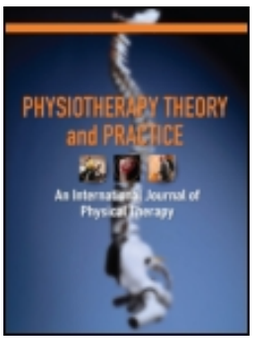

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RESEARCH REPORT

Effects of kinesiologic taping on epidermal–dermal distance, pain, edema and inflammation after experimentally induced soft tissue trauma

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Abstract

Purpose: In sports medicine, the use of kinesiologic tape has recently gained popularity. Although widely used, there is no study examining the effects of kinesiologic tape on soft tissue after a contusion injury. The aim of this study was to examine the effects of kinesiologic taping on epidermal–dermal distance, edema, pain and inflammation after experimentally induced contusion injury. *Methods*: Twelve adult female Wistar albino rats were divided into two groups: (1) 30 min group: $n = 6$, weight range: 182.0–199.4 g; and (2) 6 h group: $n = 6$, weight range: 186.9–200.8 g. After soft-tissue trauma, tape was applied to the right sides of each rat. In one group, tape was applied for 30 min while 6 h in the other. To assess the epidermal–dermal distance and edematous area, tissue sections were stained with hematoxylin and eosin and examined. Tissue sections were stained with nerve growth factor (NGF) and B-cell lymphoma 2 (Bcl-2) immunohistochemically to evaluate the effect of taping on pain and inflammation respectively. *Results*: Epidermal–dermal distances were found to be significantly higher than controls' in both groups ($p < 0.05$). Notable decreases were seen in edematous areas in both groups ($p < 0.05$). NGF and Bcl-2 immune reactivity were decreased in all tape applied sides. *Conclusions*: After soft-tissue trauma, it was histologically shown that kinesiologic taping increases epidermal–dermal distance, and may reduce the sensation of pain, edema and inflammation. For better, faster and comfortable tissue healing with protection of soft-tissue integrity, kinesiologic taping may be a valuable treatment after contusion injury. However, these results should be supported by clinical studies.

Keywords

Contusion injury, histology, inflammation, rat, taping

History

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Introduction

Contusions caused by blunt trauma are common athletic related injuries. These injuries can affect performance and participation in sports (Delos et al, 2014). A contusion injury not only damages the muscle cells, but may also lead to capillary rupture, infiltrative bleeding, inflammation, oxidative stress, pain, edema and fibrosis, depending on the extent of the injury (George, Smith, Isaacs, and Huisamen, 2015). One of the protective and supportive treatment modalities used after a contusion injury is taping (Hewetson, Austin, Gwynn-Brett, and Marshall, 2010).

There are many tape types and taping techniques (Hewetson, Austin, Gwynn-Brett, and Marshall, 2010; Ristow et al, 2014). Kinesiologic tape (KT) (Kinesio®tape, Kinesio USA, Albequerque, NM) is a therapeutic elastic tape that has recently been used in various pathologies (Morris, Jones, Ryan, and Ryan, 2013). After application, KT pulls on the skin, altering contour. The stretched elastic tape retracts back to its original length and forms convolutions in the underlying skin. It is claimed that these convolutions increase the interstitial space between the skin and underlying connective tissue. Main functions of KT include: normalizing muscle function; improving lymphatic and blood flow; reducing pain and inflammation; and improving joint malalignment and joint proprioception according to the manufacturer's claims (Kase, 2005; Kase, Willis, and Kase, 2003).

Kinesiologic tape (KT) has been suggested to affect lymphatic drainage, pain and the inflammatory response (Bialoszewski, Wozniak, and Zarek, 2009; Gonzalez-Iglesias et al, 2009; Stedge, Kroskie, and Docherty, 2012; Tsai, Chang, and Lee, 2010). Despite the growing body of literature assessing the efficacy of KT, there is no study assessing KT after a muscle contusion injury. In addition, the literature is unclear as to if or how tape may affect an acute inflammatory response and how it alters the cellular environment after a contusion injury. Therefore, this study was undertaken to investigate the effects of KT on epidermal–dermal distance, acute inflammatory response, edema and pain in an animal model following an experimentally induced contusion injury.

Methods

Prior to the study, a statistical power analysis was performed for sample size estimation. With an alpha = 0.05 and power = 0.80, the projected sample size (GPower 3.1) was approximately $n = 9$

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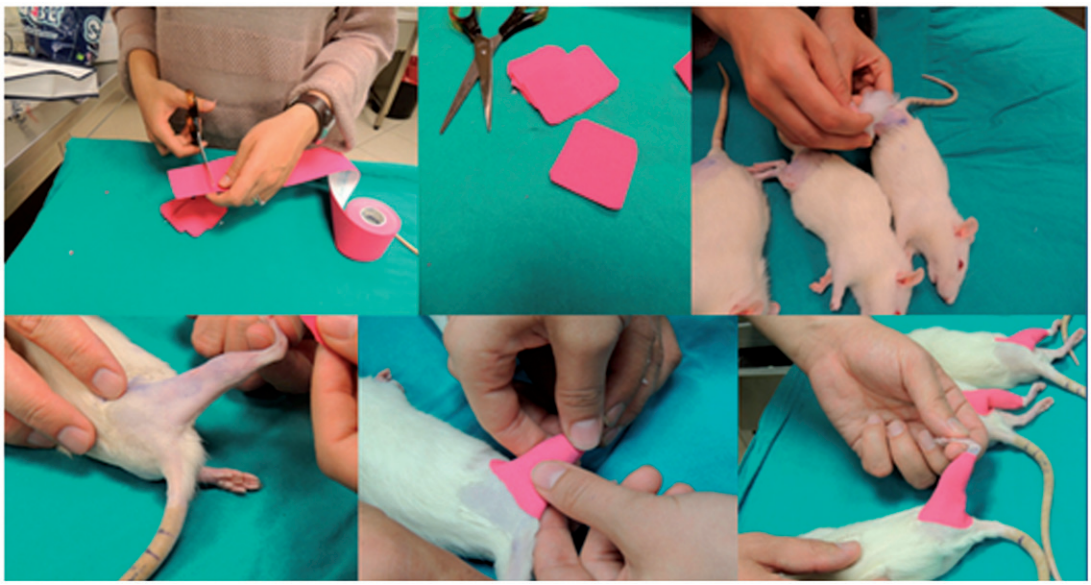

Figure 1. Application steps of kinesiologic tape.

for group comparisons. Although our proposed sample size was 9 rats for each group, the Animal Experiments Ethics Committee at our university only allowed a maximum of 6 rats. In the current study, calculated 1 beta (power) using group means and standard deviations was 0.59. Although power value of the study between groups did not reach the desired level, differences between the means or medians were clinically significant and quite large (effect size = 1.45). Therefore, data obtained from measurements were regarded as reliable.

Fourteen adult (8-week old) female Wistar Albino rats were obtained from the Laboratory of Animal Breeding and Experimental Research Centre of Gazi University, Ankara. A pilot study was initially performed on two rats. The remaining 12 rats were included in the study. The experimental protocol was approved by Gazi University Animal Experiments Local Ethics Committee (G.U.E.T.-13.013, Ankara).

Before starting the experiment all rats were anesthetized intraperitoneally with 50 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride. After anesthesia, all rats' rear legs were shaved and placed on a platform. A 2.45 cm diameter metal ball weighing 55 g was previously stained with ink to specify the region that the ball was dropped on. A ball with a diameter of 3.45 cm and a constant height of 135 cm was dropped from a pipe on to the mid-belly of gastrocnemius muscle (Khattak et al, 2010). The muscle contusion made by this contusion model was used because it was a high-energy blunt damage that created a large hematoma and inflammation was followed by healing processes that are very similar to those in humans (Diaz et al, 2003; Nozaki et al, 2008). The animals were kept in a 12-h light, 12-h dark cycle at 20 ± 2 °C. The animals were fed *ad libitum* without limitation, and access to water was provided freely. Seventy-two hours after injury, the rat's ankle was placed in a 90° dorsal flexion, thus gastrocnemius could be hold in a stretched position. Then ''I'' shaped KT was applied in the direction of insertion to origin on the gastrocnemius muscle (Kase, 2005) (Figure 1).

The rats were randomly divided into two groups. One group received KT for 30 min while the other received KT for 6 h on the traumatized tissue. The left sides of the rats were used as controls. After 30 min and 6 h of KT application, rats were anesthetized intraperitoneally. The skin and underlying tissues were harvested, sectioned and stained without the removal of the tape by a 20-year experienced veterinarian. After tissue samples were taken, the rats were sacrificed by an intracardiac blood collection method.

The collected tissue samples were fixed in 10% zinc–neutral formalin for 72 h and embedded in paraffin after routine histological procedures. Four micrometer sections were obtained from each paraffin block and stained with hematoxylin and eosin (H&E). Histological analysis was performed using a light microscope (Leica DM 4000B, Germany). The images were evaluated with the Leica Q Win 3 Software (Cambridge, UK). All of the histological analyses were performed by the same histologist who was blinded to the treatment allotment.

Specimens stained with H&E were assessed to observe the effect of KT on epidermal–dermal distance and edema. For the epidermal–dermal distance measurements, six randomly selected distances were measured for each animal on a series of cross-sections. The mean epidermal–dermal distances for each group were determined by obtaining the mean value of these six measurements. Also, to evaluate edema, a series of cross-sections were analyzed at 40× magnification. Dermis areas of cross-sections were measured first then two edema regions per unit in each series of cross-sections were measured. After all the measurements were taken mean edema areas and edema percentages were calculated.

Nerve growth factor (NGF) is a small protein secreted by tissues, which controls the excitability of nociceptor fibers by changing their expression of key sodium channels, receptors and neuropeptides involved in transmission of painful stimuli (Atherton et al, 2006). In a wide variety of experimental inflammatory conditions, many pain mediators were secreted and one of them is NGF. NGF levels rapidly increase in inflamed tissue (McMahon, 1996). After an injury, administration of exogenous NGF has been shown to produce hyperalgesia in rats and humans, and also the most direct evidence for sensitization of cutaneous afferents by NGF was demonstrated in electrophysiological recordings of nociceptors. Therefore, in the adult, NGF is essential for the induction of inflammatory pain, and therefore it is used in pain research (Wu et al, 2009). In this study, nerve growth factor antibodies were used for pain assessment. In addition, during tissue repair, it is essential for the elimination of

