7). They injected ^{133}Xenon into the maxillary artery and monitored radioactivity in a dog canine pulp with a specially designed scintillation probe. They reported a blood flow of 53 ml/min/100g in canine pulps of the dog.

In addition to the differences in vascularity of these tissues, vasoconstrictors induce different responses. A study undertaken by Gray et al (8) compared the success rate for achieving analgesia using lidocaine 2% with epinephrine 1:80,000 to plain 2% lidocaine, when administered via the periodontal ligament using the ligma jet syringe. Satisfactory analgesia was defined as an absence of painful sensation during the operative procedure. Their results demonstrated 91.6% success rate of achieving analgesia using 2% lidocaine with 1:80,000 epinephrine in the periodontal ligament injection method. The success rate of injection with plain 2% lidocaine was only 42%.

Malamed (9) found a difference in anesthetic success rate related to the type of anesthetic used, and whether or not it contained a vasoconstrictor. He measured a 81.8% success rate with plain 4% prilocaine, but a 93.1% success rate using 2% lidocaine with 1:100,000 epinephrine. In a clinical evaluation study, Miller (10) suggests that to reduce the amount of anesthetic needed, the solution should contain a vasoconstrictor. By using 2% lidocaine hydrochloride with 1:80,000 epinephrine, he achieved a success rate of 96.1%.

Olgart and Gazelius (11) reported that a supraperiosteal injection of 2% lidocaine with 1:100,000 epinephrine in the apical area of a tooth caused almost complete cessation of blood flow in the tooth pulp. Hellner (12), in 1927, suggested that a marked reduction in blood flow resulting from epinephrine in dental anesthesia could result in tissue injury.

Kim and associates (13) showed the effects of local anesthetic on pulpal blood flow in dogs. Using various local anesthetic techniques—mandibular block, infiltration, and intraseptal injection—they administered 2% lidocaine with 1:100,000 epinephrine in each of the ten dogs. The blood flow was determined by using the 15um radioisotope-labeled microsphere injection method. Their results showed that local anesthetic injections of

2% lidocaine with epinephrine 1:100,000 were capable of reducing blood flow significantly in teeth in the area of, or distal to, the injection site. In some samples blood flow was reduced by 65% and took up to 75 minutes for blood flow in the pulp to return to near normal. Furthermore, they found that injection of 2% plain lidocaine caused an increase of pulpal blood flow in some dog pulps.

Pitt Ford and associates (14) examined the duration of anesthesia and the reduction of blood flow in the dental pulps of maxillary central incisor teeth in 10 human subjects by a laser doppler flowmeter. They demonstrated that the addition of adrenaline 1:80,000 to local anesthetic increased the duration of anesthesia by four fold, and reduced pulp blood flow by 31% over a period of 90 minutes.

Epinephrine 1:100,000 in conjunction with lidocaine anesthetic produces vasoconstriction by stimulating the alpha adrenergic receptors in blood vessels, thus prolonging the duration of anesthesia and hemostasis for 60-90 minutes (15). However, as the level of epinephrine gradually decreases to a level that no longer produces an alpha adrenergic effect, the blood flow begins to increase to a rate well beyond normal. This vasodilation can last up to two hours in duration (16-18). This effect is termed a rebound phenomenon or hyperemic effect. Lindorf (17)

demonstrated the effect of epinephrine with the aid of infared thermography on humans. He showed that lidocaine with 1:100,000 epinephrine causes immediate vasoconstriction following infiltration. Ischemia of the local tissue subsides after 60 minutes, then the epinephrine slowly reverses the vasoconstriction to a reactive hyperemia which could last as long as 180 minutes. He postulated that due to the metabolic effect of epinephrine, local acidosis and reduced oxygen tension occur resulting in a secondary vasodilation of the blood vessels. He refers to the secondary vasodilation as reactive hyperemia and this condition may predispose to bacterial wound infection and delayed wound healing.

There are currently few studies available in the dental literature quantifying blood flow in gingival tissues undergoing surgical trauma. The objectives of this investigation are twofold:

1. To develop a cat model quantifying normal blood flow in maxillary muco-gingival tissue using the radiolabeled microsphere method and
2. To quantify and compare the blood flow in maxillary muco-gingival flaps which are treated with sterile saline, 2% plain lidocaine and 2% lidocaine with 1:50,000 epinephrine.

REVIEW OF ANATOMY AND VASCULAR SUPPLY

The primary function of the blood circulation is to transport nutrients to the various cells, and to remove metabolic waste products for elimination from the body. The development of the vascular system structurally and functionally is directly related to the needs of the tissues. The blood vessels and the connective tissue form a single functioning system. Thus, the physiology of the tissue depends on the circulatory transport system, and any alteration of the circulation can contribute to pathological conditions in the tissues (19).

The gingival tissue receives its blood supply mainly through supraperiosteal blood vessels, which are terminal branches of the sublingual artery, the mental artery, the greater palatine artery, the infra-orbital artery and the posterior superior dental artery. These dental arteries branch into intraseptal arteries which anastomose in the periodontal ligament, together with numerous terminal branches in the alveolar crest bone, forming a subepithelial plexus located beneath the oral epithelium of the attached gingiva. In reality, there are numerous anastomoses present among the different arteries and arterioles that supply the periodontium (20).

These features of blood supply have been well documented in vasculature studies. Egelberg (21) studied

the arrangement of the blood vessels at the dento-gingival junction in dogs after perfusion with a carbon-gelatin mixture. In healthy gingiva, a plexus of blood vessels was observed close to the crevicular epithelium, whereas in chronically inflamed gingiva, the plexus of blood vessels seen in healthy gingiva was replaced by a vascular bed with loop-like formations. Kindlova (22) examined the blood vessels supplying the periodontium in monkeys with the use of corrosive preparations and found that the vessels were arranged in networks with anastomoses in many sites. The blood vessels of the periodontium of the rat, mouse, hamster, guinea pig, cat and dog were studied by Carranza and coworkers (23). They observed that gingival blood vessels were, to a certain extent, independent of the blood supply of the periodontal ligament and alveolar bone and their histological studies revealed the blood vessels arranged in a layered plexus that is characteristic of the oral gingiva. In gingivitis studies, Hansson et al (24), Hock & Nuki (25), Hock (26) demonstrated gingival blood vessels in cats and dogs as convoluted, looped and a random vascular plexus via the use of vital microscopy.

