INDUCED COLLATERAL MICROVASCULATURE OF THE TONGUE.

SEP 78  J G WEBB,  J F NELSON,  B M KOUDELKA
**INDUCED COLLAGERAL MICROVASCULATURE OF THE TONGUE**

The right common carotid artery was ligated in twenty-five rabbits. These animals were sacrificed at intervals and their vasculature perfused with a silicone latex material. Animals sacrificed one hour after ligation showed a greatly reduced blood flow to the right side of the tongue. Only a slightly reduced flow was observed in animals sacrificed at the six hour and twenty-four intervals. Animals in the one and two week groups displayed a microvascular perfusion pattern similar to that of the control animals which underwent no ligation.

**KEY WORDS**

1. Collateral Circulation
2. Microvasculature of Tongue
INDUCED COLLATERAL MICROVASCULATURE OF THE TONGUE

Joseph G. Webb, Jr., D.M.D., M.S.*
John F. Nelson, D.D.S., MEd.**
Brent M. Koudelka, D.D.S., M.S.*

*Senior Resident, Division of Pathology, United States Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, D.C. 20012

**Chief, Department of Oral Pathology, United States Army Institute of Dental Research, Walter Reed Army Medical Center, Washington, D.C. 20012

The opinions and assertions contained herein are those of the authors and are not to be construed as official or representing the Department of Defense.
ABSTRACT

Previous studies have contained little substantial evidence relating to the microvascular effects induced by unilateral elimination of the primary blood supply to one-half of the tongue. This study was designed to ascertain the microvascular pattern in the collateral circulation of the tongue after unilateral ligation of the common carotid artery. Results indicate that blood flow to the ligated side of the tongue was substantially reduced in the one hour experimental group. The six hour and twenty-four hour groups however, demonstrated only a slightly reduced flow. Animals in the one and two week groups displayed a microvascular perfusion pattern similar to that of the control animals who underwent no ligation.

These findings indicate that if ligation of a major vessel is necessary to control hemorrhage in the tongue, collateral circulation is adequate to maintain the health of the tissue and a "normal" vascular pattern returns within one week after ligation.
INTRODUCTION

Every dentist, and especially the oral surgeon, should have a functional knowledge of the vascular pattern of the tongue. He or she is the professional most responsible not only for routine oral care, but also for the diagnosis and treatment of trauma and tumors of the oral cavity. If severe trauma occurs, or extensive surgery is necessary in the tongue or floor of the mouth, the lingual artery or one of its major branches may be involved or even severed. Hemorrhage from a damaged or severed major artery can be life-threatening and emergency procedures necessary for its control should be familiar to the dentist. Ligation of the artery at the site of hemorrhage or at some point proximal to it may be the indicated treatment. The tissue normally nourished by this artery subsequently becomes dependent upon collateral circulation for its survival. It is evident, therefore, that a knowledge of the vascular pattern of the tongue is essential for the surgeon.

This study is designed to ascertain the microvascular pattern of the collateral circulation of the tongue after unilateral ligation of the common carotid artery.

REVIEW OF LITERATURE

Very little research has been done to delineate the microvascular pattern of the collateral circulation of the tongue after ligation of the common carotid or lingual arteries. Maloney et. al. injected Acid Blue and Renografin-60 dyes in the contralateral lingual artery after ligation of one lingual artery in living dogs. They found that the contralateral unligated lingual artery was the major source in the development of the collateral circulation in 71% of their cases, and in 29% of their cases the collateral circulation was supplied by the ipsilateral common carotid artery.
Shepherd et al. observed the effects of ligation in controlling lingual artery hemorrhage in dogs, and found that bilateral ligation of the common carotid arteries was the only consistently effective method of controlling the hemorrhage. They stated that this was not a practical approach and suggested that ligation be done as close to the bleeding site as possible. This study suggests that collateral filling is both extensive and rapid in the head and neck areas.

