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Effect of postoperative doxorubicin administration on ischemic wound healing

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ABSTRACT

Some patients undergo postoperative chemotherapy despite showing impaired wound healing after a major surgery. We speculated that postoperative chemotherapy further delays wound healing in these patients. This study aimed to compare the effects of doxorubicin (DXR) in ischemic skin flap and normal incisional wound models after surgery. A 2-cm incisional wound was made in group 1 rats, and saline was injected intravenously, following surgery on the same day. Incisional wound was made in group 2–5 rats, and 8 mg/kg DXR was injected intravenously, following surgery on the same day and after 7, 14, and 21 days respectively. H-shaped double flaps were made in group 6 rats, and saline was injected intravenously, following surgery on the same day. Flaps were made in group 7–10 rats, and 8 mg/kg DXR was injected intravenously, following surgery on the same day and after 7, 14, and 21 days respectively. On days 7, 14, 21, and 28 after surgery, the suture wounds were removed, tensile wound strengths were measured, and tissue samples were collected for histopathological evaluation. The tensile strength was significantly lower in the DXR-treated groups than in the control groups for both ischemic skin flaps and incision wounds. Additionally, the cross effect between DXR and ischemia was not significant. On pathological examination, DXR showed atrophic skin changes and degeneration of skin appendages on days 14–21 after the surgery in both the models. DXR decreased the wound tensile strength and caused an atrophic change in the ischemic wound.

Keywords: doxorubicin, postoperative chemotherapy, wound tensile strength, normal incision wound, ischemic skin flap

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INTRODUCTION

Recently, cancer treatments have been well developed under a multidisciplinary approach.[1] Therefore, strict treatment schedules are often developed systematically. For instance, postoperative adjuvant chemotherapy is usually performed 3–4 weeks after surgery in malignant limb tumors.[2] However, we incidentally encountered disorders associated with wound healing, such as skin flap ischemia, after surgery. The time for initiating adjuvant chemotherapy in such complicated cases remains debatable. Clinically, adjuvant chemotherapy is often administered according to the usual protocol. However, there are few clinical reports on the optimal timing of chemotherapy administration in complicated cases. In addition, few experimental studies have examined the effects of chemotherapeutic drugs on impaired wound healing.

Doxorubicin (DXR), a typical chemotherapeutic agent, is generally used in breast cancer and malignant limb tumors. DXR has an inhibitory effect on normal wound healing.[3,4] However, the effect of DXR on ischemic wound healing is unclear. Hence, this study was conducted to investigate the effect of DXR on an ischemic skin flap model compared with normal incisional wound after surgery.

MATERIALS AND METHODS

Animals

Animals received human care in compliance with the Nagoya University Guidelines for Animal Care and Use, which are based on the U.S. National Research Council's criteria outlined in the Guide for the Care and Use of Laboratory Animals. In total, 84 8-week-old male Sprague-Dawley rats weighing 230–280 g were purchased from Chubu-Kagaku-Shizai (Nagoya, Japan). They were housed in a cage in groups of three in an air-conditioned room at a temperature of 23°C and humidity of 60%, with 12:12-h light/dark cycle. The rats were given standard laboratory rat food and running water ad libitum, except during fasting periods before and after the surgery.

Animal preparations

The rats were anesthetized using 2% isoflurane inhalation before the procedures and were restrained in the supine (prone) position. Their dorsal hair was shaved, and the surgical area was sterilized with 70% ethanol. Normal incisional wound (experiment 1) and H-shaped double flap [ischemic skin flap (experiment 2)] were used as wound models.

Experiment 1: Incisional wound model

A 2-cm linear, full-thickness incision was made in the median plane, beginning 1 cm below the inferior edge of scapula, through the skin and panniculus carnosus (Fig. 1A). The wound was sutured with 4-0 nylon sutures in an interrupted fashion. In group 1, a 2-cm normal incisional wound was made, and saline was injected intravenously, following surgery on the same day. In groups 2–5, an incisional wound was made, and 8 mg/kg DXR[5] was injected intravenously, following surgery on the same day and after 7, 14, and 21 days respectively (Fig. 2A).

