Int J Clin Exp Pathol 2019;12(4):1468-1477
www.ijcep.com /ISSN:1936-2625/IJCEP0091860

Original Article

Effect of cold and hot compress on neutrophilic migration to the site of doxorubicin extravasation

Natsuko O Miura[1,2], Naing Ye Aung[2], Mitsunori Yamakawa[2]

[1]*Department of Basic Nursing, Iwate Prefectural University Faculty of Nursing, Takizawa, Iwate Japan;* [2]*Department of Diagnostic Pathology, Yamagata University Faculty of Medicine, Yamagata, Japan*

Received January 26, 2019; Accepted February 20, 2019; Epub April 1, 2019; Published April 15, 2019

Abstract: In the present study, we investigated a part of the mechanism responsible for the effects of hot and cold compresses for extravasation of doxorubicin. We injected 20 μl of doxorubicin (DOX) (1 μg/μl) subcutaneously into the dorsal area in mice and observed the resulting skin lesions macroscopically and histologically from day 1 to day 14 thereafter in groups treated with a cold pack (18-20°C) and a hot pack (38-40°C) or left untreated (control). Immunofluorescence and RT-PCR for C5a receptor (CD88), interleukin-8 receptor (IL-8RA), and transient receptor potential cation channel subfamily V member 1 (TRPV1) were also performed. Macroscopic observation showed that the area of the skin lesion was significantly smaller in the cold group than in the control group, but was significantly larger in the hot group. The neutrophil count in the lesion was significantly higher in the hot group than in the cold (3 hrs) and control groups. The numbers of inflammatory cells expressing CD88 and IL-8RA were significantly lower in the cold group than that in the other groups at almost time points and in the hot groups at later time points, respectively. The number of nerve fascicles expressing TRPV1 was higher in the hot group than in the cold group on days 1, 3 and 14. mRNA for CD88, IL-8RA and TRPV1 was detectable by reverse transcription-polymerase chain reaction in both the cold and hot pack groups. Consequently, these results suggested that the cold pack for the extravasation of DOX might reduce inflammation.

Keywords: Hot and cold compresses, doxorubicin, C5a receptor (CD88), interleukin-8 receptor, transient receptor potential cation channel subfamily V member 1

Introduction

Doxorubicin (DOX) is a commonly used anthracycline anticancer drug. Since its introduction in 1975, DOX has been used in rituximab plus cyclophosphamide, DOX, vincristine, and prednisolone therapy [1], adriamycin, bleomycin, vinblastine and dacarbazine therapy, and modified cyclophosphamide, vincristine, doxorubicin and methotrexate therapy for lymphomas. The reported frequency of extravasation of infused anticancer drugs is 0.01-6.5% in Europe and America [2]. DOX is classified in a vesicant drug that can cause serious injury to skin tissue when it leaks out of blood vessels. DOX extravasation causes pain and necessitates cessation of treatment for wound healing, sometimes for a long period, and skin grafting and flap surgery may be necessary [3]. Application of a hot compress is also recommended for treatment of extravasation [4, 5]. Other reported therapies have included infusion of saline and hyaluronidase [6] or wash-out at the leakage site [7]. Local injection of steroid and application of dexrazoxane have been also recommended for extravasation of DOX [8, 9]. Furthermore, local cooling reduces the area that needs to be surgically resected, and decreases a recall phenomenon [10]. Animal experiments have demonstrated that cooling decreases ulceration [11], but other reports have indicated that neither cooling nor warming reduces the ulcer size or healing period [12].

Cooling causes local vasoconstriction, thus limiting diffusion of the drug into tissue, whereas warming causes local vasodilatation, and promotes absorption of the drug [13]. For drugs that bind to DNA, local cooling has been reported to reduce absorption of the drug by skin tissue after leakage. However, the period of cooling has varied, with sometimes the cold com-

press being applied for 15-30 min every 1-2 days, or sometimes continuously for 1-3 hrs [13, 14], and in fact the mechanism of the effect of such compresses remains unclear.

The purpose of the present study was to determine a part of the mechanism responsible for the effect of compress application by examining the migration of neutrophils to the site of DOX leakage, and also the intracellular expression of C5a receptor 1 (CD88), interleukin-8 receptor (IL-8RA), and transient receptor potential vanilloid type 1 (TRPV1) at the leakage site.

Material and methods

Mice

Thirty-nine BALB/c mice (male, 6 weeks, 22-25 g) were obtained from Nippon Clea (Tokyo, Japan) and housed at constant temperature (22-23°C) under a 12: 12-hrs light-dark cycle in sawdust floor cages with free access to standard laboratory chow and tap water. The mice were anesthetized with isoflurane (Abbott Japan, Tokyo, Japan) and their backs were depilated with a commercial cream (Kanebo, Tokyo, Japan).

This study was approved by the Research Ethics Committee (22091) of Yamagata University Faculty of Medicine, Yamagata, Japan.

Hot and cold compress application

Using a syringe (Nipro, Osaka, Japan) with a 30-gauge needle, 20 µl of DOX (Kyowa Hakkou Kirin, Tokyo, Japan) (1 µg/µl) was injected subcutaneously into the backs of the mice. Using a Cold/Hot Pack (3 M Healthcare, Tokyo, Japan), the site of injection was then either cooled (18-20°C) or warmed (38-40°C) while measuring the skin surface temperature with a digital thermometer (CE-309, Sato Shoji, Kawasaki, Japan). The mice were placed in a supine position. The compress was applied 3 hrs or 20 min, four times every 6 hrs [13, 14]. The mice were divided into a control group (n = 15) not subjected to compress application after DOX injection, a cold pack group (n = 15), and a hot pack group (n = 15). The lesioned skin was photographed before treatment and its area was measured using Image J (Texel Studio, Tokyo, Japan).

A picture of the lesion, including skin swelling around redness considered as the greatest dimension of inflammation was taken on the first and third days after DOX administration, and its area was measured using the image processing software Image J-Win Japanese edition. After photography on the third day, we resected the skin tissue and fixed it in 10% formaldehyde for histological examination. Skin specimens were cut into parasagittal slices, dehydrated, and embedded in paraffin using standard procedures to prepare sections 5 µm thick. The paraffin sections were stained with hematoxylin and eosin and assessed by light microscopy. We photographed 6 fields (×400) of the specimen, confirmed the presence of segmented leukocytes, and measured the neutrophil count per 1 mm^2.

Measurement of vascular permeability

On the basis of a report by Yamaki [15], we examined vascular permeability. A caudal vein was injected with 100 mg/kg body weight of fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (Inter-Cell Technologies, Jupiter, FL), and then 20 µl of DOX was injected into six sites in the panniculus carnosus muscle. We subsequently applied one compress for 30 min (n = 15) or 3 hrs (n = 6). We divided these mice into a control group (n = 7) untreated after DOX injection, a cold pack group (n = 7), and a hot pack group (n = 7). After application of the compress, we collected blood from the abdominal vena cava and centrifuged it (3,000 rpm, 10 min) to obtain serum after leaving it at room temperature for 60 min. We resected the skin tissue using an 8-mm biopsy punch (Kai Industries, Gifu, Japan), and placed it in 24-well tissue culture plates (Falcon, 3047, Beckton Dickinson Japan, Tokyo, Japan), followed by incubation with 1 ml of formamide (Kanto Chemical, Tokyo, Japan) at 50°C for 2 hrs for measurement of the fluorescence intensity using a fluorescence plate reader (Gemini EM, Japan Molecular Devices, Tokyo, Japan). In addition, we added 50 µl of serum to 24-well plates and added 950 µl of formamide to measure the intensity of fluorescence after warming treatment, similar to that for the skin tissue.

Immunofluorescence for CD88, IL-8RA and TRPV1

For this experiment 75 mice were used. We injected 20 µl of DOX (1 µg/µl) into the panniculus carnosus muscle, and then treated the

injection site with a compress for 3 hrs using an appliance. After DOX injection, we divided the mice into an untreated control group (n = 15), a cold pack group (n = 15), and a hot pack group (n = 15) as described previously.

We took pictures of the injection site on days 1, 3, 5, 7 and 14 after DOX administration and measured the area of the lesion using Image J-Win Japanese edition. We then resected the tissue on days 1, 3, 5, 7 and 14 after DOX administration. Formalin-fixed tissue was used for histologic examination and fresh frozen tissue for immunofluorescence for CD88, IL-8RA and TRPV1. Frozen tissue samples were mounted in Tissue Tek OCT compound (Sakura, Tokyo, Japan), quickly frozen in liquid nitrogen, and stored at -80°C. We obtained frozen sections 5-10 μm thick using a Cryostat (CM-1850, Leica, Tokyo) and mounted them on MAS glass cover slips (Matsunami Glass Industry, Tokyo, Japan), followed by air-drying for 1 hr. We then fixed the sections with cold acetone (4°C) for 10 min, followed by addition of 3% skim milk (Morinaga Milk Industry, Tokyo, Japan) dissolved in PBS after washing in 0.01 M phosphate-buffered saline, pH 7.4 (PBS), for 15 min. For double immunofluorescence, slide-mounted tissue sections were incubated with a primary antibody [anti-CD88 (C5aR) antibody (rabbit, polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA), anti-rat IL-8RA antibody (recognizing mouse IL-8RA, goat polyclonal, Santa Cruz Biotechnology), anti-rat TRPV1 (recognizing mouse TRPV1, rabbit, polyclonal, Alomone Labs, Jerusalem, Israel) and mouse monoclonal anti-α-SMA antibody (Clone 1A4, mouse IgG2a, Dako, Glostrup, Denmark)] overnight at 4°C. The tissue sections were washed in PBS, and then incubated with the secondary antibody [FITC-conjugated goat anti-rabbit IgG (H+L) antibody (Beckman Coulter, Brea, CA), FITC-conjugated donkey anti-goat IgG (H+L) antibody (Beckman Coulter) and tetramethylrhodamine (TRITC)-conjugated donkey anti-mouse IgG (H+L) antibody (Jackson Immuno Research, West Grove, PA) at room temperature for 3 hrs. Finally, the tissues were washed in PBS, and mounted in Fluoromount (Diagnostic BioSystems, Pleasanton, CA). As a negative control, we used PBS, non-immune rabbit IgG, mouse IgG, and goat IgG (Dako) in place of the primary antibody.

After mounting, we observed CD88 and IL-8RA in the specimen using a fluorescence microscope and took photographs of 6 fields (400×). In addition, we performed double staining using TRITC and FITC for TRPV1 and α-SMA to exclude the erector pili muscle, which includes TRPV1. Subsequently, we photographed 6 fields (400×) including the TRPV1-positive funiculus, and measured the number of cells positive for CD88 and IL-8RA per 1 mm^2.

Reverse transcription-polymerase chain reaction (RT-PCR) for CD88, IL-8RA and TRPV1

A fresh frozen section of the lesion tissues with a compress for 3 hrs on days 1, 3, 5, 7 and 14 after DOX was also obtained for RT-PCR. mRNA was purified using WaxFreeTM RNA (TrimGen, Sparks, MD). cDNA was synthesized with PrimeScript reverse transcriptase (TaKaRa Bio, Tokyo, Japan) and Random 6 mers. RT-PCR was performed using EmeraldAmpTM PCR Master Mix (TaKaRa Bio) and a Veriti thermal cycler (Applied Biosystems, Foster City, CA). The cDNA was amplified using the following PCR primers: CD88 [16], sense: 5'-TATAGTCCTGCCCTCGCTCAT-3', anti-sense: 5'-TCACCACTTTGAGCGTCTTGG-3', IL-8RA [17], sense: 5'-GACACTCTCTTAGGAGCCCACTTG-3', anti-sense: 5'-CAGGGCCTGGTCAATGTCA-3', TRPV1 [18], sense: 5'-TTGGATTTTCCACAGCCGTAGT-3', anti-sense: 5'-GAACTTGAACAGCTCCAGACATGT-3', glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [19], sense: 5'-ATCAAGAAGGTGGTGAAGCAG-3', anti-sense: 5'-GAAGGTGGAAGAGTGGGAGTT-3'.

PCR amplification was performed as follows: denaturation for 10 min at 95°C; followed by 40 cycles of PCR amplification, with each cycle consisting of 40 s of denaturation at 95°C, 1 min of annealing, and 40 s of elongation at 72°C; and with one final cycle of elongation at 72°C for 7 min. The annealing temperature was set at 60°C for CD88, IL-8RA and GAPDH, and 65°C for TRPV1. The amplified PCR products were separated by electrophoresis on a 4.0% agarose gel containing ethidium bromide, and then visualized with UV illumination.

Statistical analysis

The data of vascular permeability are presented as the mean ± standard deviation (SD). For the other data, the median of each group is shown as a box plot with whiskers from minimum to maximum. The interquartile range shows as box with the median marked as a horizontal line, and minimum and maximum

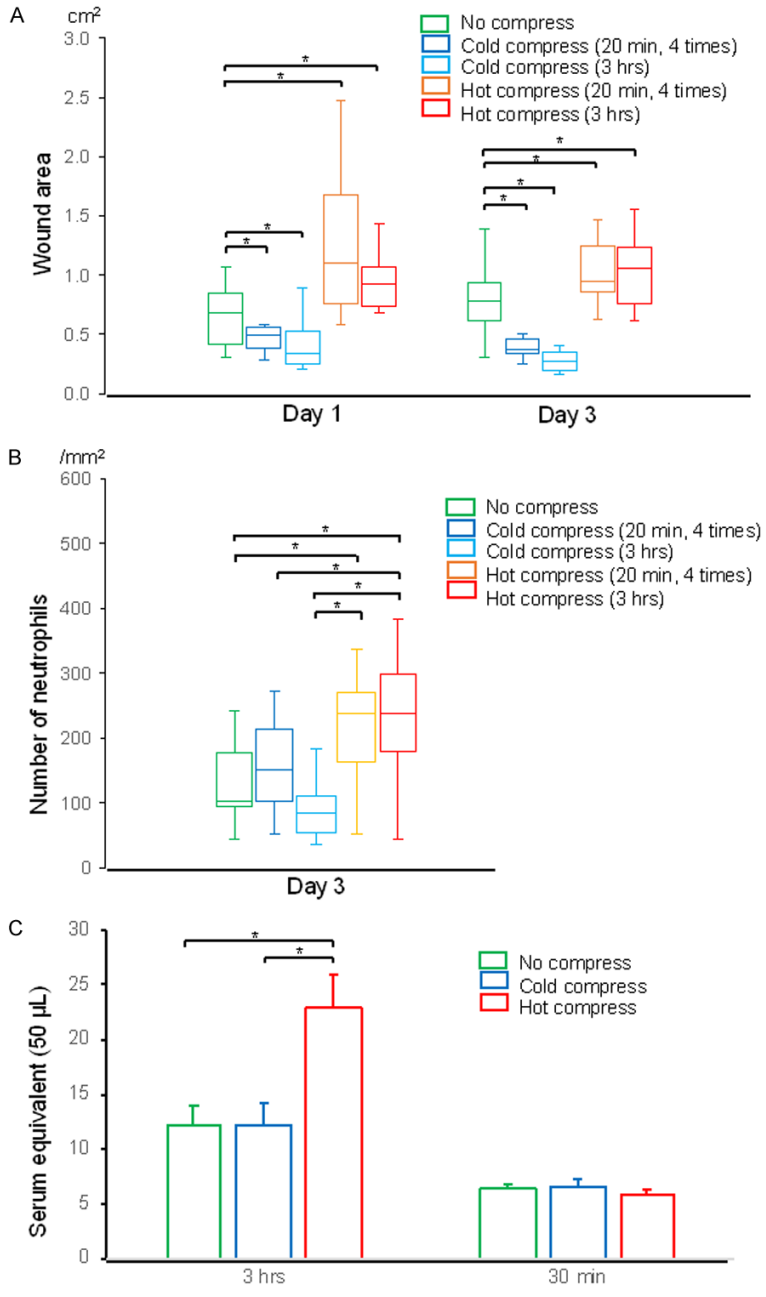

Figure 1. Effects of compress application on the size of lesion area, neutrophilic infiltrate, and vascular permeability. Both cold and hot compresses were applied 20 min or 3 hrs four times every 6 hrs. Box plot explanation: The median for each group is shown as a box plot with whiskers from minimum to maximum. The interquartile range is shownas a box with the median marked as a horizontal line; minimum and maximum from lower and upper quartile represent error bar. A. The macroscopic lesioned area after cold and hot compresses. After administering of doxorubicin into the dorsal skin of the mouse, the lesioned areas (cm^2) on the 1st and 3rd days were measured and compared in each group. B. Relationship between neutrophilic infiltration and cold or hot compression time. After administering of doxorubicin into the dorsal skin of the mouse, followed by cold or hot compression during 20 min or 3 hrs (4 times), the number of neutrophils ($/mm^2$) was counted on the third day. C. Vascular permeability: After doxorubicin administering into mouse dorsal skin followed by 30-min or 3-hrs compress, the intensity of fluorescein isothiocyanate-labeled bovine serum albumin was measured in a fluorescence plate reader. *: $P < 0.05$.

from lower and upper quartile represent error bar. The Kruskal-Wallis and Bonferroni significant difference tests were used to analyze the results, and differences at $P < 0.05$ were considered significant. All tests were performed using SPSS 16.0 for Windows.

Results

Effects of compress application on the size of lesioned area, neutrophilic infiltrate, and vascular permeability

We compared the area of swelling after application of each type of compress on the first and third days following administration of DOX. This showed that, relative to the control group, the area of the lesion in the cold pack group was significantly smaller at both time points, whereas that in the hot pack group was significantly larger (**Figure 1A**). There was an evident tendency to have higher neutrophil count in the hot pack group on the third of 3 days than in the other groups (**Figure 1B**). Vascular permeability was significantly increased at 3 hrs of hot pack application relative to the other groups (**Figure 1C**).

Effects of compress application to the prolongation of inflammation

Next, we evaluated the prolongation of inflammation on days 1 to 14 after DOX administration followed by a cold or hot compress. Because the backs of mice had resumed hair regrowth by day 14, the area of the lesioned skin was measured up to day 7. The lesions in the cold pack group were significantly smaller than those in the other groups on days 1, 5 and 7 (**Figure 2A**, **2B**).

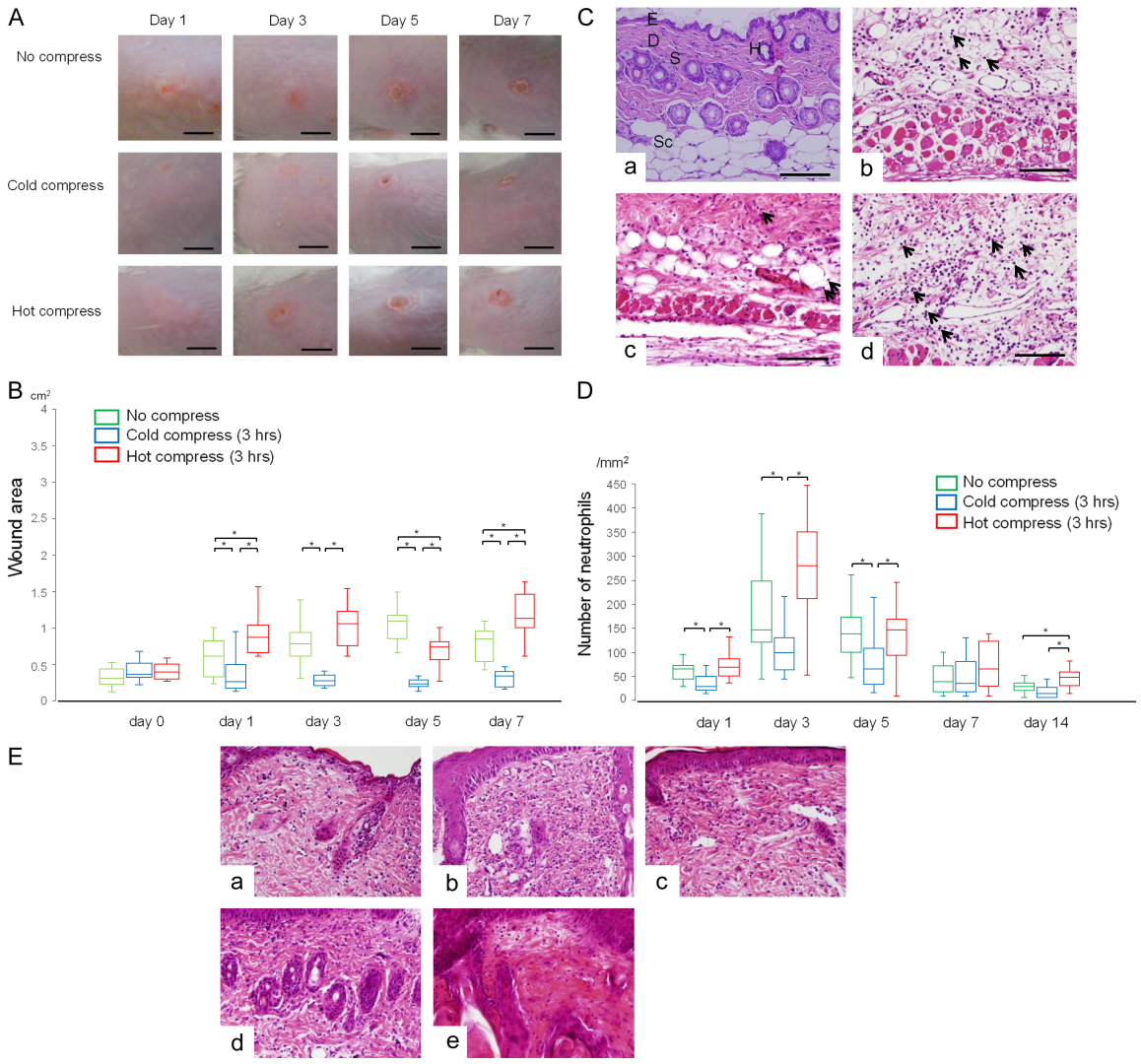

Figure 2. Histologic examination of prolongation of inflammation after administration of doxorubicin into mouse dorsal skin followed by 3-hrs compress. (A) Macroscopic pictures. Greatest dimensions of lesions were measured including edema. Bar = 10 mm. (B) Extent of the lesioned area after doxorubicin administration and 3-hrs cold or hot compresses. Box plot explanation: The median for each group is shown as a box plot with whiskers from minimum to maximum. The interquartile range is shown as a box with the median marked as a horizontal line, minimum and maximum from lower and upper quartile represent error bar. *: $P < 0.05$. (C) Histologic findings (Hematoxylin and eosin stain). (a) The dorsal skin of healthy mouse: epidermis (E), dermis (D), and subcutaneous tissue (Sc), sebaceous glands (S), the hair follicle (H). (b) Control group: neutrophils (arrowheads). (c) Cold group: neutrophils (arrowheads). (d) Hot group: neutrophils (arrowheads). Bar = 50 μm. (D) The number (per mm^2) of neutrophils after 3-hrs cold or hot compresses. Box plot explanation: The median for each group is shown as a box plot with whiskers from minimum to maximum. The interquartile range is shown as a box with the median marked as a horizontal line; minimum and maximum from lower and upper quartile represent error bar. *: $P < 0.05$.

The epidermis, dermis, subcutaneous tissue and panniculus carnosus muscle were evident in the dorsal skin tissue of the healthy mouse, and folliculus pili and sebaceous glands appeared in the dermis (**Figure 2C**). Infiltration of neutrophils into areas of epidermal thinning and flattening, the dermis, and subcutaneous tissue was evident on the first day after DOX administration (**Figure 2E**). An ulcer had formed by the third day, and with local epidermal hypertrophy and extension of the rete ridge. Collagen fibers in the deep dermis showed fine tears, and the panniculus carnosus muscle showed edematous injury with inflammatory cell infiltration (**Figure 2C**). Neighboring epidermal growth with sloughing of the skin was

found on the fifth day (**Figure 2E**), with many lymphocytes and macrophages other than neutrophils in the dermis and subcutaneous tissue. Re-epithelialization of the epidermis around the lesion was evident on the seventh day, but many inflammatory cells were still present in the dermis. Re-epithelialization under the crust was advanced on day 14, and the epidermis continued, and it was localized, and subepidermal bullae including lymphocytes and red blood cells were found. The lesion became fibroblast-rich, and granulation tissue was reduced.

The neutrophil count was maximal on the third day and decreased gradually thereafter. It had an evident tendency to be lower in the cold pack group in comparison with the other groups, and there was a significant difference between the cold and hot groups on days 1, 3, 5 and 14 (**Figure 2D**).

Immunofluorescence for CD88, IL-8RA, and TRPV1

We observed autologous fluorescence of hairs on the dorsal skin of mice. Because positivity was found in the stratum corneum and follicullus pili in the negative controls, these structures were omitted in subsequent examinations. The number of inflammatory cells showing positivity for CD88 and IL-8RA was a tendency to be lower in the cold pack group than the hot pack groups on any days and days 5, 7 and 14, respectively (**Figure 3A-D**). There was also a tendency to observe less positivity for TRPV1 in the funiculus in the cold pack group than in the other groups, and there was a significant difference between the cold and hot groups on days 1, 3 and 14 (**Figure 3E**, **3F**).

RT-PCR for CD88, IL-8RA, and TRPV1

RT-PCR of frozen tissue revealed the expression of mRNA for CD88, IL-8RA, TRPV1 and GAPDH in the positive control. Similarly, in skin tissue on the first day after DOX administration, expression of mRNA for CD88, IL-8RA, TRPV1 and GAPDH was detected, but was absent in the negative control (**Figure 4**).

Discussion

Currently there is no consensus regarding the optimal period of treatment with hot or cold compresses for anticancer drug extravasation [4, 20, 21]. A method involving repeated application of compresses for 20 min has been adopted empirically [12], but this was originally based on PRICE therapy for sprains [22] and cryotherapy for ankle strain [23]. In the present cold pack group, the neutrophil count at 20 min did not differ significantly from that in the control group, but was decreased at 3 hrs; also, the neutrophil count in the hot pack group was found to be increased regardless of the period of compress application. Repeated application of a cold pack every 20 min inhibits neutrophil infiltration relative to application of a hot pack, but it is thought that continuous cold pack application for 3 hrs is better. Furthermore, application of a hot pack for 3 hrs increases vascular permeability. A 30-min compress application has been performed empirically, but in the present study there was no significant difference in vascular permeability between the cold pack and hot pack groups. With regard to the effect of 30-min cooling, a laboratory study of rats has shown that inflammatory cell infiltration and edema resulting from extravasation of an antibiotic agent were inhibited [24]. However, no study prior to the present one has investigated leakage of an anticancer drug. Because anticancer drugs are more injurious to skin tissue and the resulting inflammation tends to be protracted, brief cooling or warming seems to have little effect. In addition, we followed the protocol from preliminary research in the injury of the skin with the doxorubicin administration by this experiment andchose suitable animal models [25]. Based upon these previous findings, we studied the subcutaneous leakage of 20 μl of DOX (1 μg/μl) on the panniculus carnosus muscle in the backs of mice, and examined the mechanism of the effect of 3-hrs compress application.

We examined the effects of compresses applied for 3 hrs on injury resulting from leakage of doxorubicin into subcutaneous tissue. We found that, in comparison with the cold pack group, the lesions were exacerbated in the hot pack group, and that the neutrophil count was increased. In the cold pack group, inflammatory cells positive for CD88 and IL-8RA were significantly decreased on any days and days 1, 5, 7 and 14, respectively, as were funiculi positive for TRPV1 and TRPV1 in the epidermis, relative to the hot pack group. Furthermore, we were

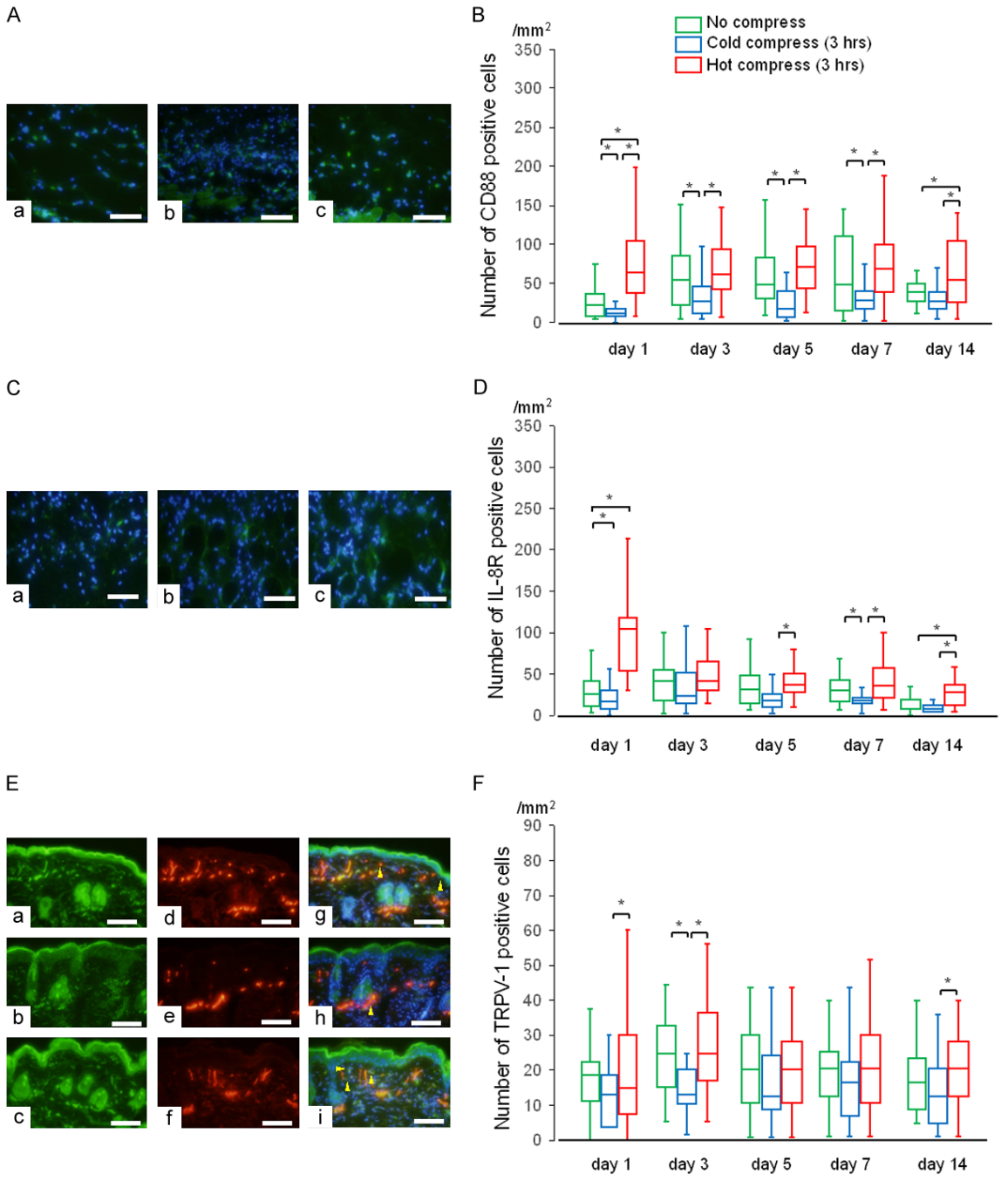

Figure 3. Immunofluorescence for CD88, interleukin-8 RA (IL-8RA) and transient receptor potential cation channel subfamily V member 1 (TRPV1) on 3rd day after administration of doxorubicin into mouse dorsal skin followed by 3-hrs compress. Box plot explanation: The median for each group is shown as a box plot with whiskers from minimum to maximum in (B, D and F). The interquartile range is shown as a box with the median marked as a horizontal line, minimum and maximum from lower and upper quartile represent error bar. (A & B) Immunofluorescence for CD88 (A) and IL-8RA (C), and the number (per mm^2) of CD88 (B) and IL-8RA (D)-positive cells after 3-hrs cold or hot compresses. Immunofluorescence of TRPV1-positive cells (E) and the number (per mm^2) of TRPV1-positive nerve fascicles (F) after 3-hrs cold or hot compresses. [(a) TRPV1 (fluorescein isothiocyanate; FITC), (d) α-smooth muscle actin (α-SMA) (tetramethylrhodamine; TRITC), (g) merge TRPV1 (arrowheads); (b) TRPV1 (FITC), (e) α-SMA (TRITC), (h) merge TRPV1 (arrowheads); (c) TRPV1 (FITC), (f) α-SMA (TRITC), (i) merge TRPV1 (arrowheads)]. (a) control group. (b) cold compress group. (c) hot compress group in (A, C and E). Bar = 50 μm, *: $P < 0.05$.

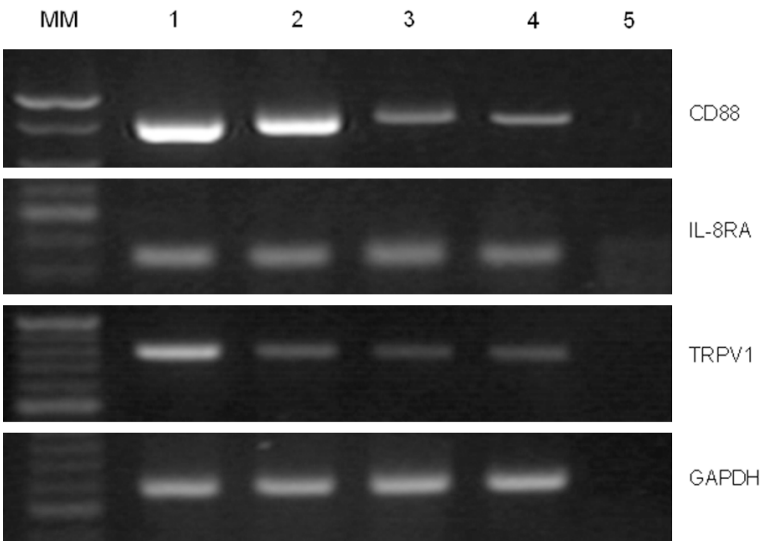

Figure 4. Reverse transcription-polymerase chain reaction (RT-PCR) for CD88, interleukin-8 RA (IL-8RA), and transient receptor potential cation channel subfamily V member 1 (TRPV1). mRNAs of CD88, IL-8RA, and TRPV1 including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were expressed in skin tissue on the first day after doxorubicin administration. MM: molecular marker, Lane 1; positive control (brain), Lane 2; control (no compress), Lane 3; cold compress, Lane 4; hot compress, Lane 5; negative control.