The microcirculatory vessels are the arterioles, the capillaries, and the venules. The arterioles measure approximately 50 microns in diameter and have several layers of smooth muscle. They regulate the blood supply to

specific tissue areas by means of their sphincter-like mechanisms. The arterioles divide into smaller vessels called metarterioles, or precapillaries, which give off capillaries which are about 8-10 microns in diameter. From the capillary bed, blood passes into post capillary venules and then into progressively larger venules before entering the internal or external jugular vein. The medium size venules measure approximately 50 microns in diameter. The various vessels are often considered to supply certain defined regions; however, there are numerous anastomoses present among the different vessels. Thus, the entire system of blood vessels, rather than individual groups of vessels, should be regarded as the unit supplying the soft and hard tissue of the maxilla and the mandible (27-29).

The peripheral circulation is essentially under the dual control, centrally through the central nervous system and locally in the tissues by the environmental conditions in the immediate vicinity of the blood vessels. The blood supply to any given area is controlled by nerve impulses and humoral agents. Arteries and arterioles are innervated with both sympathetic and parasympathetic nerve fibers and stimulation of these fibers are mediated predominantly through alpha or beta receptor effects. The lumen of the vessels is increased or decreased to control the amount of blood circulating in the area and this regulation of blood

flow is mediated by the smooth muscles located in the walls of the vessels. A hormonal mechanism also is involved in the control of blood flow. Epinephrine, which is liberated from the adrenal medulla, stimulates alpha-1, beta-1, and beta-2 receptors resulting in a predictable dose related response (19,30).

MICROSPHERE METHOD TO ANALYZE BLOOD FLOW

The availability of isotope labeled microspheres has made it possible to evaluate circulatory physiology. In 1958, Sapirstein (31) described a technique using an intravenous injection of ^{42}Potassium radioisotope to measure blood flow and a percentage of cardiac output going to a particular organ. The radiolabeled microsphere technique is based on the assumption that when labeled indicators are injected intravenously, spheres are distributed to all the organs of an experimental animal in proportion to its blood flow during the time period in which the number of spheres in the venous drainage is negligible. During this period, the fractional uptake of the isotope by the organs will correspond to the fraction of the cardiac output to the organ. The blood flow to any organ can be described as the product of the cardiac output and the fractional uptake of the total injected isotope going to the organ. In 1968, Rudolph and Heymann (32)

introduced a method of measuring blood flow with radioactive microspheres. These were injected into the circulation of lambs, sheep and dogs. The spheres traveled to the small peripheral vessels where they were trapped; the organs were removed and their radioactivity measured. Repeated measurements were made by injecting multiple nuclides in each animal and separating the radioactive spheres by gamma spectrometry. The distribution pattern of microspheres was used to determine relative distribution of blood. From their study, experimental evidence provided the following: 1) there is no significant recirculation of microspheres; 2) the distribution of spheres is proportional to flow; and 3) circulatory physiology is not altered by injection of the spheres.

The precision in which blood flow can be measured by the microsphere method is mainly determined by the number of spheres trapped in the tissue and blood sample (33). Buckberg and co-investigators (34) measured coronary and renal blood flow with the microsphere injection method in dogs, sheep, and lambs. They concluded from the study that to obtain flow calculations within a 10% error with 95% confidence, this technique requires thorough mixing of the microsphere solution and each sample contain 400 or more microspheres.

Squier and Nanny (35) used labeled microspheres to measure blood flow to 15 defined areas of the oral mucosa in monkeys. After injection of 1.5 million microspheres (15 microns in diameter), thirteen mm diameter punch biopsies were removed, weighed and counted for radioactivity. The highest blood flow was recorded in the maxillary free gingiva of 121.98 ml/min/100g and the lowest flow was 5.94 ml/min/100g in the hard palate. The tissue weights and counts were used to calculate blood flow as ml/min/100g of tissue by means of the following relationship (36):

$$\frac{\text{Sample tissue counts} \times 100 \times \text{Reference blood flow rate}}{\text{Reference blood sample counts}}$$

where reference blood flow rate = a constant, and reference blood sample counts = radioactive counts of arterial blood withdrawn over a period of time.

Meyer (1) studied the distribution of cardiac output to the oral tissue of dogs. His results indicated a greater flow to maxilla by 30% when compared to mandible. Kim (13) demonstrated similar results with microspheres measuring pulpal blood flow changes in dogs due to epinephrine in local anesthetic. Other studies have measured blood flow in gingival and osseous oral tissue using the radiolabeled microsphere method (2,37). The radiolabeled microsphere method is also used in other

tissues as well throughout the body, confirming the reliability of calculating blood flow with this method (36,38-43).

Advantages of the radiolabeled microsphere method include: (1) measurement of blood flow can be made in undisturbed tissue; (2) measurement of regional blood flow can be determined; (3) the results can be compared to the flow in other tissues; and (4) the use of different radiolabeled spheres allows for comparison of blood flow to a specific tissue following introduction of a drug or following a procedure. Disadvantages include: 1) the inability to assess transient changes in blood flow occurring over short periods of time; 2) the limitation of the number of measurements per animal; 3) the method cannot be used for experiments in humans. Additional considerations in using the radioactive microsphere method include: the necessity to sacrifice the animal following the experiment; the use of radioactivity; and cost considerations (44).

