Effect of Celecoxib on Fasciocutaneous Flap Survival and Revascularization

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Objectives: To study the effect of celecoxib (Celebrex; Pfizer, Cambridge, Mass) on (1) primary ischemic time and (2) revascularization of fasciocutaneous free flaps in a rat model.

Methods: In the ischemia study, 50 male Sprague-Dawley rats were divided into 2 groups of 25 rats each, a control group and a celecoxib group. Five rats in each treatment group were exposed to ischemic times of 4, 6, 8, 10, and 12 hours. Survival of the flap was assessed 7 days after reversal of the ischemia. Probit curves and the critical ischemic time were calculated. In the revascularization study, 30 male Sprague-Dawley rats were divided into 2 groups of 15 rats each. One group was fed celecoxib, while the other was fed a normal diet. All rats had a 3 × 6-cm fasciocutaneous flap based on the inferior epigastric artery elevated and exposed to 2 hours of primary ischemia. The flap was then sutured back into the wound bed. Each of these groups was then divided into 3 groups of 5 rats whose pedicles were divided on postoperative day 5, 6, or 7. Percentage survival of the flap was measured 7 days later. In both parts of the study, the experimental group was fed celecoxib, 1500 ppm, throughout the interoperative period. In each animal, a 3 × 6-cm ventral fasciocutaneous groin flap based on the left superficial epigastric artery was elevated.

Results: In the ischemia study, respective flap survival rates from the control and celecoxib groups at the various ischemic times were as follows: 4 hours, 100% and 100%; 6 hours, 80% and 100%; 8 hours, 80% and 80%; 10 hours, 60% and 60%; and 12 hours, 20% and 10%. The median lethal ischemic times were 9.7 and 9.6 hours, respectively. There was no statistical difference in flap survival between the celecoxib and control groups. In the revascularization study, ligation of the flap pedicle on day 5, 6, or 7 did not result in any difference in the percentage of flap survival among the 3 groups.

Conclusion: Celecoxib appears to have no deleterious effect on free tissue transfer survival or healing, as evidenced by revascularization in a fasciocutaneous free flap model.

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Composite free tissue transfer has proven to be the best technique for reconstructing complex bone and soft tissue defects in the head and neck. Many different donor sites are available for harvesting these composite tissue components. The multitude of available flaps allows one to design a donor site that offers the best composite tissue match. Free tissue transfer is available in most centers and has a success rate of between 90% and 100%.

A multitude of factors can complicate the outcome of microsurgical free tissue transfer. Many of these occur during the initial surgical procedure and are preventable or correctable by meticulous surgical technique. Other intraoperative or postoperative complications, such as seroma, hematoma, or fistula formation, may contribute to flap failure or make the flap more susceptible to an ischemic insult. Flaps that are less tolerant of ischemia may fail with devastating results to the patient.

The cyclooxygenases (COXs) are involved in the conversion of arachidonic acid to prostaglandins. Cyclooxygenase 1 is constitutively expressed in most tissues; COX-2 is an inducible enzyme whose activity is increased in response to a variety of stimuli including growth factors such as epidermal growth factor, vascular endothelial growth factor, and basic fibroblast growth factor.1,2 Cyclooxygenase 2 is expressed in macrophages and endothelial cells in response to inflammation.3,5 Nonsteroidal anti-inflammatory drugs (NSAIDs) such as COX-2 inhibitors have been used as anti-inflammatory, analgesic, and antipyretic agents because of their specificity and reported decreased gastrointestinal tract and renal toxic effects. Studies have shown that both NSAIDs and COX-specific inhibitors inhibit angiogenesis in solid and epithelial tumors.4,6,7

Initially, free flaps are dependent on their vascular pedicle for survival. Long-term survival is facilitated by revascular-
ization from the surrounding tissue bed. Factors that interfere with this revascularization can contribute to long-term wound-healing problems and decrease the ability of the flap to tolerate division of its vascular pedicle or pedicle thrombosis. The antiangiogenic affects of COX-specific inhibitors could adversely effect flap revascularization and contribute to increased patient morbidity, including late flap loss.

The objectives of this study were to evaluate the effects of celecoxib (Celebrex; Pfizer, Cambridge, Mass) on (1) primary ischemic time and flap survival and (2) revascularization of the rat fasciocutaneous flap.

METHODS

The study was divided into 2 parts. In the first part, the effect of celecoxib on primary ischemic time was examined, while in the second part, the effect of celecoxib on revascularization was studied. In both parts, adult male Sprague-Dawley rats weighing between 200 and 300 g were used. The National Institutes of Health guide for the care and use of laboratory animals was followed throughout the study. This protocol was approved by the institutional animal care and use committee at Oregon Health and Science University. Rats were housed in cages in pairs with access to food and water ad libitum, and their condition was observed daily.