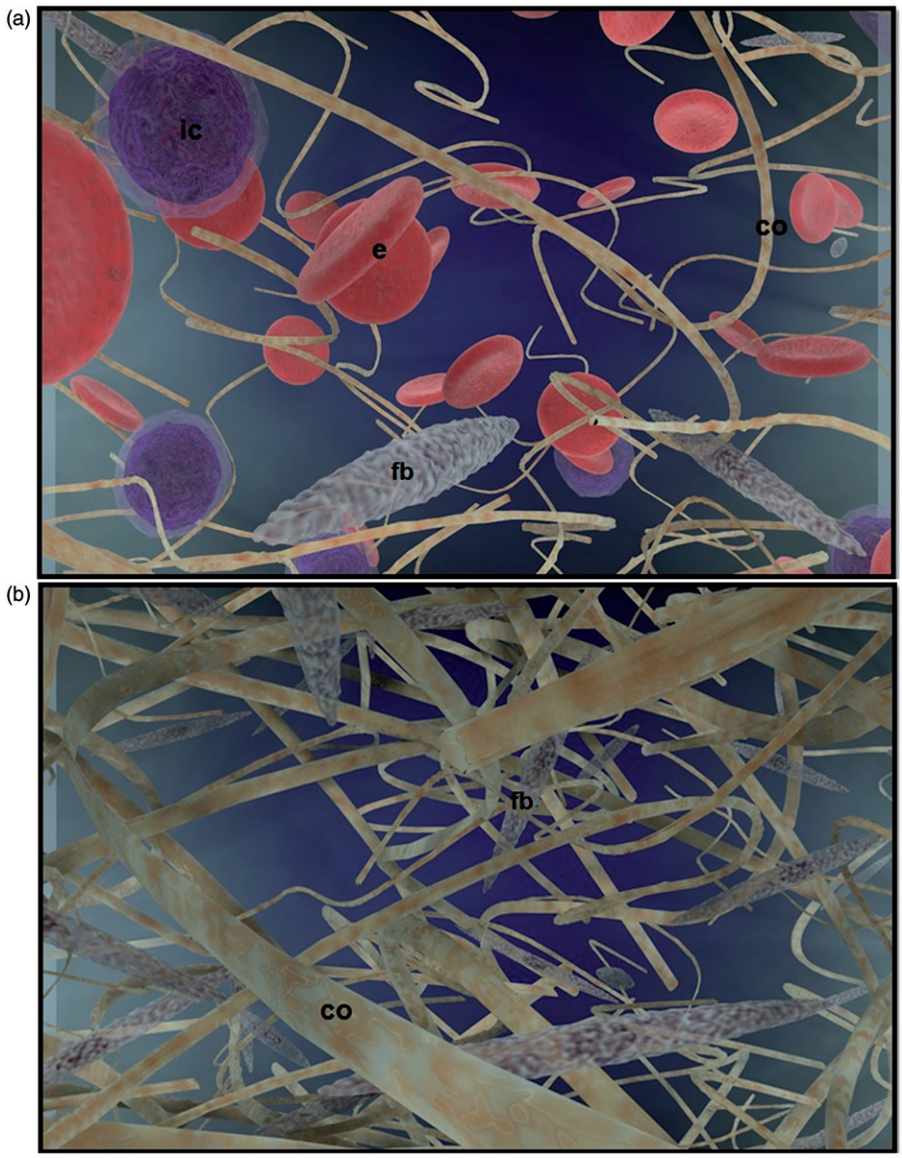

Figure 2. Three-dimensional image from the group received for 6 h: At the side without tape, collagen fibers were thinner and disordered (co). Additionally, hemorrhagic infiltration (e) and lymphocyte infiltration (ic) were observed (a). At the side with tape for 6 h, it could be observed that three dimensional structures of collagen fibers were protected (co) and there was not any infiltration hemorrhagic and lymphocyte infiltration (b). fb, fibroblast.

inflammatory cells in the early phases of healing. Regulation of these cell populations must be present to down-regulate the process and avoid complications of ''excessive repair''. This regulation is provided by B-cell lymphoma 2 and p53 proteins. Regulation of the proliferative response of inflamed tissue could be assessed by these proteins in immunohistochemical evaluations (Kane and Greenhalgh, 2000). An antibody of B-cell lymphoma was also used to assess the inflammation process. Therefore, obtained tissues were stained immunohistochemically using the avidin–biotin peroxidase method with NGF, and B-cell lymphoma 2 (Bcl-2) antibodies to measure the effects of KT on pain and the tissue healing process.

Results

When KT applied sides were compared to controls, it was observed that following both 30 min and 6-h tape application, the integrity of the dermis and the three-dimensional alignment of the collagen fibers was preserved (Figure 2). The epidermal–dermal distance was found to be significantly increased in KT applied sides in both groups compared to control sides ($p < 0.05$; Table 1 and Figure 3). Epidermal–dermal distance in the 6-h application group was also significantly higher than in the 30-min group ($p < 0.05$; Table 1).

Table 1. Intra and inter group comparisons of the epidermal–dermal distances (μm).

	Control side Median (IQR)	Taped side Median (IQR)	*p*
30 min group	45.81 (28.36–69.80)	64.35 (46.37–84.53)	0.001*
6 h group	58.50 (49.28–87.91)	152.26 (111.22–273.72)	0.001*
p	0.07	0.001*	

IQR: Inter quartile range, Mann–Whitney U test.
*$p < 0.05$.

In all the groups, the edematous areas were found to be decreased compared to the sides without KT. However, it was observed that the decrease in the edema area was statistically significant both in the 30-min and 6-h KT applied group ($p < 0.05$; Figure 4).

When we evaluated the immunohistochemical findings, there was a proportional decrease in Bcl-2 relative expression of KT applied sides after trauma. The most significant decrease was found in the 6-h KT applied group (Figure 5). In addition, when compared to controls, NGF relative expression showed a

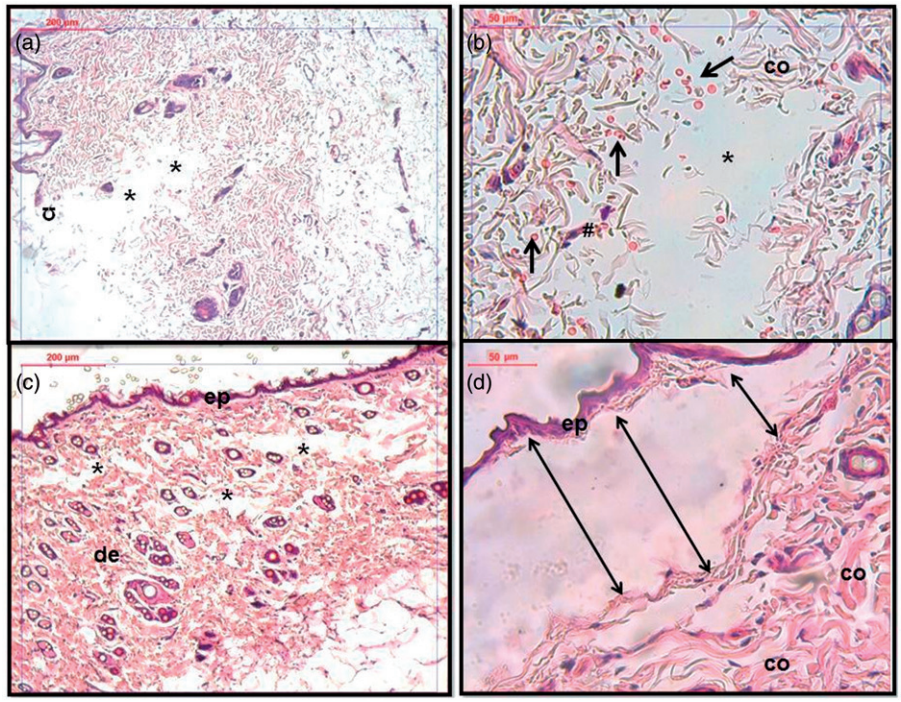

Figure 3. After tissue injury, group received 6 h tape: at the control side, there were edematous areas (*) (a). Hemorrhagic infiltration and lymphocyte infiltration at various levels of the dermis (#) was observed. Collagen fibers were thinner and disordered (co) (b). At the taped side, the integrity of the epidermis (ep), dermis (de) was preserved. There were minimal edematous areas (*) (c). Also the alignment of the collagen fibers (co) were preserved and there was an increase in epidermal-dermal distance (↕) (d) (Haematoxylin and Eosin).