Gotze and Lierse studied the efficacy of the contralateral anastomoses in the tongue of a rabbit by ligating the left lingual artery and sacrificing the animal after seven days. They fixed the tissue in formaldehyde, enclosed it in paraffin, and stained the tissue with Azan. Their results showed absolutely no change in the connective tissue, muscle, epithelium, or vasculature of either half of the tongue. Only one animal, however, was studied in this manner.

These findings indicate that although there have been limited studies of the vascular patterns of the tongue, the literature contains little substantial evidence as to the effects of unilateral elimination of the blood supply to the tongue.

MATERIALS AND METHODS

A total of thirty-three (33) New Zealand White male rabbits weighing approximately seven pounds each were used in this study. A dosage of 0.5 cc per kilogram of body weight of 2% sodium pentobarbital was injected in the vein of one ear for the purpose of attaining operative anesthesia. A 3-centimeter midline incision was made extending from the general area of the thyroid cartilage to the suprasternal notch. Exposure of the right common carotid artery was accomplished by blunt dissection (Fig. 1). The artery was then ligated with 3-0 silk suture one centimeter below the level of the thyroid cartilage.

Twenty-five (25) animals were operated. To assure having specimens
demonstrating early changes in vasculature, three (3) groups of five (5) experimental animals were sacrificed at intervals of one (1) hour, six (6) hours, and twenty-four (24) hours. Two additional groups were included to demonstrate the finally established vascular pattern at the one week and two week intervals. Only ten animals were used in the control group since the resultant vascular pattern was compared to the "normal" distribution of vasculature of the rabbit tongue as described by Gotze and Lierse and others. Two control animals were sacrificed with each of the five groups of experimental animals. After being sacrificed, all animals were perfused with a solution of silicone latex "Microfil".*

The perfusion technique used in this study is similar to the one reported by Cutright and Bhaskar. Each animal was anesthetized with 1.5 cc of 2% sodium pentobarbital using the vein of one ear. The abdominal and thoracic cavities were opened, the ascending aorta isolated (Fig. 2), and a cannula inserted and ligated into the aorta. A small drainage vent was made in the right atrium. A 100 cc syringe was used to flush heparinized saline into the vasculature of the head and neck until the exiting fluid in the atrial vent became clear. A solution of silicone latex Microfil* was then perfused into the vascular system. After complete perfusion, the cannula and atrial vent were clamped shut and the animals refrigerated for 24 hours to allow vulcanization to take place. The desired tissue was then removed and processed for study as follows.

Each tongue was processed using the following alcohol-methyl salicylate clearing sequence:

- Day One - Immerse in 25% ethyl alcohol for 24 hours
- Day Two - Immerse in 50% ethyl alcohol for 24 hours

Day Three - Immerse in 75% ethyl alcohol for 24 hours
Day Four - Immerse in 95% ethyl alcohol for 24 hours
Day Five - Immerse in absolute ethyl alcohol for 24 hours
Day Six - Immerse in methyl salicylate

The methyl salicylate replaces the fluids of the treated tissue. This alters the index of refraction and renders all of the soft tissue transparent, so that only the latex in the vessels is readily visible.

METHOD OF ANALYSIS OF DATA

The specimens were quantitatively evaluated clinically on the basis of observed collateral circulation. Any circulation to the side of the tongue with the ligated common carotid artery was considered to be collateral circulation.

Each specimen was transilluminated and the degree of perfusion of the ligated side was graded by three independent researchers using a scale of 0 to 3, with 0 indicating no perfusion and 3 denoting complete perfusion. The collected data was then analyzed by comparison of mean values (Table I).

RESULTS

One Hour: This group of tongue specimens exhibited the least amount of perfusion on the ligated side of all groups studied. There was, however, some degree of perfusion evident in each specimen (Fig. 3).

