Reduction of adhesion formation and promotion of wound healing after laminectomy by pharmacological inhibition of pro-inflammatory cytokines: an experimental study in the rat

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Abstract In a previous experiment using TNF inhibition in the rat it was accidentally found that adhesion and scar formation was reduced compared to previous experience. Wound and bone healing also seemed enhanced. The present study was conducted to assess if this observation could be verified in a controlled setting using a standardized laminectomy in the rat. Five rats received doxycycline and five other rats received saline and served as control. Macroscopic blinded evaluation 1 week after the laminectomy revealed that adhesion and scar formation was less in doxycycline-treated animals than in control animals. Wound and bone healing was found to be better in doxycycline-treated animals. The mechanisms for the observed effects cannot be fully understood but the data indicate that further research may lead to opportunities to design pharmacological modalities to reduce adhesion and scar formation, maybe in combination with suitable barriers.

Keywords Wound healing · Adhesion · Cytokine · Spine · Rat

Introduction

Spinal surgery may usually induce various degrees of scar tissue and adhesion formation in the epidural space. This may induce problems if repeated surgery is needed [1], and the scar tissue per se has even been suggested to be able to

induce compression of the intra spinal nervous tissues [2]. Various attempts have been undertaken to control and reduce such scar tissue formation. Most common is the intra-operative placement of certain barriers, either by other tissues such as fat grafts [3] or by applying specially developed compounds [4]. The end results are, however, not entirely satisfactory and may even produce adverse effects per se [5]. When performing animal surgery with simultaneous treatment with an inhibitor of tumor necrosis factor (TNF), it was noticed that epidural scar tissue formation and adhesions were remarkably reduced when compared to previous experimental experience. It even seemed that wound healing had been enhanced, particularly bone healing. TNF is a pro-inflammatory cytokine and inhibition of this cytokine would induce an anti-inflammatory action. It seems surprising that inhibition of the normal inflammatory response, which has been considered crucial for normal wound healing, would induce the observed effects. The present pilot study was undertaken in order to study if inhibition of pro-inflammatory cytokines in general would induce reproducible reduction of scar tissue formation and adhesion formation as well as to study the effects on wound healing in a controlled experimental setting. Thus, instead of using a selective TNF inhibitor, the non-selective cytokine inhibitor doxycycline was chosen.

Materials and methods

Ten female Sprague–Dawley rats with an average body weight of 225 g were housed in groups with free access to food (B&K Rat/mouse standard, BeeKay Feeds & Beddings, Sollentuna, Stockholm) and tap water. Temperature was kept at 21°C, light schedule was 12 h daylight starting

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at 6:00 a.m. and 12 h darkness starting at 6:00 p.m., and the humidity was kept at 50%. The rats were anesthetized by inhalation of isoflurane and the experimental protocol was approved by the local animal ethics committee.

The skin on the back was shaved. Through a midline incision, a laminectomy of the fourth lumbar vertebra was performed with a rongeur. Letting the width of the rongeur to decide the width of the defect, the laminectomy defect was standardized. The spinal muscle fascia and skin was sutured. Five rats received an intra-peritoneal injection of 3.0 mg/kg doxycycline (Doxyferm[®], Nordic Drugs, Limhamn, Sweden). This dose corresponds to the dose of doxycycline for clinical use in humans. The other five rats received an intra-peritoneal injection of saline (control). After 1 week the rats were re-anaesthetized and wound healing, scar formation and adhesions were macroscopically graded according to a semi-quantitative scale by a person being unaware of the experimental protocol.

Results

The data from the macroscopic evaluation are shown in Table 1. In general, the saline-treated (control) animals displayed similar scar formation and wound healing as known from previous experimental experience. In the doxycycline-treated animals, however, skin, fascia and muscle healing seemed to be clearly improved compared to the saline-treated animals. In saline-treated animals there was usually a scar in the center of the laminectomy defect that was more organized than in the doxycycline-treated animals (Table 1; Fig. 1a). This scar was sometimes adherent to the underlying dura (Fig. 1b). The epidural fat seemed to be “reactive” and harder in the saline-treated animals. Since this was not anticipated, this finding was never graded. In doxycycline-treated animals there was a clear impression that the laminectomy defect was almost healed, contrary to the saline treated animals where the defect seemed to have the same width as at the day of surgery (Fig. 1c). Similar to the fat changes, this was not anticipated and was thus not graded. When extending the laminectomy in doxycycline treated animals, the soft “scar” usually formed a fat “droplet” that was not adherent to the dura (Fig. 1d).

Discussion

Data from the present study indicate that a reproducible reduction in scar formation/adhesion and an enhanced wound healing may be obtained following an experimental laminectomy after treatment by a non-specific inhibitor of pro-inflammatory cytokines in a rat model.