able to determine the expression of mRNA for CD88, IL-8RA and TRPV1 by RT-PCR. Chemokines and C5a, including IL-8, are closely associated with migration of neutrophils into inflammatory tissue. IL-8 is classified as a CXC chemokine and is a chemotactic factor that causes neutrophils to migrate into inflammatory tissue after activation of its receptor, IL-8R [26]. C3a and C5a stimulate mast cells by activation of the complement system, bringing about vascular hyperpermeability, followed by migration of neutrophils and macrophages into inflammatory tissue [27]. It inhibits the migration of neutrophils into skin tissue and decreases their expression of CD88 and IL-8R, thus decreasing any unnecessary injury.

In the present study, application of a cold pack for 3 hrs tended to decrease the expression of TRPV1 in peripheral nerves at the injury site on days 1, 3 and 14, relative to that in the hot pack group. TRPV1 belongs to the TRP superfamily and is activated by capsaicin, being a channel having high Ca^{2+} permeability. It is a thermosensor expressed on dorsal root ganglia and C-fibers, which are sensory nerves releasing neuropeptides such as substance P and calcitonin gene-related peptide [28]. It is activated by high fever (> 43°C) and nitric monoxide, but when inflammation occurs, it is activated within the normal temperature range and is associated with inflammatory pain [29]. In addition, in colitis, TRPV1 increases the number of neutrophils migrating into areas of inflammation, and increases myeloperoxidase activity [30]. In the present study, expression of TRPV1 was found in peripheral nerves from the first day after DOX extravasation. Expression of epidermal TRPV1 was also observed from day 1. Expression of CD88 was found from the first day of inflammation especially with the hot compress, but a marked inflammatory reaction was promoted by many neutrophils that migrated into the area of tissue injury, and these were thought to express TRPV1. In addition, the expression of TRPV1 and CD88 continued after the first day, and skin injury caused by DOX continued. DOX that leaks into skin tissue binds to DNA, and not only causes local cell death, but also release of DNA-anthracycline complexes from dead cells. These are taken up by neighboring normal cells by endocytosis, thus increasing the degree of cytotoxicity. However, in this study, the expression of CD88 mRNA did not seem to be consistent with the expression level of immunofluorescence. Future work should quantify these factors by real-time PCR and quantify the amount of protein by western blotting.

In this study, it was suggested that CD88 and IL-8RA were expressed on neutrophils, and TRPV1 was expressed on peripheral nerves, and these were associated with expansion of the injury. Three-hrs cooling inhibited the expression of these molecules, suggesting that this would have been effective for inhibition of the inflammatory reaction, whereas warming worsened the lesion. In addition, application of a hot pack for 3 hrs significantly enhanced vascular permeability. These results suggest that as well as inducing local vasodilatation [13], a

hot pack does not promote healing, but rather causes sudden edema.

In the present study, we were able to reveal the following using mouse model for the first time: (1) Application of a cold pack (20°C) for 3 hrs inhibited the expression of CD88 and IL-8RA, and decreased the neutrophil count. In addition, it significantly reduced the extent of the lesion evident macroscopically until day 7. (2) Application of a hot pack (40°C) aggravated vascular permeability and increased the degree of swelling. In addition, it increased the expression of CD88 and IL-8RA and raised the neutrophil count, significantly exacerbating the lesion. In relation to risk management, it is important to monitor the occurrence of cytostatic drug extravasation in order to improve the quality of nursing care provided to cancer patients. Extravasation of anticancer drugs can cause severe skin lesions, and is usually detected clinically during routine patient care.

When infused anticancer agents leak from blood vessels, both cold and hot packs are commonly used to treat the skin surface. However, application of a hot pack to damaged skin tissue results in further injury, and our findings suggest that such lesions might be aggravated. In addition, increased expression of TR-PV1 causes pain, and the excessive inflammatory reaction may prolong the healing period. We think that the present findings have shed some light on the effects of application of cold and hot packs that are used routinely in clinical practice, and the underlying mechanisms involved.

Acknowledgements

We would like to thank Hiromi Murata and Junko Takeda (Department of Pathological Diagnostics, Yamagata University Faculty of Medicine) for their valuable assistance during this study.

Disclosure of conflict of interest

None.

Address correspondence to: Natsuko O Miura, Department of Basic Nursing, Iwate Prefectural University Faculty of Nursing, 152-52, Takizawa, Iwate 020-0693, Japan. Tel: +81-19-694-2236; Fax: +81-19-694-2236; E-mail: natsuko@iwate-pu.ac.jp

References

[1] Ali K, Sial AA, Baig MT, Ansari SH, Adil SO, Shamsi TS. Detection of the incidence of infections and acute biochemical changes in diffuses large B-cell lymphoma patients treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) with and without rituximab. Curr Drug Saf 2018; 13: 102-106.

[2] Schulmeister L. Extravasation management: clinical update. Semin Oncol Nurs 2011; 27: 82-90.

[3] Roe H. Anthracycline extravasations: prevention and management. Br J Nurs 2011; 20: S18-22.

[4] de Lemos ML, Walisser S. Management of extravasation of oxaliplatin. J Oncol Pharm Pract 2005; 11: 159-162.

[5] Boonruab J, Nimpitakpong N, Damjuti W. The distinction of hot herbal compress, hot compress, and topical diclofenac as myofascial pain syndrome treatment. J Evid Based Integr Med 2018; 23: 2156587217753451.

[6] Schrijvers DL. Extravasation: a dreaded complication of chemotherapy. Ann Oncol 2003; 14 Suppl 3: iii26-30.