Six Hour: As a group, these specimens exhibited a higher degree of perfusion in the ligated tissue than the one hour group, but less than seen in the one week and two week groups (Fig. 4). There was some individual variation evident in these specimens, however, with one specimen being almost completely perfused.
Twenty-four Hour: The degree of perfusion in the ligated tissue of these specimens was judged to be very close to that seen in the six-hour group (Fig. 5). Individual variation was not a prominent feature in this group.

One Week: All specimens in this group had a perfusion pattern that very closely approximated that found in the control group, in that no significant differences between the ligated and nonligated sides were noted (Fig. 6).

Two Week: These tongues were judged to be bilaterally perfused and could not be significantly differentiated from those of the control group (Fig. 7 and 8).

DISCUSSION

Collateral circulation in the tongue has been shown to be sufficient for maintenance of tissue viability even when one common carotid artery is ligated. This would indicate that if the management of hemorrhage necessitates the ligation of a common carotid artery, it might be performed without undue concern about anoxia and subsequent tissue necrosis in the rabbit tongue.

Although variables such as possible naturally-occurring individual variation in the experimental animals could not be completely eliminated, our results indicate that there is a significant difference in the degree of perfusion between the early and later experimental groups. The specimens recovered from the animals that were perfused one hour after ligation of the common carotid artery demonstrated the most dramatic reduction in number of perfused vessels, while tongues from the one and two week groups were essentially bilaterally normal and equal. The six hour and twenty-four hour experimental groups demonstrated more perfusion than the one hour group, but less perfusion than the one week and two week groups.

While perfusing the control animals, it was observed that the initial
vascular filling occurred in the base of the tongue in the dorsal lingual and sublingual arteries. This was followed by filling of the tip, with the lateral borders of the middle third of the tongue being the last areas to be filled with latex. The half of the tongue contralateral to the ligated common carotid artery demonstrated a complete perfusion pattern in all animals.

Contralateral anastomoses in the rabbit tongue, via the right communicantes superficiales and profundi arteries, apparently provide much more efficient collateral circulation than is seen in the human tongue. Nevertheless, the pattern of the arteries and veins in each half of the tongue of these two species is nearly identical and similar reactions could be expected in each.

Further study is indicated to determine whether the established vascular pattern is a result of new capillary budding, a reversal of blood flow in some vessels, or a combination of these processes.

**SUMMARY AND CONCLUSION**

This study was undertaken to ascertain the microvascular pattern of the collateral circulation of the tongue after unilateral ligation of the common carotid artery. The right common carotid artery was ligated in twenty-five New Zealand White male rabbits. These animals were sacrificed in groups of five at intervals of one hour, six hours, twenty-four hours, one week, and two weeks. The head and neck vasculature of these animals was flushed with heparinized saline and perfused with latex.

The results demonstrate that circulation was present but significantly reduced in the one hour group, slightly reduced in the six hour and twenty-four groups, and "normal" in the one and two week groups.

This study indicates that the collateral circulation of the tongue is extensive and that a "normal" vascular pattern returns within one week after unilateral ligation of the common carotid artery.
In conducting the research described in the protocol, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.
LEGEND

Fig. 1 Exposure of the right common carotid artery.
Fig. 2 Ligature being placed under the aorta prior to insertion of the cannula.
Fig. 3 One hour specimen with severely impaired perfusion unilaterally.
Fig. 4 Six hour specimen with impaired perfusion unilaterally.
Fig. 5 Twenty-four hour specimen with impaired perfusion unilaterally.
Fig. 6 One week specimen with nearly complete perfusion.
Fig. 7 Two week specimen with complete perfusion bilaterally.
Fig. 8 Control specimen with "normal" perfusion pattern.

Table I. Comparison of Mean Values
REFERENCES


<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>degree of perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>8</td>
<td>2.9</td>
</tr>
<tr>
<td>ONE HOUR</td>
<td>5</td>
<td>0.66</td>
</tr>
<tr>
<td>SIX HOURS</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>TWENTY FOUR HOURS</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>ONE WEEK</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>TWO WEEK</td>
<td>5</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* 0 = No Perfusion  
3 = Complete Perfusion