Table 1 Macroscopic evaluation of tissue changes

Skin	Fascia	Muscle	Consist.	Adh. dura	Adh. bone	Inflam.
Control						
0	0	++	++	++	++	(+)
(+)	(+)	++	+	0	++	0
(+)	(+)	++	+	(+)	++	(+)
(+)	(+)	++	++	++	++	(+)
0	0	++	+	(+)	++	(+)
Doxycycline						
0	0	0	(+)	0	(+)	(+)
0	(+)	++	(+)	0	(+)	0
0	0	(+)	(+)	0	(+)	0
0	0	++	(+)	0	(+)	0
0	0	(+)	(+)	0	(+)	0

Skin (healing of the skin incision): 0, good healing; (+), slight diastasis; +, pronounced diastasis; ++, infection

Fascia (healing of fascia) and muscle (healing of muscle): 0, good healing; (+), slight diastasis; +, clear diastasis; ++, hematoma or infection with loss of contact

Consist. (consistency of the scar in the laminectomy space): 0, like water; (+), a gel-like clot that easily breaks; +, gelatinous but could be handled without breaking; ++, soft scar; +++, hard scar

Adh. Dura (adhesion of the scar to the spinal dura) and Adh. bone (adhesion of the scar to the bone): 0, no adhesion; (+), only slight adhesion that breaks with minimal force; +, pulling of the scar will move the dura but the adhesion will easily break; ++, adhesion to the dura/bone that will not break using slight force; +++, scar firmly adhered to the dura/bone and difficult to separate

Inflam. (Inflammatory reaction in the laminectomy space): 0, no inflammation; (+), slight inflammation; +, intra spinal structures hyperemic and swollen

Wound healing is generally a positive physiological event that may restore the anatomy and function of various tissues following trauma. The ideal end result of wound healing would be to restore the tissues to the situation, before the trauma. One important part of the wound healing process is to form connective tissues or scar tissue that may support the healing tissues during wound healing and regeneration. However, in many cases during wound healing the newly formed connective tissues (scar tissue) may interfere negatively with the normal function of the healing tissues. Following abdominal and gynecologic surgery it is not uncommon that the surgical procedure per se may induce adhesions that may both make later surgery more difficult and even induce pathological conditions such as ileus or infertility [6–8]. Spinal surgery may often result in a dense scar formation called epidural fibrosis. This may in certain case induce significant difficulties for repeated surgery and has even been suggested to induce compression of the adjacent nerve tissue per se [2, 9]. A method for controlling wound healing, particularly the formation of scar tissue and adhesions, would be of a great

Fig. 1 **a** The opened spinal canal one week after surgery in a control rat. On the dura mater *1* there is a visible soft scar *2* that was formerly in the laminectomy defect. The cut pedicles *3* are visible for orientation. **b** When light force is applied to the scar (from **a**) by a forceps (an *arrow* is indicating the direction of the force) the scar remains attached to the dura. The epidural fat surrounding the scar seems to be “reactive” and harder than found in naïve rats (*asterisks*). **c** The laminectomy defect in a doxycycline treated rat. The dura and

spinal cord *4* may be seen through the gelatinous filling of the laminectomy defect. A bridging of bone over the laminectomy defect has occurred *5*. The two *white lines* represent the former borders of the laminectomy. Central to these lines newly formed bone may be seen *6*. **d** When the spinal canal is exposed by removing newly formed bone on the lower aspect (left side in the animal) the gelatinous scar forms a fatty droplet *7* that is not attached to the dura. The *black line* indicates the former border of the laminectomy defect

value in most of the cases of post-traumatic and post-surgical wound healing.

Many attempts have been done in order to control scar formation. There are a number of methods relying on barriers of various properties and there has been administration of a substantial number of bioactive substances [3, 4, 10]. However, the results have not been entirely satisfactory. Since the fibroblasts are responsible for producing collagen much attention has been drawn to the regulation of the fibroblasts in order to reduce scar formation [11–13]. Transforming growth factor (TGF), which is an anti-inflammatory cytokine, and fibroblast growth factor (FGF) are known to stimulate the fibroblasts to produce collagen [14–16]. Attempts have been made to administer various TGF-inhibitors for this purpose with promising results [17–19]. However, there is at date no established treatment with an anti-TGF compound. TNF is generally considered to reduce collagen production from fibroblasts in vitro systems [20, 21] but may interact with other pro-inflammatory cytokines such as IL-1 and IFN-gamma to change its activity [12, 22, 23].

The present study is a preliminary pilot study performed in order to assess if the accidental observation that wound

healing rate seemed increased and that scar formation seemed reduced following simultaneous treatment with an inhibitor of a pro-inflammatory cytokine, TNF. The data certainly support this initial observation but the information is too limited to allow for any general conclusions. Doxycycline is basically an antibiotic but since it has metal-ion binding properties it may inhibit the action of matrix metalloproteinases that activates certain cytokines by shedding them from the cell membrane in their former inactive form [24, 25]. In this sense, doxycycline also acts as a non-specific inhibitor of other pro-inflammatory cytokines such as TNF, IL-1, IL-6 and IFN-gamma [26, 27].

The mechanisms for the observed effects cannot be fully understood. As previously discussed inflammation is generally considered to be beneficial to the wound healing. It is also known that pro-inflammatory cytokines inhibit the action of fibroblasts in vivo. To inhibit the inflammatory response in order to enhance wound healing and reduce adhesion formation may therefore seem contradictory. The mechanism for the observed effects should probably not be sought for at the level of the fibroblasts and collagen production. One may instead speculate that an inhibition of

certain pro-inflammatory cytokines may reduce the local inflammatory response in the wound area. This would theoretically result in a reduced extravasation of inflammatory cells and probably a subsequent less pronounced activation of the fibroblasts by, for instance various growth factors. This would in turn lead to less production of collagen. Another not so elaborate explanation would be based on the balance between growth factors and inflammatory cytokines. It is known that there is a balance between these two groups of substance in the way that they counteract each others activity [28, 29]. