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Orgınal Article

Investigation of the Effects of Cyclooxygenase I and Cyclooxygenase II Inhibitors on Angiogenesis in a Random-Pattern Rat Abdominal Skin Flap

Random Bazlı Sıçan Karın Derisi Flebinde Siklooksijenaz 1 ve Siklooksijenaz 2 İnhibitörlerinin Anjiyogenez Üzerine Etkilerinin Araştırılması

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Abstract

Background: Cyclooxygenase (COX) inhibiting agents are already in use before, during and after flap surgery due to their analgesic and anti-inflammatory properties and they are known to improve flap viability. We have investigated the influence of COX1 and COX2 inhibitor drugs in the neoangiogenesis of random pattern flaps.

Methods: Forty-eight rats were divided into 4 groups and their lateral based inguinal flaps measuring 3x3 cm were used as the experimental flap models. Group 1 was the control group so no agents were administered. In groups 2, 3 and 4; Celecoxib (selective COX2 inhibitor), Metamizole Sodium (nonselective COX1 and COX2 inhibitor) and Paracetamol (selective to COX2 in the periphery) were administered, respectively. Clinical observations, radiological studies and histopathological analyses were undertaken in order to demonstrate and compare the individual agents' influence in flap viability and neoangiogenesis. **Results:**Groups 2 and 4 displayed lesser neoangiogenesis when compared with the control group by the end of the first week. The vessel intensities of groups 3 and 1 were not significantly different at the end of the first week; however, by the end of the third week, group 3 yielded a significantly better neoangiogenesis rate ($p<0,05$). Metamizole sodium is proven to significantly enhance vascularization by the end of 3 weeks.

Conclusion: This study has shown that Metamizole Sodium, a collectively inhibiting COX1 and COX2 significantly improves flap vascularity, and when postoperative analgesia is required following a flap surgery, it is a suitable drug of choice.

Keywords: Random pattern flap, Neoangiogenesis, Celecoxib, Paracetamol, Metamizole Sodium

ÖZ

Giriş: Siklooksijenaz (COX) inhibe edici ajanlar, analjezik ve antiinflamatuar özellikleri nedeniyle flep cerrahisi öncesinde, sırasında ve sonrasında sıkça kullanılmaktadır ve onların flep viabilitesini etkilediği bilinmektedir. Biz çalışmamızda random paternli flep beslenmesi üzerine COX-1 ve COX–2 inhibisyonu yapan ilaçların etkilerini araştırırdık.

Materyal ve Metot: 48 rat 4 gruba ayrıldı ve lateral tabanlı 3x3 cm boyutlarındaki inguinal flepler deneysel flep modeli olarak kullanıldı. Birinci deney grubu kontrol grubu olduğundan dolayı herhangi bir ilaç kullanılmadı. Sırasıyla 2. 3. ve 4. gruplarda Selekoksib (selektif COX–2 inhibitörü), Metamizol Sodyum (COX–1 ve COX–2 nonselektif inhibitörü), Parasetamol (periferal COX–2 selektif inhibitörü) uygulandı. Sonrasında bu belirtilen ajanların flep canlılığı ve neoanjiyogenez üzerindeki etkisini göstermek ve karşılaştırmak için klinik gözlemler, radyolojik çalışmalar ve histopatolojik analizler yapıldı.**Bulgular:** Grup 2 ve 4'te birinci haftanın sonunda kontrol grubuyla karşılaştırıldığında daha az neoanjiyogenez görüldü. Birinci haftanın sonunda grup 1 ve 3'ün damar yoğunlukları arasında anlamlı bir fark yoktu; ancak grup 3'te üçüncü haftanın sonunda neoanjiyogenez açısından anlamlı olarak fark görüldü. ($p<0,05$). Metamizol sodyumun 3 haftanın sonunda vaskülarizasyonu önemli ölçüde arttırdığı gözlendi. **Sonuç:**Bu çalışma COX1 ve COX2'yi birlikte inhibe eden Metamizol Sodyum'un flep vaskülaritesini önemli ölçüde iyileştirdiğini ve bir flep cerrahisini takiben postoperatif analjezi gerektiğinde, tercih edilebilir uygun bir ilaç olduğunu göstermiştir.