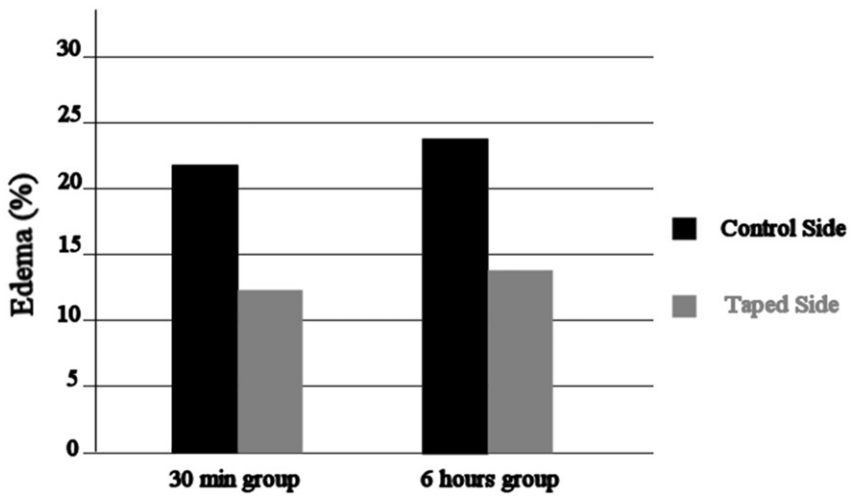

Figure 4. Percentage of area of edema in the dermis.

significant decrease in KT applied sides (Figure 6) in both groups. Also, the largest decrease in NGF relative expression was found in the 6-h KT applied group.

Discussion

In this study, it was histologically shown that after KT application on animal subjects following trauma, the integrity of the dermis and the three-dimensional alignment of the collagen fibers was preserved. The epidermal–dermal distance increased and the width of the edematous area was found to be decreased in taped sides compared to controls. It was also shown that application of KT decreased NGF Bcl-2 immune reactivity, and that these responses were greater with a longer tape application.

Following soft tissue trauma, all layers of the rats' skin (epidermis, dermis and hypodermis), the integrity of the dermis, and the three-dimensional alignment of the collagen fibers were preserved in the sides that received KT compared to controls. According to these results, KT application after soft tissue trauma seems to provide protection of structure of connective tissue in animal subjects. As there is no study that assesses the effect of tape on skin and underlying connective tissue on humans, these findings were not discussed with the literature.

It is indicated that, KT creates convulsions that are theorized to lift the skin. Kase, Willis, and Kase (2003) reported that KT provides a constant pulling force to the skin and it provides a restoration of superficial and deep fascia function by increasing epidermal–dermal. However, they did not provide any original data to support this theory and also there is no study to prove this effect. In this study, convulsions on the epidermis were only observed on the taped sides. With the application of KT, the distance between the dermis and epidermis was found to significantly increase at 30 min approximately 1.5 times; and at 6 h approximately three times compared to the sides without tape. This result supports the hypothesis that tape increases the distance