LOCAL ANESTHETIC

History

Karl Koller clinically introduced cocaine, an alkaloid, as a topical anesthetic in medicine in 1884. Later that year, Halstead used cocaine as a local

anesthetic for a mandibular block. In 1903, Braun demonstrated that addition of epinephrine to local anesthetic solutions greatly prolonged and intensified the action. Lofgren and Landquist, in 1943, synthesized lidocaine. It was introduced into dentistry by Bjorn and Halted in 1947 (15,45,46).

Chemistry of Local Anesthetics

The local anesthetic formula is basically one of an aromatic lipophilic group, an intermediate chain ester or amide, and a hydrophilic group, which is usually a secondary or tertiary amine. Local anesthetics may be divided into an ester-linked group and an amide-linked group. Examples of esters include cocaine, procaine, and tetracaine. Examples of amides include lidocaine, prilocaine, mepivicaine, bupivicaine and etidocaine. The aromatic group of a local anesthetic is the largest part of the molecule and is commonly derived from an aniline or benzoic acid; it functions to define anesthetic potency and activity. The intermediate chain maintains the proper spacing between the aromatic and hydrophilic portions of the molecule and determines the metabolism. Chemically, local anesthetics are weak bases that are poorly soluble or unstable in solution. Salts of local anesthetic forming stable soluble quartenary amines enable the storage and use of these agents (15,47).

The physiochemical properties that determine anesthetic activity are lipid solubility, protein binding and pKa. Lipid solubility appears to determine anesthetic potency because the highly lipophilic molecule diffuses into nerve tissue more readily. Protein binding appears to be directly related to the duration of anesthetic activity. The pKa dictates the amount of base form available at a given pH. Increasing the pH of a solution decreases the hydrogen ion concentration, and the equilibrium shifts toward the free base form. Therefore, more of the local anesthetic agent will exist in the free base form, which is important because the amount of free base form is directly related to the onset of action (47).

All of the clinically effective injectable local anesthetics possess some degree of vasodilating activity. The increase in blood flow following the injection of a local anesthetic leads to increased rate of absorption of the local anesthetic into the blood, decreased duration of action and decreased effectiveness of the local anesthetic because it diffuses away from the injection site more rapidly, and increased bleeding at the site of administration due to increased perfusion (16).

Lidocaine

Today, lidocaine is the most widely used local anesthetic in dentistry compared to its predecessor

procaine. It possesses more rapid onset of action, produces more profound anesthesia, a longer duration of action, and greater potency (46). Lidocaine is currently used as the standard of comparison. Properties of lidocaine include: amide classification, metabolization in the liver and excretion via the kidneys, vasodilating properties, pKa of 7.9, and a 90 minute half-life. Dental formulations are 2% plain lidocaine, 2% lidocaine with 1:50,000 epinephrine concentration, and 2% lidocaine with 1:100,000 epinephrine concentration (16).

VASOCONSTRICTORS

The purpose of adding a vasoconstrictor to local anesthetics is to decrease the rate of vascular absorption, thereby allowing more molecules to reach the nerve membrane and to improve the depth and duration of anesthetic. Also, the vasoconstrictor counteracts the vasodilating effect of the local anesthetic solution by constricting the local blood vessels through the alpha receptor effects (30).

The vasoconstrictors used in conjunction with local anesthetics are chemically identical to the sympathetic nervous system mediators--epinephrine and norepinephrine. The adrenergic action of the vasoconstrictors resembles the response of adrenergic nerves to stimulation, so therefore they are classified as sympathomimetic or adrenergic drugs.

The most useful and popular sympathomimetic amine used as vasoconstrictor is epinephrine. Dose-dependant epinephrine is a potent stimulator of both alpha and beta adrenergic receptors mediated through the autonomic nervous system. Activation of alpha receptors by epinephrine produces contraction of smooth muscle in blood vessels, primarily in smaller arterioles and pre-capillary sphincters. Activation of beta receptors produces smooth muscle relaxation (vasodilation and bronchodilation) and cardiac stimulation (increased heart rate and strength of contractions) (15,16,46,47).

Epinephrine produces vasoconstriction in local anesthetics through its alpha effects thus prolonging the duration of anesthesia and preventing minimal blood loss in dental procedures. However, Malamed (16) has postulated that vasoconstrictors possess an action of producing a vasodilatory effect on tissues after the epinephrine has decreased to a level that no longer produces an alpha adrenergic effect. He states that the restricted tissue blood flow slowly returns to normal, but then rapidly increases to a rate well beyond normal flow resulting in a beta adrenergic effect and refers to this occurrence as a rebound phenomenon. Although the effect is the same, Lindorf (17) states that this hyperemic effect results from

localized tissue hypoxia and acidosis due to prolonged vasoconstriction.

HEMODYNAMIC STUDIES

Hemodynamics may be defined as the study of the physical parameters of blood flow and the factors that determine, modify, and regulate these parameters. Poiseuille's law states that flow and intravascular pressure show a direct relationship, provided that the flow resistance remains constant. Flow (volume moved per unit time) is directly proportional to the pressure drop across a tube and to the conductance (reciprocal of the resistance to flow) of the tube system (48). However, there are other related physical variables that influence the blood circulation as stated in Starling's law. The law states that the net filtration pressure across the capillary wall is dependant on capillary pressure, tissue pressure, osmotic pressure in the plasma, and the osmotic pressure in the tissue (28,48,49).

Microcirculatory studies have been done using various flow methods in examining healthy and diseased states of different tissues. Gangarosa (50) compared capillary blood flow in the oral mucosa and subcutaneous tissue of cats using the isotope clearance method, and showed that isotope clearance was at least two times more rapid from oral

mucosa than from subcutaneous tissue. Epinephrine added to the isotope solution caused a dose-related depression of clearance from both tissues. The relationship between epithelial thickness and blood flow was examined in oral mucosa and skin regions of the cat by Johnson and coworkers (2). Blood flow to these tissues was determined using the radiolabeled microsphere method, and thickness of the epithelium was calculated from histologic sections. The results of their study found that oral tissues had significantly higher blood flow than the skin regions, and that the palate had the thickest epithelium. When epithelial thickness was related to blood flow there was a significant positive correlation indicating that a thicker epithelium is associated with a higher blood flow. The authors suggest that this finding may reflect the greater metabolic demands of the thicker epithelia.