Anahtar Sözcükler: Random bazlı flep, Neoanjiogenez, Selekoksib, Parasetamol, Metamizol Sodyum

Highlights

- The objective of flap surgery is to close complex tissue defects with similar tissues in a functional and aesthetically pleasing manner and with minimal donor area deformity
- Analgesic and antiinflammatory drugs are frequently used during the perioperative period in patients undergoing flap repair.
- A common feature of most NSAIDs is that they inhibit cyclooxygenase (COX)-1 and COX-2 enzymes, which catalyze the formation of prostaglandins and some other eicosanoids from arachidonic acid in tissues.
- In this experimental study MS did not affect neoangiogenesis in the seventh day but significantly increased it in the twenty- first day.
- Non-selective NSAIDs should be preferred in patients who have undergone flap surgery, especially in delayed flap surgery, if there is no contraindication.

Introduction

Flaps are frequently used in plastic surgery in the reconstruction of tissue defects to achieve the most appropriate functional and aesthetic result. The objective of flap surgery is to close complex tissue defects with similar tissues in a functional and aesthetically pleasing manner and with minimal donor area deformity (1,2).

Flap surgery is constantly evolving, and new approaches are being introduced. So far, experimental studies have focused on the effects of delayed procedures, microsurgical methods of additional arterial and vein anastomoses, physical applications, anticoagulants, antiadrenergic drugs, sympathetic receptor blockers, vasodilating drugs acting directly on smooth muscles, agents that change the rheological properties of blood, and agents that increase ischemia tolerance on skin flap viability (3).

Analgesic and antiinflammatory drugs are frequently used during the perioperative period in patients undergoing flap repair. A common feature of most NSAIDs is that they inhibit cyclooxygenase (COX)-1 and COX-2 enzymes, which catalyze the formation of prostaglandins and some other eicosanoids from arachidonic acid in tissues (3-8).

In the present study, the selective COX-2 inhibitor celecoxib, metamizole sodium (MS), which non-selectively inhibits COX-1 and COX-2, and paracetamol, which is used non-selectively but is selective for COX-2 enzyme in the periphery, were used to investigate the effects on angiogenesis in the random-pattern flap model.

The effects of celecoxib, MS, and paracetamol on neoangiogenesis were compared based on clinical, radiological, and pathological findings in a random-pattern skin flap model. The aim of the study was to recommend or not recommend different drug treatments during flap surgery depending on the positive or negative effects of different antiinflammatory drugs on the random-pattern flap.

Materials and Methods

Approval for this study was obtained from the Animal Care and Ethics Committee of 19 Mayıs University (HADYEK-PYO.TIP.1904.12.009). A total of 48 adult female Sprague–Dawley rats weighing 200–250 grams were used. All the rats were kept in the same room and fed the same diet.

Anesthesia was induced in rats via intraperitoneal administration of Ketamine HCl 75 mg/kg and xylazine 0.2 mL/kg. The animals were placed in the supine position, and their abdominal wall hair was shaved. Flap dimensions were drawn with a skin pen. A 3 x 3 cm rectangular flap was designed

in the left inguinal region of the rat with a lateral base distally parallel to the midline and top and bottom borders 90 degrees to this line **(Figure 1)**. After drawing the flap, the surgical area was stained with povidone–iodine solution. The surgical procedure was started after proper surgical dressing was done. Superficial epigastric artery and the descending branches of lateral thoracic artery were cut to make the flap random-based.

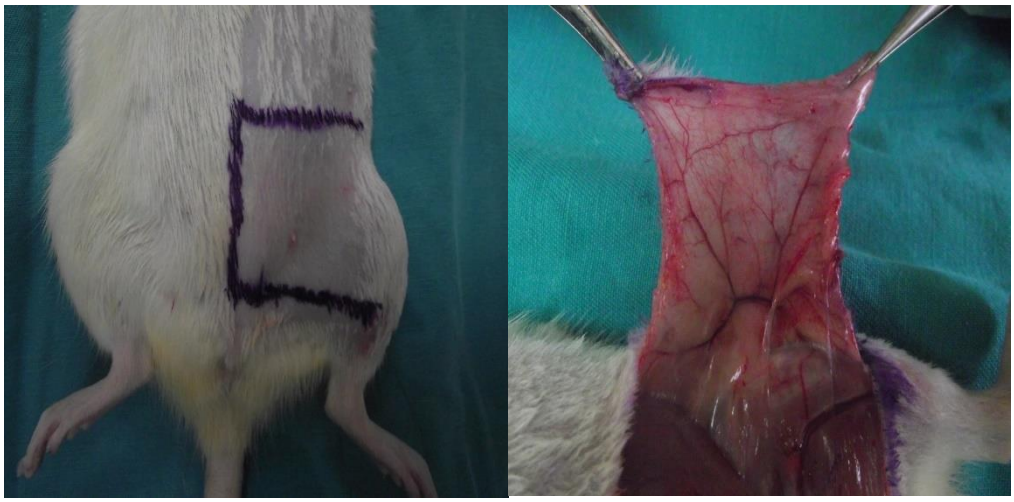


Figure 1. **A)** Photo of flap design. **B)** Photo of flap dissection

For 7 and 21 days, orogastric gavage was used to administer 50 mg/kg/day of celecoxib in distilled water, 100 mg/kg/day of MS in distilled water, and 200 mg/kg/day of paracetamol dimethyl sulfoxide solution (9-11). The rats were divided into four main groups. Each group was further divided into two (7 and 21 days).

Group 1a (Control Group): A random-pattern abdominal flap was performed without the use of any medical agents. Rats were monitored for 7 days.

Group 1b (Control Group): A random-pattern abdominal flap was performed without the use of any medical agents.Rats were monitored for 21 days.

Group 2a (Celecoxib Group): Starting 1 day before the operation, a single daily dose of 50 mg/kg of celecoxib was given in distilled water for 7 days, followed by the application of a random-pattern abdominal skin flap.

Group 2b (Celecoxib Group): Starting 1 day before the operation, a single daily dose of 50 mg/kg of celecoxib was administered for 21 days, followed by the application of a random-pattern abdominal skin flap.

Group 3a (MS Group): Starting 1 day before the operation, a single daily dose of 100 mg/kg of MS was administered for 7 days, followed by the application of a random-pattern abdominal skin flap.

Group 3b (MS Group): Starting 1 day before the operation, a single daily dose of 100 mg/kg of MS was administered for 21 days, followed by the application of a random-pattern abdominal skin flap.

Group 4a (Paracetamol Group): Starting 1 day before the operation, a single daily dose of 200 mg/kg of paracetamol was administered for 7 days, followed by the application of a random-pattern abdominal skin flap.

Group 4b (Paracetamol Group): Starting 1 day before the operation, a single daily dose of 200 mg/kg of paracetamol was administered for 21 days, followed by the application of a random-pattern abdominal skin flap.

On the 7th and 21st days after surgery, 1 x 1 cm skin specimens were taken from the living part of the flaps of three rats from each group.

The samples were fixed in 10% buffered formalin solution, trimmed after 24 hours, and post-fixed. Samples were evaluated under a Nikon Eclipse E600W light microscope, and microscopic photographs were taken using a Nikon DS Camera Head DS-5M. Hematoxylin-Eosin was used for histopathological evaluation. Immunohistochemical staining was done with CD31.

For angiographic evaluation, the vascular bed was washed with heparinized SF, and a solution containing 75–100 mL of contrast material (lead oxide and gelatin) was given for 3–5 minutes.