Flaps were elevated in the following manner: General anesthesia was achieved using an isoflurane nonrebreathing inhalation mask. Once anesthetized, the animal was placed in the supine position on a warming pad at 40°C. The abdomen was shaved to remove all hair. The skin was then prepared with a povidone-iodine solution (Betadine; Purdue Pharma LP, Stamford, Conn). A standard 3 x 6-cm fasciocutaneous flap based on the left superficial epigastric artery and vein was raised. A standard template was drawn for each animal. Vascular dissection included the femoral vessels both proximal and distal to the origin of the epigastric pedicle. The distal femoral vessels were tied and transected to shunt all femoral flow to and from the raised flap. The deep femoral artery and vein were ligated and transected. The femoral nerve was left intact throughout the procedure. Rats in each part of the study were divided into control and celecoxib groups. The rats in the control group were fed water and food ad libitum. The rates in the celecoxib group were given 1500 ppm of celecoxib via oral administration by dissolving 200 mg in 267 mL of water 7 days prior to surgery. This was continued throughout the protocol time period. All animals received the same preoperative and postoperative care and were treated with 0.1 mg/kg of buprenorphine hydrochloride in gelatin cubes twice a day for 3 days for analgesia.

ISCHEMIC TIME

In the first part of the study, 50 rats were used. The rats were divided into a control group and a celecoxib group of 25 animals each. Following elevation of the flap, all animals had a 20-g Heifitz clip applied to the vein and artery of the vascular pedicle for 4, 6, 8, 10, or 12 hours. During this ischemic time, the flap was sutured back into place; the animals were allowed to ambulate and given food and water ad libitum. At the end of the 2-hour period of ischemia, the animals were reanesthetized, the corner of the flap was elevated, and the Heifitz clip was removed. The flaps were then stitched back into place using 4-0 sutures, and the animals were awakened. After awakening, the animals were placed in the supine position on a warming pad at 40°C. The abdomen was shaved to remove all hair. The skin was then prepared with a povidone-iodine solution (Betadine; Purdue Pharma LP, Stamford, Conn). A standard 3 x 6-cm fasciocutaneous flap based on the left superficial epigastric artery and vein was raised. A standard template was drawn for each animal. Vascular dissection included the femoral vessels both proximal and distal to the origin of the epigastric pedicle. The distal femoral vessels were tied and transected to shunt all femoral flow to and from the raised flap. The deep femoral artery and vein were ligated and transected. The femoral nerve was left intact throughout the procedure. Rats in each part of the study were divided into control and celecoxib groups. The rats in the control group were fed water and food ad libitum. The rates in the celecoxib group were given 1500 ppm of celecoxib via oral administration by dissolving 200 mg in 267 mL of water 7 days prior to surgery. This was continued throughout the protocol time period. All animals received the same preoperative and postoperative care and were treated with 0.1 mg/kg of buprenorphine hydrochloride in gelatin cubes twice a day for 3 days for analgesia.

In the ischemia analysis, the primary outcome measure was survival of the flap. Survival in all groups was either 100% or 0%. The data were analyzed using SPSS software, version 11 (SPSS Inc, Chicago, Ill). The difference in survival was analyzed using a 1-way analysis of variance. A Tukey and Scheffe post hoc test was used to identify differences between periods of ischemia. A probit analysis of the probability of flap survival based on the variable ischemic times was calculated for the 3 groups of animals. The predicted ischemic time when 50% of the flaps became totally necrotic was calculated as critical ischemic time.

In the revascularization analysis, the primary outcome measure was percentage survival of the flap. Differences in sur-
In the ischemia study, the overall survival was not statistically different between the rats that received celecoxib and those that did not (Table 2). Probit curves were similar in both groups (Figure 3), and no statistically significant difference was detected. The calculated critical ischemic time for the control and celecoxib-treated groups were 9.7 and 9.6 hours and were not significantly different (P = .99) (Figure 3). A power analysis was performed and revealed that more than 500 animals per group would have to be used to detect a difference.

In the revascularization study, all flaps survived the initial 2-hour ischemic period. The mean ± SD percentages of flap survival between groups are listed in Table 3. Within the control group, there was a significant difference in flap survival rates between pedicles severed on days 5 and 6 (P = .02) and between pedicles severed on days 5 and 7 (P = .02) (Figure 4). There was a statistically significant flap survival difference between pedicles severed on days 5 and 7 (P = .02) and between those with pedicles severed on days 6 and 7 (P = .05) within the celecoxib group (Figure 4). There was no statistically significant difference in the flap survival rates between the control and celecoxib groups for each of the pedicle transection groups at day 5, 6, or 7 (Figure 5).

**COMMENT**

Free tissue transfer is often used to restore form and function to the patient following head and neck surgery. Successful transfer depends on a patent vascular pedicle until revascularization from the periphery of the surgical bed can occur. Revascularization is an integral component of wound healing and long-term flap survival. One component of this revascularization is the migration and proliferation of capillary endothelial cells. Endothelial cells in the wound bed and flap undergo migration and mitosis toward angiogenic factors. Ingrowth of vessels and the development of interconnections between the flap and its wound bed decrease the dependency of the flap on its vascular pedicle. As these vascular connections proliferate, there will be a point in time when the transferred tissue is no longer dependent on its vascular pedicle to survive. One method of determining the time course and extent of the revascularization process is to ligate the vascular pedicle at various times and measure how much of the flap survives. Belmont et al demonstrated that if the pedicle is transected at 5 days after surgery, roughly 30% of the flap will survive. At 7 days, more than 80% will survive. Our control data are similar to those previously reported, with a mean of 30% survival at 5 days and 80% survival at 7 days.

Many factors may interfere with the revascularization process. One example of iatrogenic interference with flap revascularization is that which occurs following photodynamic therapy (PDT). Photodynamic therapy results in rapid vascular stasis, thrombosis, hemorrhage, and anoxia-induced tumor cell death. Damage to the vascular endothelium results in impaired release of nitric oxide–related, endothelial-derived relaxing factor and subsequent arterial vasoconstriction. In the pedicled flap model, PDT has been shown to decrease wound healing and lead to an increase in seroma formation and infectious complications. Belmont et al showed that in a rat free flap model, PDT caused decreased percentage of flap survival compared with control animals. The effects on flap survival of hemostatic agents such as Tisseel (Baxter, Deerfield, Ill) and FloSeal (Baxter) have also been studied. These agents could potentially form a barrier or incite inflammation that could interfere with flap revascularization. However, Jorgensen et al did not find an effect on flap survival in rat free flaps treated with these agents compared with controls.

Numerous studies of epithelial and solid tumors, including head and neck cancer, have found that COX-2 appears to be up-regulated during tumorigenesis. Evidence supports the notion that COX-2 promotes tumor-specific angiogenesis and induces proangiogenic factors such as vascular endothelial growth factor. Animal studies have shown that NSAIDs and COX-specific inhibitors have inhibitory effects on tumor growth and tumor
These effects have been well studied in the human colon as well as head and neck cancer. These drugs are therefore being studied as potential chemotherapeutic agents. A first step in determining the safety profile of a new drug in free flap surgery is to assess its potential effects on flap survival. Theoretically, COX-2 inhibitors should not affect the primary survival of a free tissue transfer. Our study was able to confirm this. Comparison of the 2 probit curves generated from the control and celecoxib groups (Figure 3) demonstrates that they were almost identical. The same can be said about the median lethal ischemic times of 9.6 and 9.7 hours. This data would demonstrate that COX-2 inhibitors have no deleterious effect on immediate free flap survival.

To our knowledge, no study has analyzed specifically the effects on flap revascularization caused by drugs that inhibit prostaglandin synthesis such as NSAIDS and COX-2 inhibitors. While the antiangiogenic properties of NSAIDS and COX-specific inhibitors are potentially useful in modifying tumor growth, these same properties may adversely affect flap revascularization and wound healing. Jones et al. showed that both NSAIDS and COX-specific inhibitors restrict angiogenesis through direct effects on endothelial cells. They challenged the notion that this would impair ulcer and wound healing. However, Blomme et al. found no difference in wound healing in full-thickness skin wounds in mice treated with either nonselective or selective COX inhibitors. Other studies have failed to find effects on either skin or mucosal healing in mice treated with COX inhibitors.

### Table 1. Numbers of Animals in Each Revascularization Group

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Postoperative Day Pedicle Severed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 5</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

*Data are given as No. of surviving flaps/total No. of flaps.

### Table 2. Number of Surviving Flaps for Each Ischemic Period*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Ischemic Period, h</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4/5</td>
<td>4/5</td>
<td>3/5</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>5/5</td>
<td>4/5</td>
<td>3/6</td>
<td>1/5</td>
<td></td>
</tr>
</tbody>
</table>

*Data are reported as mean ± SD percentages.

### Table 3. Flap Survival Percentages in Each of the Revascularization Groups*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Postoperative Day Pedicle Severed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 5</td>
</tr>
<tr>
<td>Control</td>
<td>29.4 ± 19.6</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>30.4 ± 19.6</td>
</tr>
</tbody>
</table>

*Data are reported as mean ± SD percentages.
Figure 5. Celecoxib vs control flap survival. This figure compares the percentage of flap survival between the celecoxib and the control groups for various time intervals.

While the present study shows that the percentage of flap survival does not change in rats treated with celecoxib, it does not specifically look at the degree of revascularization. A study to analyze vessel density with CD31 immunofluorescence could more accurately quantify the amount of angiogenesis, specifically vessel growth, into these rat flaps. This study did not specifically analyze the effects of nonspecific COX inhibitors such as NSAIDs. The small numbers in the present study also limit its ability to detect a small effect.

In conclusion, celecoxib did not demonstrate any short-term detrimental effect on flap survival in the rat fasciocutaneous free flap model. The intraoperative use of these agents for pain control or as a chemotherapeutic agent may not interfere with flap revascularization and survival, although more formal human studies would be required to definitively answer this question.

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REFERENCES