A few years later Johnson and associates (37) again examined bone blood flow in various regions of dogs using the microsphere method. Mean blood flow ranged from 3.71 ml/min/100g in mandibular cortical bone to 22.7 ml/min/100g in cancellous rib samples. The results from this study indicate that blood flow to the maxillary posterior bone is high in comparison to other oral tissues, but still blood flow is significantly less than that of cancellous rib bone.

The effects of 2% lidocaine with epinephrine (1:100,000) administered by accepted local anesthetic techniques on pulpal blood flow in dogs were determined using the 15um radiolabeled microsphere injection technique by Kim and coworkers (13). The pulpal blood flow decreased significantly with all three techniques; however, the most drastic reduction occurred in the molar teeth with the intraseptal injection of anesthetic. When 2% lidocaine without epinephrine was used in the intraseptal injection, pulpal blood flow increased significantly. Lastly, observations were presented by Nobuto et al (51) and Nobuto et al (52) of tissue sites maintaining blood circulation through gingival wound healing. They observed that the revascularization of free gingival grafts in dogs over denuded bone was mainly through the formation of new capillaries from those existing below the surrounding epithelium and periodontium.

CONCLUSION

In comparison with other tissues, quantitative studies of blood flow in oral soft tissues in experimental animals are few. There appears to be conflict in the literature in regards to the effect of injectable anesthetics on tissue blood flow. Is it the anesthetic or the vasoconstrictor agent that alters blood flow? Or, is it a neuronal

response via the autonomic nervous system which alters blood flow over a two hour time period? The proposed study will provide a model for the measurement of blood flow in oral maxillary muco-gingival tissues and compare blood flow in maxillary muco-gingival tissues over a two hour time period which have been injected with different anesthetic solutions.

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MEASUREMENT OF BLOOD FLOW IN REFLECTED MUCO-GINGIVAL TISSUE FLAPS IN CATS USING THE RADIOLABELED MICROSPHERE METHOD

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CHAPTER 2

INTRODUCTION

The most commonly performed endodontic surgery in clinical practice is periradicular surgery. This is achieved by an injection of a local anesthetic, with a vasoconstrictor to control hemostasis, and a reflection of the muco-gingival tissue from the underlying bone. Clinical observations, especially in longer surgical procedures, have shown that there may occur a sudden influx of blood flow in a surgical site that previously had good hemostasis. Vasoconstrictors are effective agents in preventing or minimizing blood loss during dental procedures. However, commonly used vasoconstrictors, such as epinephrine, possess the disturbing action of producing a vasodilatory effect after the vasoconstriction has worn off. Hence, this can apparently lead to an increased blood flow following an ordinary surgical operation. This effect in the dental literature is termed the rebound phenomenon (1-4).

In an intraoral injection of a local anesthetic solution containing the vasoconstrictor epinephrine, which possesses both alpha and beta receptor action, alpha adrenergic effects predominate locally and vasoconstriction occurs. Regional blood flow is reduced, causing the desired effects of prolonged anesthesia and hemostasis. This effect is usually immediate and persists for

approximately 30 to 90 minutes following the injection. It has been postulated that as the local tissue concentration of epinephrine decreases, the beta adrenergic effects of epinephrine will produce vasodilation. Because of this reversal, the local blood flow increases and the hemostatic effect of the vasoconstrictor is lost. This rebound phenomenon which leads to increased postoperative blood loss may last up to 2 hours (1-3).

Lindorf's study has been quoted to demonstrate the rebound phenomenon in humans (4). Using infared thermography, Lindorf (5) recorded thermal changes in human tissue resulting from alterations in vascular flow following the intraoral injection of one ml of 1:000,000 of epinephrine diluted in a saline solution. The thermograph revealed a rapid onset of vasoconstriction indicated by a decrease tissue temperature, a subsequent slow return (beginning at 60 minutes and reaching normal by 120 minutes), followed by an increase in blood flow and temperature which was still evident at the termination of the experiment (180 minutes post injection).

Although Lindorf's study (5) provided evidence of the blood flow alterations, this does not necessarily reflect the clinical situation because no surgical procedure was performed and one ml of 1:100,000 epinephrine in a saline solution was injected. Kim and associates (15) studied the

immediate effect of local anesthetics on pulpal blood flow in dogs and reported a 65% reduction of pulpal blood flow up to 75 minutes with the use of 2% lidocaine with 1:100,000 epinephrine. Furthermore, they found that injection of 2% plain lidocaine caused an substantial increase in pulpal blood flow. In contrast, Pitt Ford et al (16) injected 1-2 mls of 2% plain lidocaine into the labial sulcus and found that the pulpal blood flow of the adjacent maxillary central incisor tooth was unchanged. However, following an injection of 1 ml of 2% lidocaine with epinephrine, pulpal blood flow was significantly reduced, and the duration of decreased blood flow was 68 minutes.

There are currently few studies in the dental literature quantifying blood flow in muco-gingival tissues. Also, there appears to be no supportive evidence of the relationship between the vasopressor drug epinephrine and the rebound phenomenon onset.

The objectives of this investigation are twofold: To develop a cat model quantifying normal blood flow in maxillary muco-gingival tissue using the radiolabeled microsphere method; and to quantify and compare blood flow in maxillary muco-gingival flaps which have been injected with 2% plain lidocaine and 2% lidocaine with 1:50,000 epinephrine.