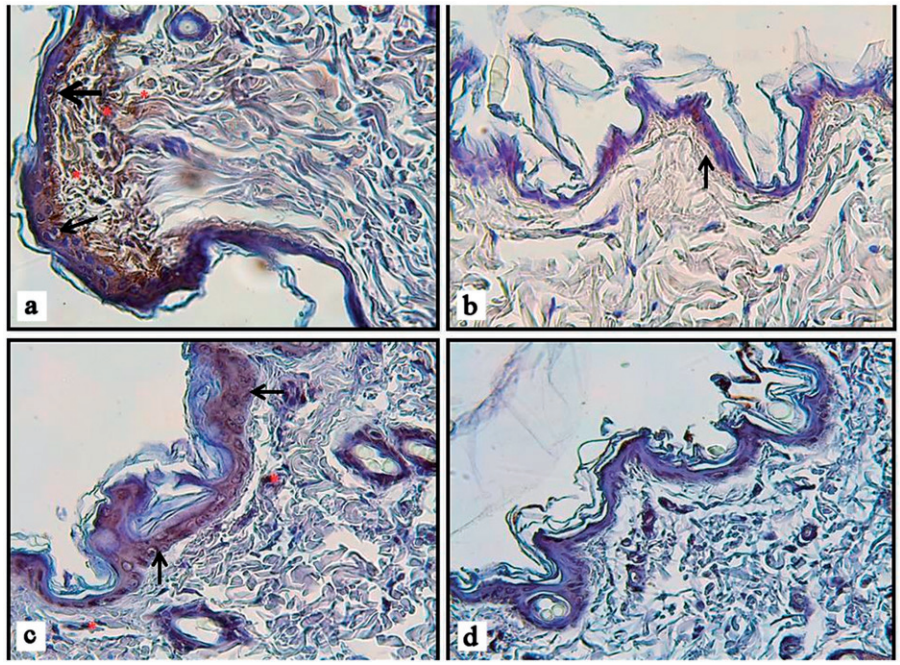

Figure 5. B-cell lymphoma 2 (Bcl-2) relative expression of the groups: at taped sides, increased Bcl-2 relative expression was seen in epidermal cell cytoplasm (↑) and dermal cells (*) (a,c). Bcl-2 relative expression was decreased in taped sides (b,d) especially after 6-h application (d). (DAB, haematoxylin 400×).

between skin and subcutaneous tissue. When taped groups are compared, epidermal–dermal distance is greater in the 6-h taped group. This suggests that the taping effect is directly proportional with time. With these results, it may be said that the KT really creates convulsions on the skin and increases epidermal–dermal distance. Therefore, this study may serve as a scientific answer to whether this hypothesis is correct. In addition, it may also be interpreted that after KT application, observed greater epidermal–dermal distance may lead to a further reduction in congestion of lymphatic fluid or hemorrhages under the skin.

Kase, Willis, and Kase (2003) indicated that KT increases the absorption of large and small molecules in the interstitial area. Shim, Lee, and Lee (2003) investigated the effect of KT with passive exercise on lymphatic flow in rabbit hind leg. The results showed that KT application with passive exercise increased lymphatic flow and they concluded that KT could be a treatment option in cases of peripheral lymphedema. In this study, less edema formation was observed on the side with the 6-h taped group. This result supports previous studies that indicate KT reduces edema effectively, especially when it is applied for 6 h. Nevertheless, there is a need for studies evaluating edema in humans.

Kase, Willis, and Kase (2003) affirmed that KT deforms and stimulates large-fiber cutaneous mechanoreceptors that may inhibit nociceptive impulses in the spinal column and decrease pain via an ascending pathway. In literature, there are clinical studies investigating the effect of KT on pain, however they have discrepancies (Aytar et al, 2011; Karatas, Bicici, Baltaci, and Caner, 2012; Montalvo, Le Cara, and Myer, 2014). In addition, there is no study investigating KT on pain following a muscle contusion injury. In this study, pain was assessed by NGF relative expression. NGF is an important pain mediator in skin (Wu et al, 2009). It was hypothesized that NGF plays a key role in nociception and that several hypo- and hyper-algesic situations directly belong to altered levels (Atherton et al, 2006; McKelvey, Shorten, and O'Keeffe, 2013). In the light of this information, after a trauma, NGF involvement and possible pain sensation are expected to increase. In this study, it was shown that NGF involvement is decreased in taped sides in both groups. Thus, KT may have a positive effect on pain reduction. However, pain is a subjective parameter and could not be directly assessed. Therefore, this result should be supported by evidence based clinical studies. Also, further clinical studies investigating the effect of KT on pain using other pain mediators are needed.