[7] Dionyssiou D, Chantes A, Gravvanis A and Demiri E. The wash-out technique in the management of delayed presentations of extravasation injuries. J Hand Surg Eur 2011; 36: 66-69.

[8] Vidall C, Roe H, Dougherty L, Harrold K. Dexrazoxane: a management option for anthracycline extravasations. Br J Nurs 2013; 22: S6-12.

[9] Kreidieh FY, Moukadem HA, El Saghir NS. Overview, prevention and management of chemotherapy extravasation. World J Clin Oncol 2016; 7: 87-97.

[10] Davis SL, Shibasaki M, Low DA, Cui J, Keller DM, Purdue GF, Hunt JL, Arnoldo BD, Kowalske KJ, Crandall CG. Cutaneous vasoconstriction during whole-body and local cooling in grafted skin five to nine months postsurgery. J Burn Care Res 2008; 29: 36-41.

[11] Harwood KV, Bachur N. Evaluation of dimethyl sulfoxide and local cooling as antidotes for doxorubicin extravasation in a pig model. Oncol Nurs Forum 1987; 14: 39-44.

[12] Soble MJ, Dorr RT, Plezia P, Breckenridge S. Dose-dependent skin ulcers in mice treated with DNA binding antitumor antibiotics. Cancer Chemother Pharmacol 1987; 20: 33-36.

[13] Wengström Y, Margulies A; European Oncology Nursing Society Task Force. European Oncology Nursing Society extravasation guidelines. Eur J Oncol Nurs 2008: 12; 357-361.

[14] Hadaway L. Infiltration and extravasation. Am J Nurs 2007; 107: 64-72.
[15] Yamaki K, Takano-Ishikawa Y, Goto M, Kobori M, Tsushida T. An improved method for measuring vascular permeability in rat and mouse skin. J Pharmacol Toxicol Methods 2002; 48: 81-86.
[16] Riedemann NC, Guo RF, Neff TA, Laudes IJ, Keller KA, Sarma VJ, Markiewski MM, Mastellos D, Strey CW, Pierson CL, Lambris JD, Zetoune FS, Ward PA. Increased C5a receptor expression in sepsis. J Clin Invest 2002; 110: 101-108.
[17] Fan X, Patera AC, Pong-Kennedy A, Deno G, Gonsiorek W, Manfra DJ, Vassileva G, Zeng M, Jackson C, Sullivan L, Sharif-Rodriguez W, Opdenakker G, Van Damme J, Hedrick JA, Lundell D, Lira SA, Hipkin RW. Murine CXCR1 is a functional receptor for GCP-2/CXCL6 and interleukin-8/CXCL8. J Biol Chem 2007; 282: 11658-11666.
[18] Ta LE, Bieber AJ, Carlton SM, Loprinzi CL, Low PA, Windebank AJ. Transient receptor potential vanilloid 1 is essential for cisplatin-induced heat hyperalgesia in mice. Mol Pain 2010; 6: 15.
[19] Ager RR, Fonseca MI, Chu SH, Sanderson SD, Taylor SM, Woodruff TM, Tenner AJ. Microglial C5aR (CD88) expression correlates with amyloid-beta deposition in murine models of Alzheimer's disease. J Neurochem 2010; 113: 389-401.
[20] Adami NP, de Gutiérrez MG, da Fonseca SM, de Almeida EP. Risk management of extravasation of cytostatic drugs at the Adult Chemotherapy Outpatient Clinic of a university hospital. J Clin Nurs 2005; 14: 876-882.
[21] Anjum S. Hot fomentation versus cold compress for reducing intravenous infiltration. Nurs J India 2007; 98: 253-254.
[22] Bleakley CM, O'Connor SR, Tully MA, Rocke LG, Macauley DC, Bradbury I, Keegan S, McDonough SM. Effect of accelerated rehabilitation on function after ankle sprain: randomised controlled trial. BMJ 2010; 340: c1964.
[23] Hubbard TJ, Denegar CR. Does cryotherapy improve outcomes with soft tissue injury? J Athl Train 2004; 39: 278-279.
[24] Oyama N, Ishida Y, Takeda T. Experimental study of the effects of applying a poultice to skin lesions produced by extravasation of antibiotics. J Facult Nurs Iwate Pref Uni 2008; 10: 1-7.
[25] Yilmaz M, Demirdover C, Mola F. Treatment options in extravasation injury: an experimental study in rats. Plast Reconstr Surg 2002; 109: 2418-2423.
[26] Moepps B, Nuesseler E, Braun M, Gierschik P. A homolog of the human chemokine receptor CXCR1 is expressed in the mouse. Mol Immunol 2006; 43: 897-914.
[27] Jang JH, Liang D, Kido K, Sun Y, Clark DJ, Brennan TJ. Increased local concentration of complement C5a contributes to incisional pain in mice. J Neuroinflammation 2011; 8: 80.
[28] Malin S, Molliver D, Christianson JA, Schwartz ES, Cornuet P, Albers KM, Davis BM. TRPV1 and TRPA1 function and modulation are target tissue dependent. J Neurosci 2011; 31: 10516-10528.
[29] Gavva NR, Bannon AW, Hovland DN Jr, Lehto SG, Klionsky L, Surapaneni S, Immke DC, Henley C, Arik L, Bak A, Davis J, Ernst N, Hever G, Kuang R, Shi L, Tamir R, Wang J, Wang W, Zajic G, Zhu D, Norman MH, Louis JC, Magal E, Treanor JJ. Repeated administration of vanilloid receptor TRPV1 antagonists attenuates hyperthermia elicited by TRPV1 blockade. J Pharmacol Exp The 2007; 323: 128-137.
[30] Szitter I, Pozsgai G, Sandor K, Elekes K, Kemeny A, Perkecz A, Szolcsanyi J, Helyes Z, Pinter E. The role of transient receptor potential vanilloid 1 (TRPV1) receptors in dextran sulfate-induced colitis in mice. J Mol Neurosci 2010; 42: 80-88.