MATERIALS AND METHODS

Sixteen adult mix breed cats of either sex weighing from 6-10 pounds were used and divided into two groups. On the day of surgery each cat was anesthetized with an intramuscular injection of 1 ml of Ketamine (100mg/ml) and a .05 ml of Prom Ace (10mg/ml). Group 1 consisted of six animals whose maxillary muco-gingival tissues were reflected on either the left or right quadrant, with the contralateral tissues left attached. Each flap had a vertical incision on the mesial line angle of the cuspid and a horizontal sulcular incision extending to the first molar. The flaps were reflected with a #7 periosteal elevator. Blood flow was measured in both intact and reflected tissue to determine the effect of tissue reflection alone. Group 2 consisted of ten cats which were used to test the effects of different solutions on blood flow in reflected tissue. In each cat, the left and right maxillary quadrants were first treated with one of the following solutions: 1) injection with .6 ml sterile saline; 2) injection with .6 ml 2% lidocaine plain (Astra Pharmaceutical Worchester, MA); 3) injection with .6 ml 2% lidocaine with 1:50,000 epinephrine (Astra); or 4) no injection. Each was injected in the vestibule of the muco-gingival tissue with a 30-gauge needle as follows: .2 ml apical to the cuspid, .2 ml apical to the second bicuspid,

and .2 ml apical to the third bicuspid for a total of .6 ml of solution per animal. With each quadrant receiving only one treatment, two treatments were assigned randomly to each cat, resulting in 5 quadrants of tissue receiving each treatment.

After injection of the solutions in ten cats in group 2, their muco-gingival tissues were reflected from both left and right maxillary quadrants. The flap design was the same as described for group 1. After raising a flap, a saline soaked gauze was placed between the muco-gingival flap and the underlying bone to prevent any contact between the two tissues. All flaps were reflected a minimum of 90 minutes to a maximum of 135 minutes before the injection of the radiolabeled microspheres.

Utilizing a sterile technique, the left femoral artery was cannulated, and Tygon formula S-54-HL microbore tubing (I.D = .040 inches, O.D =.070 inches) was inserted into the abdominal aorta for the collection of the reference blood sample in a 6 ml syringe. A PE 90 catheter (I.D.=.034 inches, O.D.=.070 inches, Intramedic, Clay Adams, Parsippany, NY) was placed in the left ventricle of the heart via the right common carotid artery to allow intraventricular injection and adequate mixing of the radiolabeled microspheres. A Cobe disposable transducer connected to a Gould Recorder 200 via the PE 90 heart

catheter monitored the arterial blood pressure. This monitoring device also assisted in proper placement of the catheter in the left ventricle. Regional blood flow was determined by the injection of ^{153}Gadolinium microspheres 15u diameter (Dupont, Boston, MA). The radiolabeled microspheres were suspended in 10% dextran solution with 0.01% Tween-80, vortexed, agitated in an ultrasonic bath for 5 minutes, and re-vortexed to insure adequate mixture of spheres. Immediately 2.5 ml, containing 2.5 million spheres, were rapidly withdrawn from the microsphere vial and injected into the left ventricle via the PE 90 catheter over a period of 20 seconds, followed by flushing the catheter with 3 ml of heparinized saline solution. Withdrawal of arterial blood at 4.05 ml/minute (Harvard infusion/withdrawal pump, Boston, MA) was started five seconds prior to injection of the spheres and was continued for 65 seconds to obtain the reference blood sample.

At the termination of the experiment, the cat was sacrificed by an injection of euthanasia solution given intravenously. The maxillary right and left muco-gingival flaps (treated and untreated) were collected, weighed and placed into separate counting vials containing 10% formalin. (Figure 1) The vials were placed in a gamma counter (Auto-gama II, Packard Co. Evanston, IL) and tissue levels of radioactivity were determined. Adrenal, kidney,

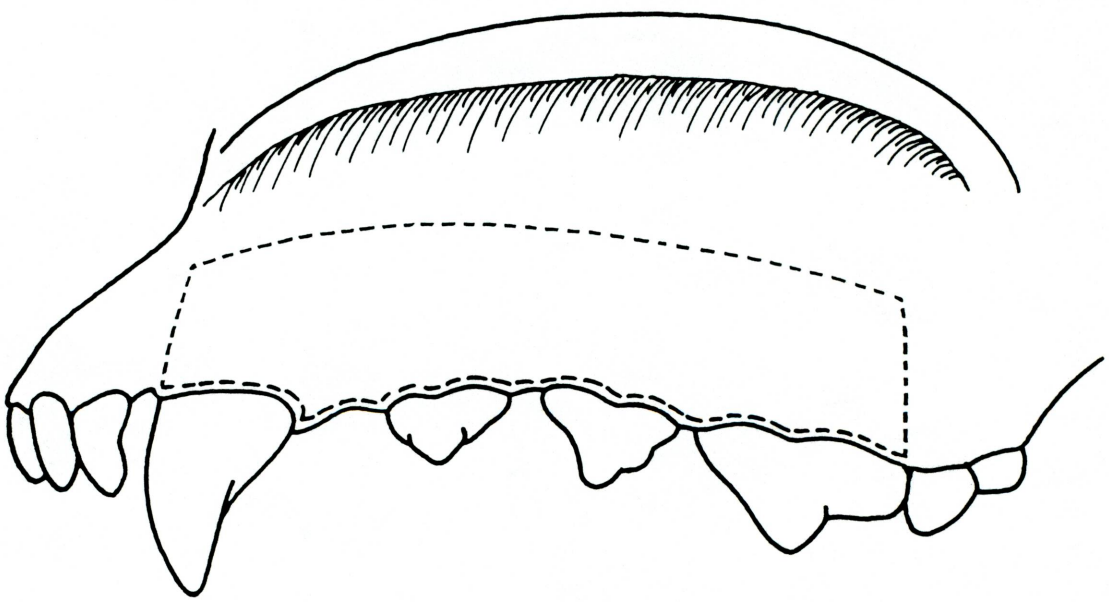

Figure 1. Drawing of a cat's maxilla depicting (by dotted lines) the muco-gingival tissue sample that was collected.

liver and mandibular gingival tissue samples were collected and weighed from each animal to check for uniform systemic dispersion of the radiolabeled microspheres. After taking the samples and counting their levels of radioactivity, the blood flow for each sample was determined and expressed as ml/min/100g of tissue by the following formula:

$$\frac{\text{Sample tissue counts X 100 X Reference blood flow rate}}{\text{Reference blood sample counts}}$$

where tissue counts = radioactive counts of sample tissue; reference blood flow rate = 4.05 ml/minute (a constant); reference blood sample counts = radioactive counts of the blood sample withdrawn from the femoral artery in 65 seconds.