During tissue repair, regulation of cell populations must be present. It is essential for the elimination of inflammatory cells in the early phases of healing. Since tissue repair involves rapid but controlled increases in cell proliferation, it makes sense that Bcl-2 would be involved because immediately after trauma, Bcl-2 increases to allow for the cellular proliferation. With time, Bcl-2 levels decrease to shut down the inflammatory process and down-regulate the proliferative response (Kane and Greenhalgh, 2000; Kolt, 2004). In the current study, the effect of taping on the post-traumatic soft tissue inflammation process was determined by Bcl-2 relative expression. Bcl-2 relative expression was shown to decrease in both taped sides. This reduction was found to be more obvious in the group that received tape for 6 h. These findings support the idea that KT reduces inflammation after soft tissue trauma. It may also be concluded that KT helps the reconstruction of damaged tissue in animal subjects. However, studies evaluating the inflammatory process in humans are needed.

Limitations

Limitations included a restricted implementation period of anesthesia in rats (maximum of 6 h), a limited number of rats allowed by the ethics committee (maximum of 6) and failure to follow effects of the tape after removal.

Conclusion

Following soft tissue trauma in animal subjects, application of KT seems to protect the integrity of the dermis and the three-dimensional alignment of the collagen fibers. In addition, KT application on rats' skin increases epidermal–dermal distance and decreases width of the edematous area. It also reduces the NGF and Bcl-2 immune reactivity, which may be interpreted as possibly reducing the sensation of pain and helping reconstruct the tissue after trauma. These findings suggest a potential benefit from the application of tape in clinical care; however, the response in a contusion model may not represent the response in other types of injuries (e.g. sprain/strain). In addition, it would not

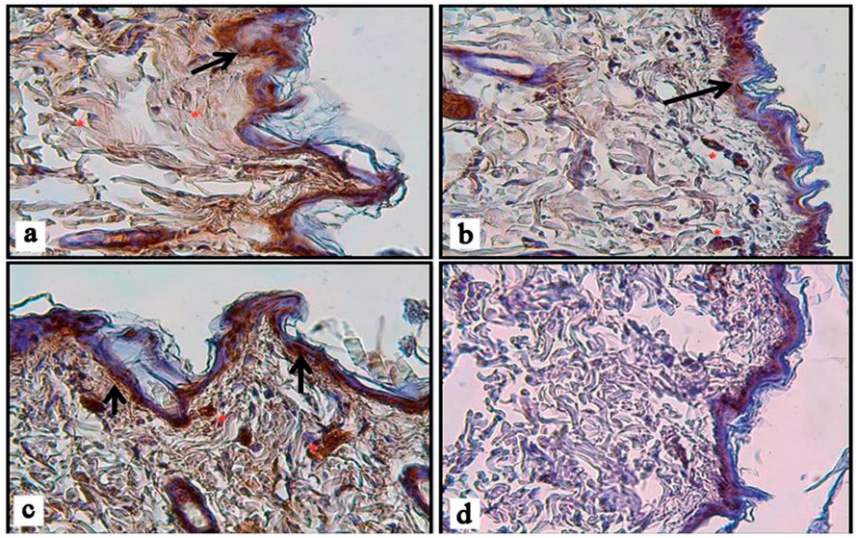

Figure 6. Nerve Growth Factor (NGF) relative expression of the groups: A positive NGF immune staining was seen in control sides (↑) (*) (a,c), NGF immune staining involvement was decreased in groups with KT (b,d) (↑) (*) especially after 6-h application (d). (DAB, haematoxylin 400×).

be possible to assess the tissue histology in humans but other markers could be used that may help further understand injury management of taping. Therefore, these results would offer plausible explanations; however, in order to generalize these results to treatment of acute musculoskeletal conditions in humans, further evidence based clinical studies are needed to obtain more clear results. Although these results may not reflect the reactions seen in clinical studies, this study is important to shed light on effects of KT on soft tissue after trauma.

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Declaration of interest

All authors have no conflicts of interest with respect to the data collected and procedures used within this study. This research was supported by Gazi University Scientific Research Projects Unit (Project no: 47/2012–01).

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