It was observed that the solution containing contrast material outflowed from the raised flap edges and the foot incision. Then, the flap pedicle was cut, placed on moist gauze, and wrapped to avoid folding. The samples were then kept in a refrigerator at +4°C for 24 hours. X-rays were taken on a mammography device (Mammo Diagnost UC, Philips, Germany) from a distance of 66 cm under 24 kV and 8 mAs.

The vessel density in the tissues was measured using the “VesSeg Tool” vessel segmentation software program **(Figure 2)**.

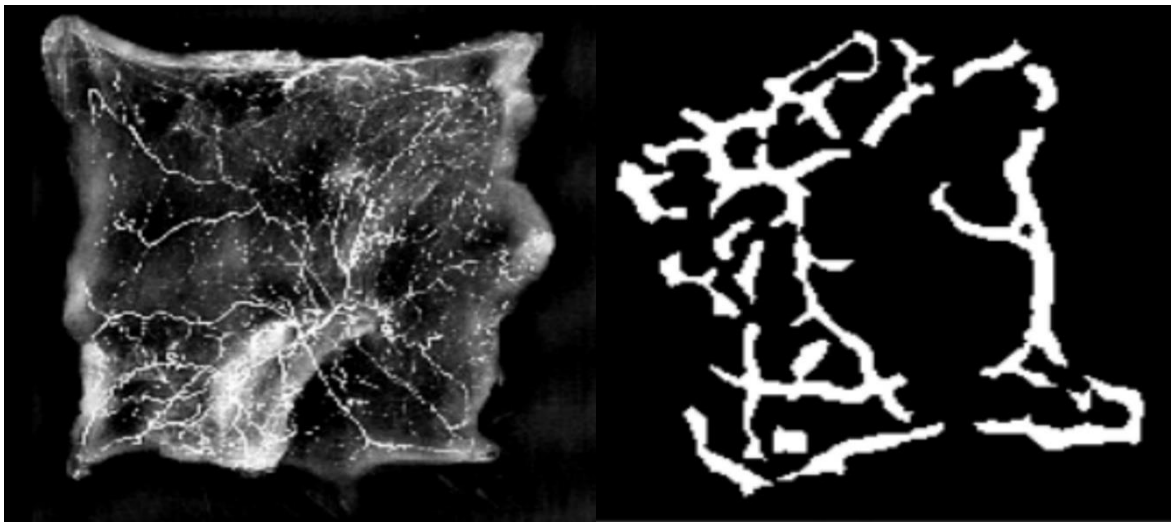


Figure 2. **A)** Photo of microangiography image of the flap of the control group. **B)** Photo of condensed area for pixel analysis

Stastical Analysis

Descriptive statistical analysis of the measurement values was performed using the "VesSeg Tool" vessel segmentation software program. To evaluate the differences between the groups, a factorial analysis of variance organized according to time was performed. The Student's t test was used to compare the effects of drugs over time. Analyses were conducted using the "SAS" statistical package program.

Results

In rats treated with celecoxib, wound dehiscence was more severe after surgery, and when specimens were taken for pathological evaluation after 21 days, excessive seroma accumulation was observed under the flap. Wound healing was rapid and uneventful in all rats treated with metamizole **(Figure 3).**

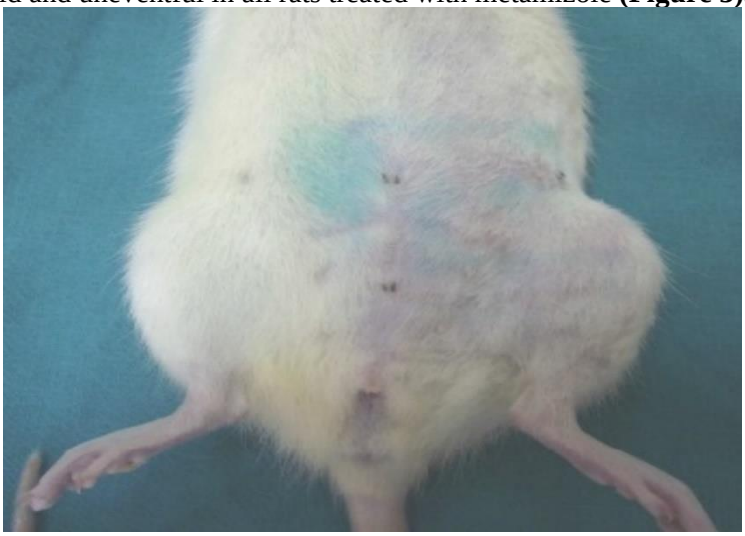
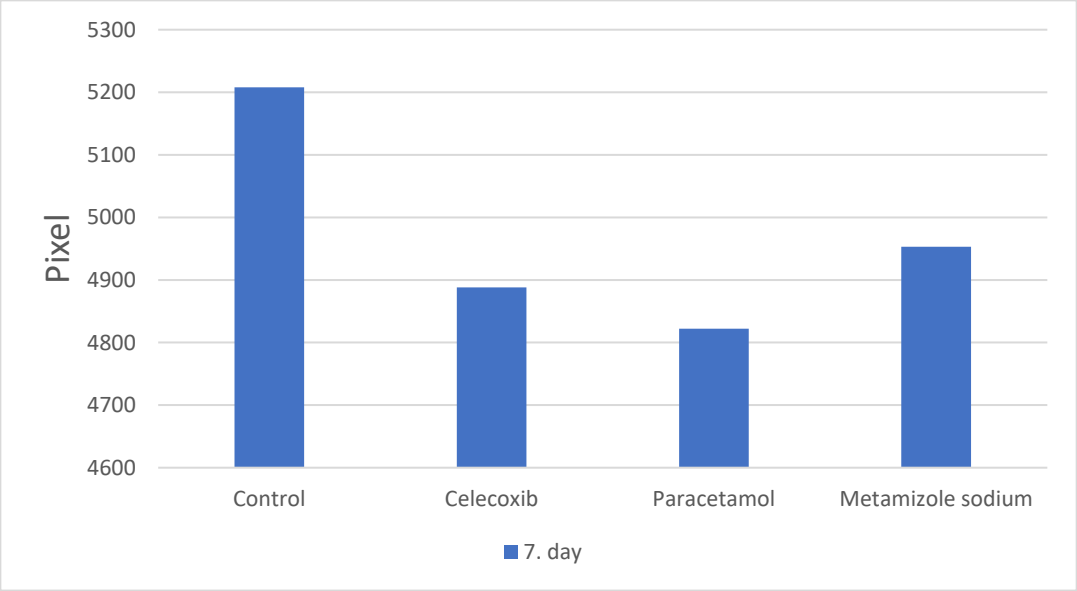


Figure 3. Photo of wound healing in a rat belonging to the Metamizole Sodium group at the end of the 21st day

Comparisons of total area values between groups on day 7:

When the groups were evaluated separately for neoangiogenesis after 7 days of follow-up, no statistically significant difference was found between the MS and control groups ($p > 0.05$, **Table 1**).

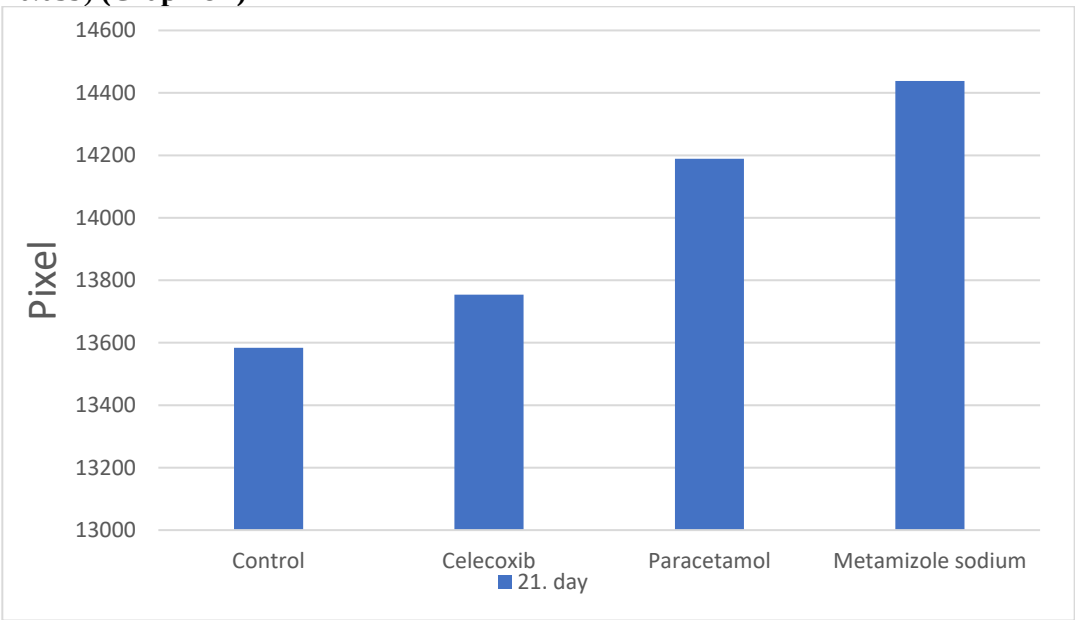
Although not statistically significant, the total vessel area was lower in the celecoxib and paracetamol groups than in the control group ($p > 0.05$, **Graphic 1**).



Graphic 1. Angiogenesis densities between groups on day 7

Comparisons of total area values between groups on day 21:

When the groups were evaluated separately for neoangiogenesis after 21 days of follow-up, a statistically significant difference was found, and vessel formation was increased in the MS group compared to the control group (p = 0.039) **(Graphic 2)**



Graphic 2. Angiogenesis densities between groups on day 21

Table 1. Angiogenesis densities in the experimental groups

Groups	**7th day**				
	Mean Area	Standard Deviation	Min/Max	C.V %	P
Control	5208	75.17	5124-5358	2.5	>0,05
Celocoxib	4888	176.168	4539-50101	6.24	>0,05
Metamizole Sodium	4953	25.67	4902-4980	0.89	>0,05
Paracetomole	4822	102.8	4628-4978	3.69	>0,05
	21st day				

Control	13584	360.14	13158-14300	4.59	>0,05
Celocoxib	13754	566.77	12621-14351	7.13	>0,05
Metamizole Sodium	14438	184.23	14078-14686	2.21	0.039
Paracetomole	14189	210.69	13787-14499	2.57	>0,05

Px: pixel c.v: coefficient of variation

Although not statistically significant, the total vessel area was higher in the paracetamol and celecoxib groups than in the control group ($p > 0.05$) **(Table 1).**

Sections taken on the 7th and 21st days after surgery were evaluated histopathologically in terms of neoangiogenesis and inflammatory cell density.

Inflammatory cell density was scored as 1–10, 11–100, 101–1000, and >1000, and the groups were compared accordingly. Neoangiogenesis was scored as 0–20, 20–40, 40–60, and >60. The "hotspot" method described by Weidner was used for neovascularization (12).

When the groups were compared in terms of neoangiogenesis, it was found that MS did not increase neoangiogenesis compared to the control group at the end of the 1st week, whereas a significant increase was observed at the end of the 3rd week compared to the control group.

On the contrary, celecoxib and paracetamol decreased neoangiogenesis compared to the control group at the end of the 1st week, whereas a slight increase in angiogenesis was observed at the end of the 3rd week compared to the control group (**Graphic 1,2**) **(Figure 4)**

No difference was found between the groups in terms of inflammatory cell density at the end of the 1st week. At the end of the 3rd week, inflammatory cell density was significantly reduced in all groups.

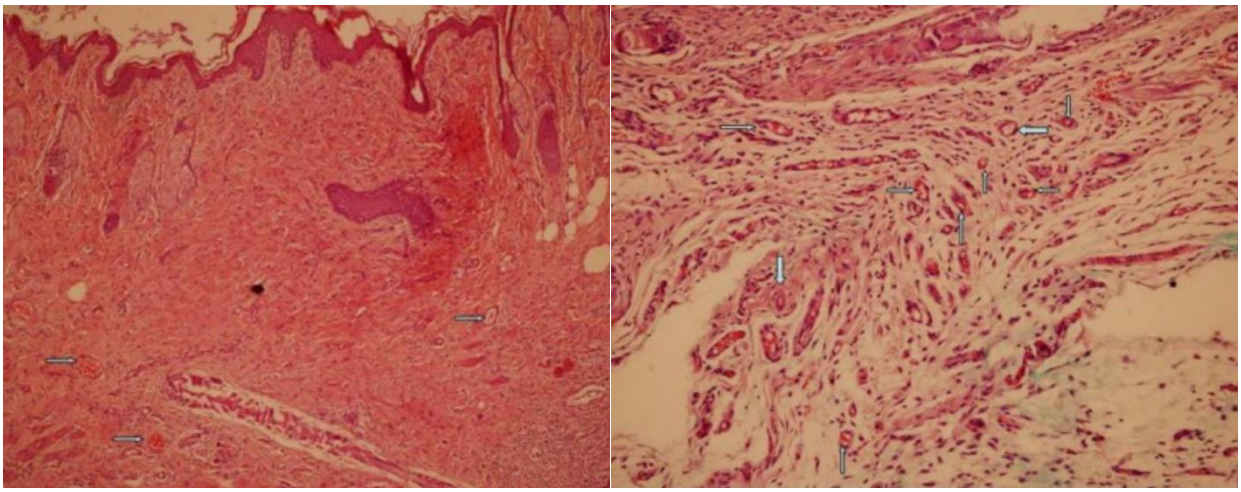


Figure 4. A) Photo of histopathological appearance of a flap in the control group after one week.
B) Photo of histopathological appearance of a flap belonging to metamizole sodium group after 21 days

Discussion

Flap surgery is constantly developing, and new approaches are being introduced. The evidence on the effects of COX-1 and COX-2 inhibitors on flap survival is increasing, but studies investigating the effects on angiogenesis are limited (4).

Repeated interventions may be required when flap necrosis occurs. This increases morbidity, length of hospital stay, and medical costs. Certain surgical and medical applications have been used for a long time to prevent these problems and improve flap success (3).

Most of the medical applications to increase skin flap viability remained in the experimental stage and were not used in routine clinical practice. Random-pattern skin flaps have been generally used in the literature to investigate the effects of medical agents on flap viability. Random-pattern flaps have no known axial vessels and are fed predominantly from the subdermal plexus (3).

The random flap model was used in this experimental study. Using this flap model, the effects of celecoxib, MS, and paracetamol on flap viability and neoangiogenesis were compared based on clinical, radiological, and pathological findings. This will allow us to determine the positive or negative effects of different antiinflammatory drugs on the random flap and to recommend or not recommend different drug treatments.

The mechanism of action of celecoxib depends on the inhibition of prostaglandin synthesis via the inhibition of the COX-2 enzyme. Celecoxib belongs to the group of selective COX-2 inhibitors. In the present study, celecoxib was used with the hypothesis that it would decrease flap viability by reducing neoangiogenesis as a result of inhibiting the COX-2 enzyme in the periphery of random-pattern flaps. When COX-2 is stimulated, the production of epidermal growth factor, vascular endothelial growth factor, and fibroblast growth factor increases (13). Studies on NSAIDs and COX-specific inhibitors showed inhibition of angiogenesis in epithelial tumors (14-16). In another study, Ballerini et al. reported that selective COX-2 inhibitors reduced vascularity, especially in cancerous tissues (17). Mark et al. investigated the effects of COX-2 inhibitors on viability and revascularization of the fasciocutaneous flap. No adverse effects were found on ischemia and flap viability, and it was reported that COX-2 inhibitors had the same effect on flap revascularization as the control group and did not cause flap loss (4). In the present study, celecoxib decreased neoangiogenesis to some extent at the end of the 1st week, but neoangiogenesis was increased at the end of the 3rd week compared to the control group. While reduced angiogenesis in the 7th day is consistent with the literature, it is increased rather than decreased by celecoxib in the 21st day. It can be considered as an option in flap surgery.