It has been suggested that at least 384 spheres be present in each sample if blood flow measurements are to be considered reliable (17). To determine the number of spheres in a given tissue sample, the total counts for the sample were divided by the specific activity of the spheres. All samples contained at least 384 microspheres.

DATA ANALYSIS

An analysis of variance (ANOVA) was used to compare blood flow values among all samples in group 1 and group 2. Further analysis of the data in group 1 (attached versus reflected) included the Wilcoxin Signed Ranks Test and the

Duncan's Multiple Range Test in group 2. A p value < .05 was accepted as significant.

RESULTS

In group 1, the mean blood flow value for the attached tissue (controls n=6) was 34.38 ml/min/100g of tissue. The mean blood flow value of the reflected tissue (n=6) was 152.297 ml/min/100g of tissue. The mean reflection time of the flap was 117 minutes ±7 minutes. Blood flow to the attached tissue of the maxillary gingival-mucosa was significantly lower (p=0.027) than that of the maxillary flapped tissue of the maxillary gingival mucosa (Figure 2).

Figure 3 shows the group 2 results of different local anesthetic treatments on blood flow in flapped maxillary tissue. The sample injected with 2% plain lidocaine had the highest blood flow rate of 90.24 ml/min/100g of tissue and the no treatment group had the lowest blood flow rate of 41.19 ml/min/100g of tissue. The 2% lidocaine with 1:50,000 epinephrine treatment group had a flow rate of 60.60 ml/min/100g of tissue while the saline treatment group was 46.81 ml/min/100g of tissue. The mean reflection time of the flap was 116 minutes ±8 minutes.

There was a significant difference between samples injected with 2% plain lidocaine and the samples injected with saline or no treatment. However, there was no

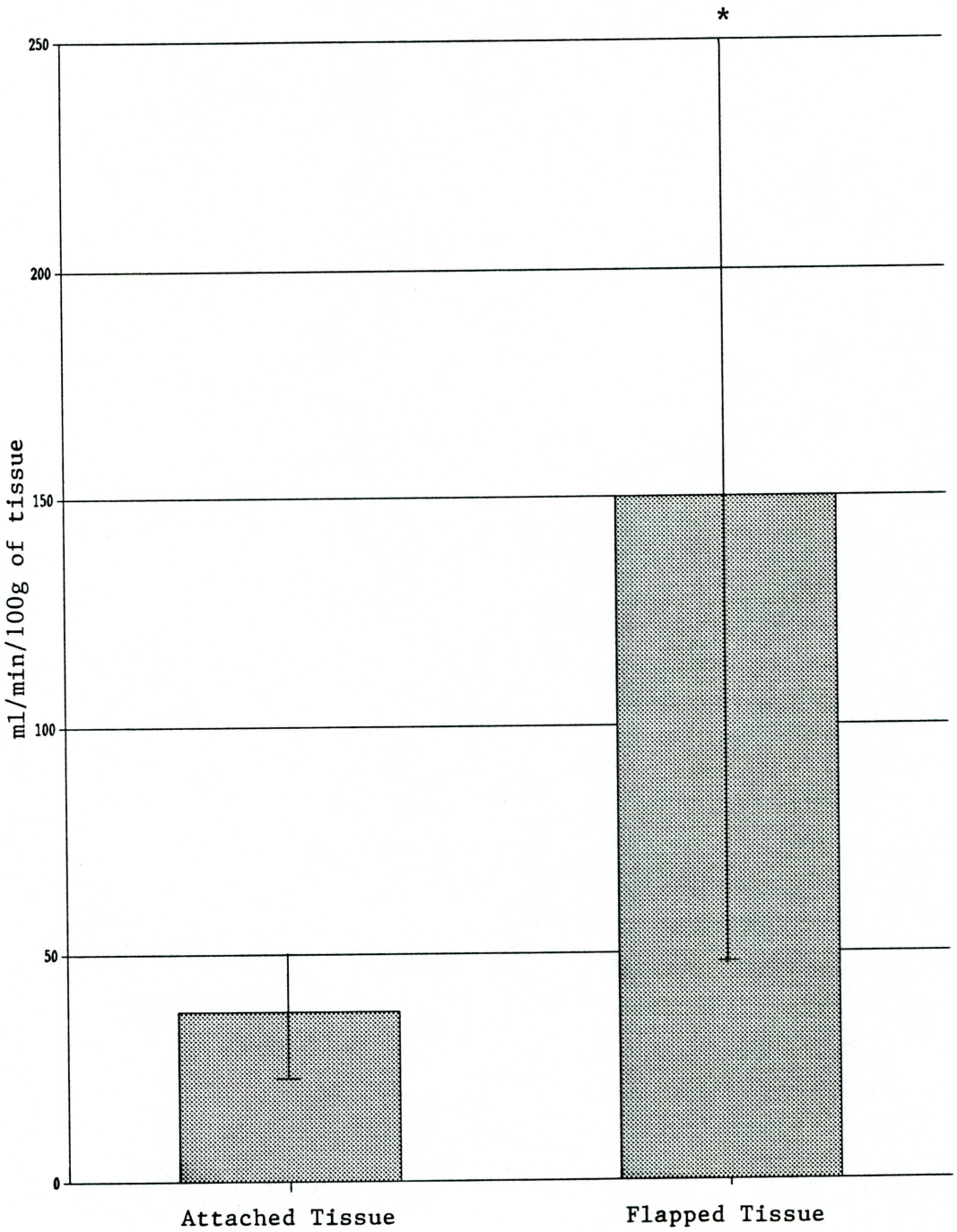

Figure 2. Blood flow in attached and flapped tissues. Data are expressed as mean plus-minus standard error of the mean.

* P<0.05 compared to attached tissue

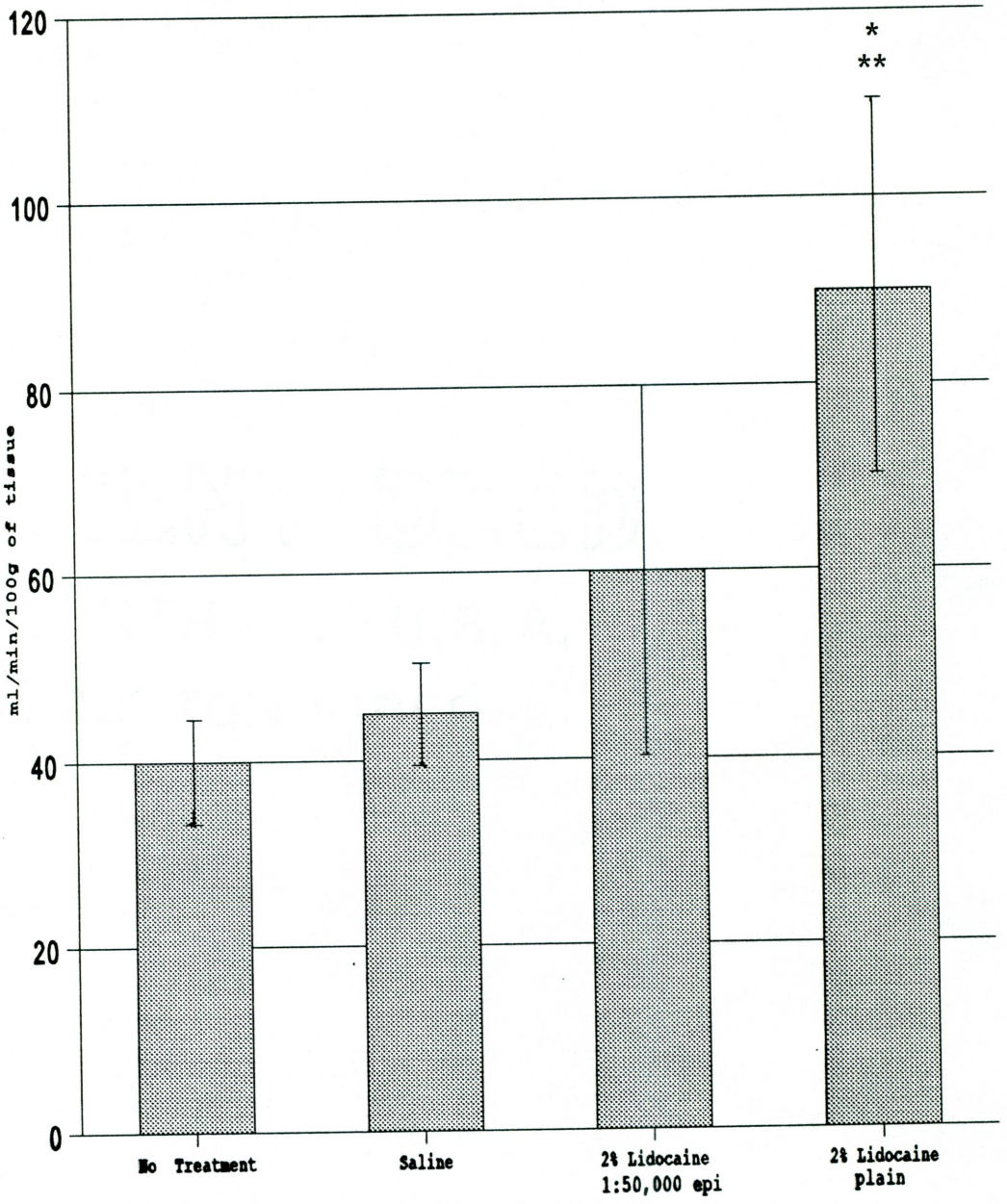

Figure 3. Blood flow shown in treatment groups; no treatment, saline, 2% lidocaine with 1:50,000 epinrphrine, and 2% lidocaine plain. Data are expressed as mean plus-minus standard error of the mean.

* P<0.05 compared to no treatment
** P<0.05 compared to saline

significant difference in blood flow samples injected with 2% plain lidocaine and those injected with 2% lidocaine with 1;50,000 epinephrine.

DISCUSSION

In the cat, the lip and buccal mucosal epithelium are among the thinnest in the oral regions, which is very different from primates, whose regions have the thickest epithelium (6).

The blood flow measurements for oral gingival tissue in cats found in this study are within range of those reported by Johnson (6) and Gangarosa (7) and generally similiar to results reported by other investigators for other species, including dogs (8,9,10,11) and monkeys (12). In this study, the mean gingival blood flow value of 34.38 ml/min/100g in group 1 agrees well with those reported by Johnson et al (6) who reported blood values ranging between 30.85-36.23 ml/min/100g in the maxillary gingiva of cats using the radiolabeled microsphere method. Gangarosa (7) measured blood flow values within the same range studying the oral submucosa along the maxillary mucobuccal fold in cats using 131I-4-Iodoantipyrine local clearance method.

Our gingival blood flow values are in agreement with those reported by Kaplan et al (9) for dogs with minimal

bone loss due to periodontal disease. Using the radiolabeled microsphere method, Kaplan measured blood flow in gingival tissues in beagles with varying degrees of periodontal disease and found blood flow values in these tissues ranging from 28 ml/min/100g in dogs with minimal bone loss (<10%) to 93 ml/min/100g in dogs with mild bone loss (10-20%).

In group 2, the samples injected with 2% plain lidocaine had a mean flow rate higher than the tissue treated with sterile saline or tissue with no treatment. There was a significant difference in blood flow rate of those injected with 2% plain lidocaine compared with those with no treatment or injected with sterile saline. This result may be due to the chemical nature of lidocaine. All local anesthetic drugs, except cocaine, are vasodilator by nature. However, the degree of vasodilation produced by various agents differs. Lidocaine has shown greater intrinsic anesthetic potency than that of mepivacaine or prilocaine and has a half-life of 90 minutes. This vasodilating activity of lidocaine can result in greater vascular absorption such that less lidocaine is available for nerve blockade. Lidocaine 2%, without a vasoconstrictor, has been listed as a poor drug for pulpal anesthesia for conventional dental anesthetic. This may be

due to its vasodilating effects producing ineffectivity as a local anesthetic (13,14).

Kim et al (15) have shown similiar results found in the present study with the use of 2% lidocaine in dogs using the radiolabeled microsphere method. They reported that the use of 2% lidocaine without epinephrine resulted in a fifty percent increase in pulpal blood flow in the dog using an intraseptal injection technique. They also reported a decrease in pulpal blood flow when injected 2% lidocaine with 1:100,000 epinephrine whereas saline injections did not produce any detectable changes in pulpal blood flow. This is in contrast to the finding of Pitt Ford et al (16), who demonstrated on human subjects that 2% plain lidocaine had no effect on pulpal blood flow as assessed by laser doppler flowmeter.

In group 2, we found no significant difference ($p>.05$) between the blood flow of samples injected with 2% plain lidocaine and those injected with 2% lidocaine and 1:50,000 epinephrine. An explanation for this may be due to the flap reflection time and the duration of the solution used in the present study. Milam and Giovannitti (1) state that following an intraoral submucosal injection of a local anesthetic solution containing epinephrine, alpha-adrenergic effects predominate locally and vasoconstriction occurs. Regional blood flow is reduced accounting for the

desired hemostasis. This effect is usually immediate and persists for approximately 30 to 90 minutes following the injection of the local anesthetic. As the local tissue concentration of epinephrine decreases to a level that no longer produces an alpha-adrenergic effect, the local blood flow increases and the hemostatic effect of epinephrine is lost. In this study, the mean time lapse between injection of anesthetic and blood flow measurement was 117 minutes. This time lapse may possibly contribute to the minimal difference that 1:50,000 epinephrine produced in blood flow compared with 2% plain lidocaine. In contrast, Pitt Ford and co-workers (16) discovered a significant drop in blood flow (31%) which lasted for 68 minutes when injecting maxillary central incisors of humans with 1 ml of 2% lidocaine with 1:80,000 epinephrine.

The radiolabeled microsphere method was used to measure muco-gingival blood flow in this study, which resulted in considerable variation in blood flow values. This variability may be due to a combination of the following factors: 1) The microsphere method requires surgical preparation of each animal. Due to the size and anatomical differences in the animals, surgical difficulties were encountered with some of the cats, thus, possibly affecting cardiac output, 2) Blood flow was determined from oral muco-gingival samples that had been

dissected free from the underlying tissue, but it is possible that the samples could contain submucosal tissues and muscle fiber, 3) The fact that a 10% error in the microsphere method is acceptable (17). Despite these potential variations, the blood flow values found in this study were consistent in the different experimental groups.

Based on our results, it appears that the flaps injected with 2% lidocaine with 1:50,000 epinephrine had no increased blood flow values after 117 minutes. Vasoconstrictors, such as epinephrine, are effective agents in preventing or minimizing blood loss during dental procedures. However, traditional thought has expressed that epinephrine possesses the action of producing a rebound dilatory effect after the vasoconstriction has worn off (half-life = 90 minutes) which results in a increased blood flow for up to two to three hours (1-4). Gutmann and Harrison (4) suggest that this sudden "opening of the flood gates" in a surgical site signals the rapid change from alpha to beta adrenergic effect. In contrast, Lindorf (5) states that due to the metabolic effect of the vasoconstrictor, hypoxia of tissue and acidosis cause a secondary vasodilation of the vessels.

The results of this study reveals that the injection of 2% plain lidocaine causes a significant increase in blood flow 117 minutes after the intial injection, while

the injection of 2% lidocaine with 1:50,000 epinephrine in the flaps results in lesser blood flow in the reflected gingival flaps. It appears that the anesthetic lidocaine and its intrinsic vascular effect may be the significant factor that is causing the prolonged vasodilation and increased blood flow for approximately two hours post injection. The epinephrine in the 2% lidocaine in this study did not result in dilation of blood vessels, contrary to current literature, but actually interfered with the vasodilation properties of lidocaine, resulting in a decreased blood flow to the tissues two hours post injection. Had the vasoconstrictor produced a rebound beta effect, as stated by other authors (1-4), this rebound phenomenon should have occurred in a two hour time period. Based on our results, it appears that epinephrine reduces the increased blood flow induced by the injection of 2% plain lidocaine and may not be responsible for the rebound effect if injected in combination with a local anesthetic.

CONCLUSION

In summary, under the conditions of this study, blood flow was significantly altered by reflecting the tissue and injecting 2% lidocaine with or without epinephrine over a two hour time period. In contrast to the current rebound

phenomenon, the findings of this study do not support this theory. This study concludes that the increased blood flow is a function of the vasodilation properties of lidocaine and that the vasoconstrictor, epinephrine, has a restraining effect on increasing blood flow post operatively in reflected muco-gingival tissues.

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