Experiment 2: H-shaped double flap (ischemic skin flap)

A rat back ischemic wound model was established according to the design from Quirinia.[6,7] An H-shaped double flap, consisting of cranially and caudally based flaps (width, 2 cm; length, 4 cm) was marked with ink. The skin and panniculus carnosus were incised. After the flaps were raised, perforating branches of the flaps were cut, and the flaps were sutured back with 4-0 nylon

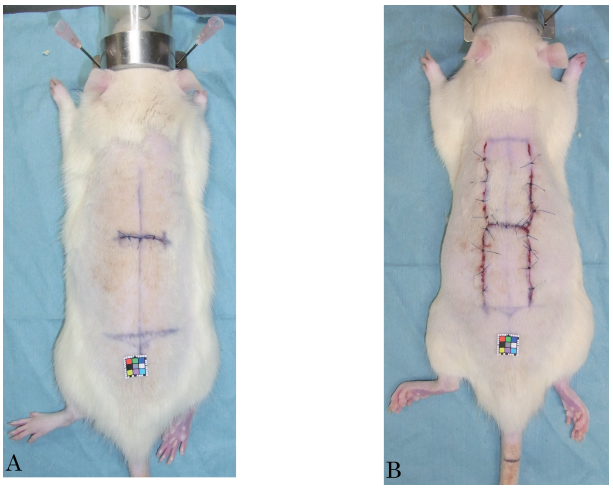

Fig. 1 Photograph of the incisional wound and ischemic skin flap model
Fig. 1A: An incisional wound model.
Fig. 1B: An ischemic skin flap model. Color reference maker: CasMatch.

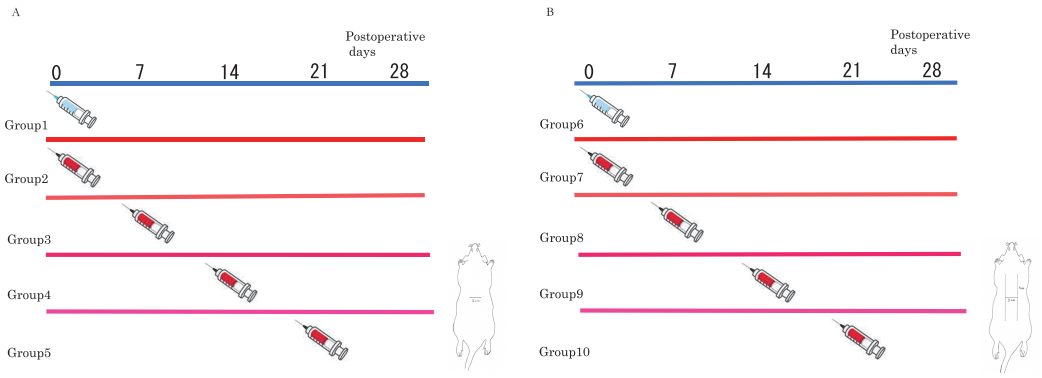

Fig. 2 Timeline of surgeries and injections
A: Experiment 1, B: Experiment 2. S: Sampling. Blue injector: Saline injection, Red injector: DXR injection.

sutures in an interrupted fashion (Fig. 1B). In group 6, H-shaped double flap was made, and saline was injected intravenously, following surgery on the same day. In groups 7–10, H-shaped double flaps were made, and 8 mg/kg DXR was injected intravenously, following surgery on the same day and after 7, 14, and 21 days respectively (Fig. 2B).

Laser Doppler perfusion imaging

Skin perfusion was evaluated using laser Doppler perfusion imaging. Skin perfusion was evaluated at the center of the flap using Omegazone OZ-2 (Omegawave, Tokyo, Japan). Measurements were obtained for the same regions on the day of surgery (baseline) and at 0, 7, and 13 days after the surgery. The tissue was penetrated with a laser beam (780 nm), and part of the incident light was scattered due to moving red blood cells in the vessels, which was detected

and processed to obtain computerized color-coded photographs.[8)]

Tensile strength test

Three rats belonging to each group were euthanized with carbon dioxide gas. Tissue samples were collected on days 7, 14, 21, and 28 after surgery, and the sutures were removed. The breaking strength of the repaired wound was tested using a skin tension meter (AGS-X, Shimadzu, Kyoto, Japan). The skin sample was vertically placed between two clamps of the tension meter. Next, the skin strips were stretched at a constant rate (1 mm/s) until disruption occurred. Breaking strength was defined as the force required to completely disrupt the wound.[9,10)]

Histopathological evaluation

Sample tissues were excised, fixed in 10% formaldehyde in phosphate buffered saline (PBS), and embedded in paraffin. The paraffin-embedded samples were sectioned into 5-μm slices, which were stained with hematoxylin and eosin and Elastica-Masson for histopathological examination under an Olympus BX50 stereomicroscope (Olympus, Tokyo, Japan).

Statistical analysis

The mean tensile strengths and standard errors of the mean of all the specimens from each group on each day were calculated. The tensile strength of each group was statistically compared with that of the control group (groups 1 and 6) using the Welch test, and the significance level was set to 0.05. Furthermore, we assumed that tensile strength linearly elevated with time after surgery, and that ischemia and DXR administration decreased the rate of its elevation. Because DXR was administrated on different days, the time-course data of tensile strength were fitted with a two-segmented linear regression model in which the slope parameters discontinuously changed at the beginning of DXR administration. This analysis was performed using R 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was determined using two-sided analysis, and the significance level was set at 0.05.

RESULTS

Suture dehiscence was not observed in any group. Rats receiving DXR experienced a transient loss of approximately 10% of their body weight, whereas control rats regained weight from day 0. Moreover , weight gain in rats receiving DXR was lesser than that in the control group. Laser Doppler images of the entire flap demonstrated lack of blood supply with marked ischemia at the edge of the head side.

Wound tensile strengths of experiments 1 and 2 are summarized in Table 1 and 2, respectively. Tensile strength in all the groups gradually increased on postoperative days 7–28. The wound tensile strengths in groups 2 and 3 tended to be significantly lower than in group 1 on days 14–28 after wounding. Moreover, the wound tensile strength in group 7 tended to be significantly lower than in group 6 on days 7–21 after the surgery.

In linear regression analysis for experiment 1, DXR significantly decelerated the restoration speed of tensile strength, and slope parameters of linear regression, in groups 2 and 3 (Table 3). Linear regression analysis for experiment 2 showed that DXR significantly decelerated the restoration speed of tensile strength when DXR administration was delayed in groups 7 and 8 (Table 4). The results of linear regression analysis of all the cases are presented in Table 5. Both DXR and ischemia significantly decelerated the restoration speed of tensile strength compared with normal incisional wound. Cross effect between DXR and ischemia was not significant;

however, the additive effect was significant. Interestingly, the effect of ischemia was greater than that of DXR.

On pathological examination, wounds in group 1 were well adhered and re-epithelialized on day 7 after the surgery (Fig. 3A). The width of the connection was greater on day 14 than on day 7 (Fig. 3B). Collagen bundles in the connection thickened on days 21 and 28 after the operation, whereas the number of cells in the connection decreased (Fig. 3C, D). DXR resulted in thinning of samples, atrophic skin changes, and degeneration of skin appendages after 14 of surgery in groups 2 and 3. The width of connection in groups 2 and 3 was lesser than that in group 1 on day 14 after the surgery, whereas the cellularity in the connection in groups 2 and 3 was lower than in group 1 (Fig. 4). There was no necrosis or skin ulcer after 28 days of the surgery in any group.

In ischemic skin flaps, the edges at both the sides, especially the head sides, were pale, with insufficient blood supply on flap elevation. On day 7 after the surgery, the edge of the head side in group 6 could be easily separated (Fig. 5A). Partial skin necrosis occurred and was partially recovered at the edge of the head side on day 14 after the surgery (Fig. 5B); the edge of the head side was regenerated after 14 days of surgery (Fig. 5B, C, D). Re-epithelialization also occurred within 21 days after partial skin necrosis (Fig. 5B, C).

The edge of the head side was well regenerated, and there were two separate zones on day 21 after the surgery in group 6 (Fig. 6A); one was a light blue zone, which recovered from necrosis and was constructed with immature collagen fibers, and the other was a strong blue zone, which survived an ischemic condition and was constructed with mature collagen fibers. DXR caused severe atrophic skin changes and wide degeneration of skin appendages on day 21 after the surgery in groups 7 and 8 (Fig. 6B, C). Although re-epithelialization occurred within 21 days in groups 7–9, epidermal thickness was observed in groups 7–9 (Fig. 6B, C, D).

Table 1 Wound tensile strength for Experiment 1

Postoperative day	Day 7 (N)	Day 14 (N)	Day 21 (N)	Day 28 (N)
Group 1	3.4 ± 0.2	16.0 ± 2.2	21.5 ± 1.3	25.1 ± 1.0
Group 2	3.3 ± 0.0	8.1 ± 2.3*	15.7 ± 3.5	22.1 ± 0.4*
Group 3		15.3 ± 2.9	19.7 ± 1.2	16.9 ± 1.1*
Group 4			20.9 ± 0.8	23.1 ± 0.6
Group 5				25.9 ± 4.3

N: Newton, mean ± SEM
*P < 0.05

Table 2 Wound tensile strength for Experiment 2

Postoperative day	Day 7 (N)	Day 14 (N)	Day 21 (N)	Day 28 (N)
Group 6	3.2 ± 0.9	4.7 ± 2.2	11.4 ± 2.4	15.8 ± 2.5
Group 7	0.9 ± 0.8*	3.0 ± 1.8	5.4 ± 0.4*	10.1 ± 0.1
Group 8		5.3 ± 1.7	9.6 ± 2.6	11.4 ± 0.5
Group 9			11.9 ± 0.3	12.4 ± 3.2
Group 10				13.9 ± 1.6

N: Newton, mean±SEM
*P < 0.05

Table 3 Results of linear regression analysis for Experiment 1

	Units	Estimate (95%CI)	P-values	Adjusted R^2
Restoration speed of tensile strength in Group 1	N/day	0.991 (0.823, 1.160)	$1.22 \times 10^{-13*}$	0.819
Changes in restoration speed (Caused by DXR)				
Group 2	N/day	−0.244 (−0.374, −0.114)	$7.71 \times 10^{-4*}$	
Group 3	N/day	−0.326 (−0.516, −0.346)	0.002*	
Group 4	N/day	−0.245 (−0.555, 0.065)	0.129	
Group 5	N/day	−0.236 (−0.881, 0.408)	0.447	
(intercept)	N	−0.219 (−3.084, 2.646)	0.882	

DXR: Doxorubicin, N:Newton, CI: confidence interval
*P < 0.05

Table 4 Results of linear regression analysis for Experiment 2.

	Units	Estimate (95%CI)	P-values	Adjusted R^2
Restoration speed of tensile strength in Group 6	N/day	0.635 (0.538, 0.733)	$5.94 \times 10^{-15*}$	0.853
Changes in restoration speed (Caused by DXR)				
Group 7	N/day	−0.230 (−0.305, −0.155)	$6.78 \times 10^{-7*}$	
Group 8	N/day	−0.177 (−0.286, −0.067)	0.003*	
Group 9	N/day	−0.168 (−0.347, 0.011)	0.075	
Group 10	N/day	−0.246 (−0.619, 0.126)	0.203	
(intercept)	N	−2.162 (−3.818, −0.507)	0.015*	

DXR: Doxorubicin, N: Newton, CI: Confidence interval.
*P < 0.05

Table 5 Results of linear regression analysis for all cases beyond groups.

	Units	Estimate (95%CI)	P-values	Adjusted R^2
Restoration speed of tensile strength in Group1	N/day	1.033 (0.942, 1.124)	$<2 \times 10^{-16*}$	0.887
Changes in restoration speed				
Caused by DXR	N/day	−0.263 (−0.166, −0.360)	$1.04 \times 10^{-6*}$	
Caused by ischemia	N/day	−0.431 (−0.516, −0.346)	$1.71 \times 10^{-15*}$	
Cross effect of DXR and ischemia	N/day	0.044 (−0.094, 0.182)	0532	
(intercept)	N/day	−1.226 (−2.768, 0.317)	0.125	

DXR: Doxorubicin, N: Newton, CI: Confidence interval.
*P < 0.05

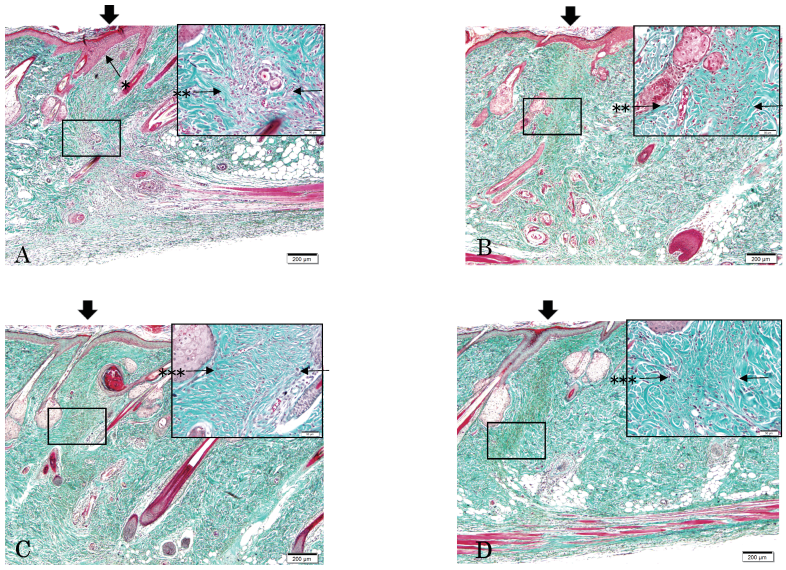

Fig. 3 Elastica-Masson staining for group 1
On day 7 after the operation, the wound was well adhered and re-epithelialized (*).On day 14 after the operation, the connection width was wider than that on day 7 (**).Collagen bundles in the connection thickened on days 21 and 28 after the surgery (***). A: at 7 days after the surgery, B: at 14 days, C: at 21 days, D: at 28 days. Point of adhesion (arrowhead).

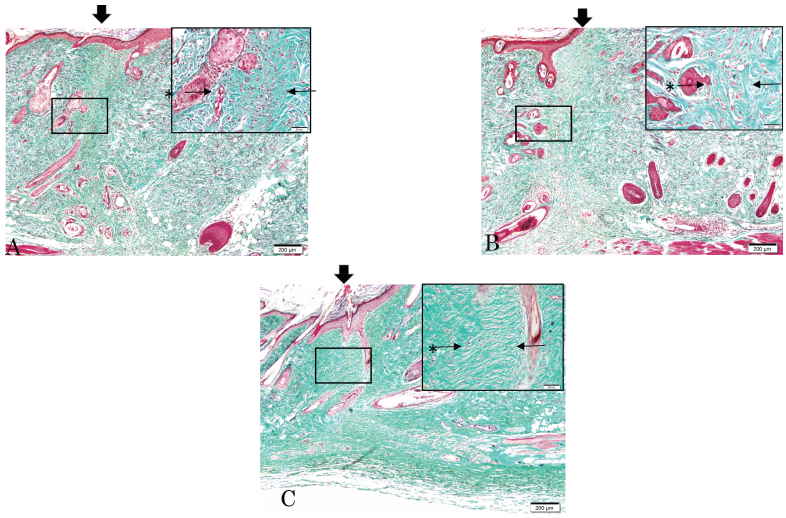

Fig. 4 Elastica-Masson staining on day 14 after the surgery
The connection widths in groups 2 and 3 were narrower than in group 1 on day 14 (*), whereas the cellularity in the connections in groups 2 and 3 were lower than in group 1 (*). A: group 1, B: group 2, C: group 3. Point of adhesion (arrowhead).

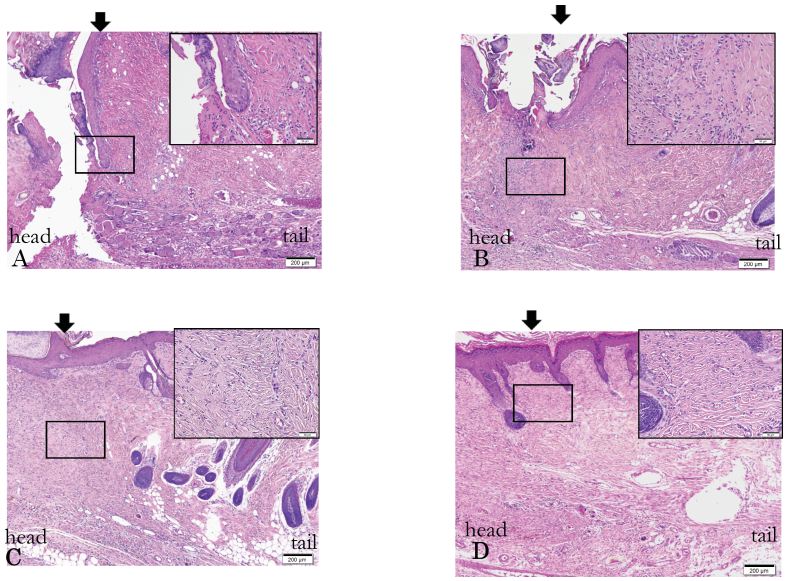

Fig. 5 Hematoxylin and eosin staining of group 6
On day 7 after the surgery, the edge of the head side was easily separated. A: The edge of the head side was regenerated on day 14 after the surgery. B, C, D: Re-epithelialization also occurred within 21 days after partial skin necrosis (B, C). A: At 7 days after the surgery, B: at 14 days, C: at 21 days, D: at 28 days. Point of adhesion (arrowhead).

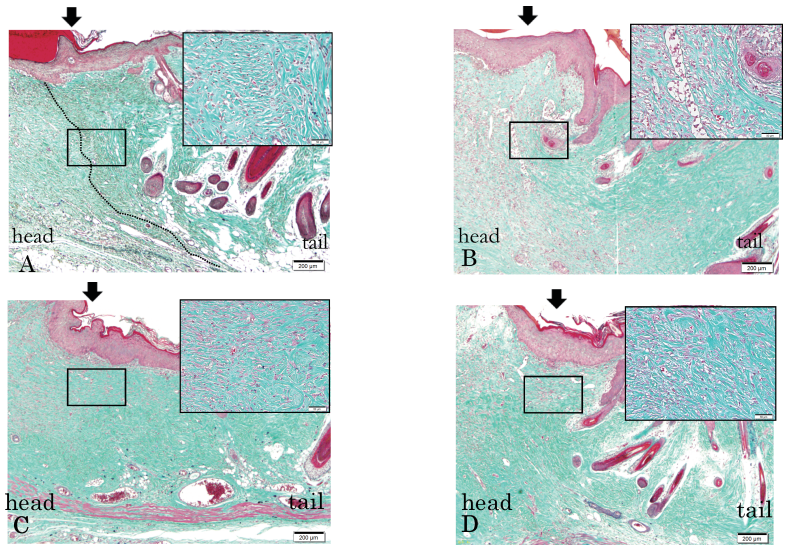

Fig. 6 Elastica-Masson staining on day 21 after the surgery
The edge of the head side was well regenerated, and there were two separate zones(dotted line) on day 21 after the surgery in group 6 A, B, C, D: Severe atrophic skin changes and wide degeneration of skin appendages were observed on day 21 after the surgery in groups 7 and 8. B, C: Re-epithelialization occurred and epidermal thickness was still observed (B, C, D). A: group 6, B: group 7, C: group 8, D: group 9. Point of adhesion (arrowhead). The dotted line shows the boundary between two separate zones.

DISCUSSION

DXR is a broad-spectrum anthracycline group chemotherapeutic agent that is widely used in cancer treatment.[4,11,12] It is often used in combination with other chemotherapeutic agents. However, DXR sometimes impairs wound healing.[3,4] De Cunzo *et al.*[13] reported that DXR may decrease wound tension, which is the primary outcome parameter for suture healing. Devereux *et al.*[14] reported a decrease in collagen fiber diameter with impaired maturation and lower hydroxyproline levels on days 14 and 21 days after DXR treatment in rats. We examined the effect of postoperative DXR administration on an incisional wound by assessing the mechanical (wound tensile strength) and histological parameters. DXR significantly decelerated the restoration rate of tensile strength within 7 days after the surgery when the administration was delayed. DXR resulted in thinning of samples, atrophic skin changes, and degeneration of skin appendage on day 14 after the surgery. Our findings supported the fact that DXR has an inhibitory effect on normal wound healing in an incisional model, similar to the findings of previous studies.[13,14]

Skin ischemia is a serious clinical problem in skin flap surgery, which is routinely used for wound coverage to prevent infection and restore the form and function of the skin.[8] Additionally, we need a replicable ischemia model to investigate the problems associated with ischemic wounds. A few studies have analyzed wound tension using an ischemic model. Using this model, we examined the effect of postoperative DXR administration on ischemic wound healing by assessing the mechanical and histological parameters. DXR significantly decelerated the restoration rate of tensile strength within 7 days after the surgery if its administration was delayed. DXR resulted in severe atrophic skin changes and wide degeneration of skin appendages on day 21 after the surgery. We found that DXR had a delayed effect on ischemic wound healing as well as on the incisional model.

Interestingly, our findings indicated that the cross effect between DXR and ischemia was not significant, although an additive effect of DXR and ischemia was significant. DXR and ischemia may share a common mechanism, which includes inhibition of fibroblast cell growth, reduction of collagen organization, and remedy by antioxidants, for exhibiting their effects. DXR may impair wound healing by direct inhibition of mitosis in local fibroblasts and keratinocytes or myelosuppression of platelets and inflammatory cells.[15] The reduced wound tensile strength in DXR-treated animals may be due to reduced scar collagen accumulation and reduced fiber diameter.[16] Moreover, allopurinol, a xanthine oxidase inhibitor, protects against DXR toxicity in wound healing.[17] On the contrary, there was less fibroblast proliferation in the ischemic wounds on days 7 and 14 than in the nonischemic control wounds.[18] Osman *et al.*[18] investigated collagen density and found that collagen organization was significantly decreased due to ischemia compared with nonischemic controls. Additionally, antioxidants partially improve healing in ischemic skin wounds.[18] Taken together, both DXR and ischemia have an inhibitory effect on fibroblast proliferation and collagen biosynthesis, which is mediated by oxygen free radicals.

We chose the maximum dose tolerated with an acceptable mortality rate of DXR and H-shaped double flap model showing moderate transient ischemia and typical partial necrosis.[5] These conditions mimic the clinical setting, suggesting that skin ischemia had more severe effects than DXR.

It is difficult to determine the optimal time of initiating postoperative chemotherapy in patients with impaired wound healing, such as flap partial necrosis and skin rejection. We found that when administered within 7 days after the surgery, DXR had a tendency to decelerate the restoration rate of tensile strength in ischemic wounds. Pathologically, DXR led to atrophic skin changes and degeneration of skin appendages on days 14–21 after the surgery in both the models. Specifically, there is a risk in initiating chemotherapy 21 days after surgery in such cases. Further studies are needed to determine the exact time of DXR administration in ischemic situations to minimize

the side effects.

CONCLUSION

We concluded that DXR decreases the tensile strength and causes atrophic changes in ischemic wounds.

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