Paracetamol causes weaker COX-1 and COX-2 enzyme inhibition than other NSAIDs (16,18). There is no study in the literature investigating the effects of paracetamol on flap viability or revascularization. The difference between paracetamol and celecoxib is that paracetamol only inhibits COX-2 in the periphery. In the present study, paracetamol's effects on neoangiogenesis by inhibiting the COX-2 enzyme in the periphery of random-pattern flaps were the same as those of celecoxib. Similarly to celecoxib, it reduced neovascularization in the 7th day and increased it in the 21st day compared to the control group. Paracetamol, which is used safely in pregnant women, children, and adults, may be an alternative drug for patients undergoing flap surgery.

MS, used in the present study, inhibits the COX enzyme and exerts analgesic, antipyretic, antiinflammatory, and antispasmodic effects. Even though metamizole sodium is commonly used in the clinical aplications, there is no clinical study in the literature about its effect of randomized flap viability. Metamizole sodium is frequently used clinically, although it can cause kidney problems such as fluid and electrolyte disorders, acute renal failure, nephrotic syndrome, tubular necrosis and interstitial nephritis. It has been shown that significant histopathological changes occur in distant organs due to oxidative stress in lower extremity ischemia-reperfusion (19). Side effects of metamizole sodium should be considered in patients undergoing lower extremity surgery. Its analgesic effect is stronger than acetylsalicylic acid. Although the cyclooxygenase inhibitor and antiinflammatory effects of MS are low, it has strong pain-relieving properties (8). MS was used in the present study with the hypothesis that it would increase neoangiogenesis by inhibiting both COX-1 and COX-2 enzymes in random-pattern flaps.

Daniel RK et al. reported that ibuprofen, a non-selective NSAID, increased flap viability in rat skin (3). For flap viability to increase, neoangiogenesis should also increase. The results obtained in the present study showed that MS had no positive or negative effect on neoangiogenesis after the 1st week. However, it significantly increased neoangiogenesis at the end of the 3rd week. The results showed that MS has similar effects to other non-selective COX-inhibiting NSAIDs in terms of neoangiogenesis. These findings are consistent with the literature. Given that MS increases angiogenesis, non-selective antiinflammatory drugs can contribute positively to flap viability by increasing neangiogenesis, especially in delayed flaps. If antiinflammatory drugs are used in patients with flaps, drugs that inhibit COX-1 and COX-2 non-selectively are preferable.

In the present study, all groups had the same inflammatory cell density values as the control group during the acute period. After 21 days, however, all groups had significantly reduced inflammatory cell density.

Conclusion

In this experimental study MS did not affect neoangiogenesis in the seventh day but significantly increased it in the twenty- first day. Non-selective NSAIDs should be preferred in patients who have undergone flap surgery, especially in delayed flap surgery, if there is no contraindication. Effective use of medication can improve flap vitality and help reduce losses and failures after surgery.

Ethical Approval: *Permission was obtained Animal Care and Ethics Committee of 19 Mayıs University (HADYEK-PYO.TIP.1904.12.009).*

Author Contributions: *Concept: AS, TŞ. Literature Review: AS, TŞ. Design: AS, TŞ.. Data acquisition: AS. Analysis and interpretation: AS, TŞ. Writing manuscript: AS. Critical revision of manuscript: TŞ.*

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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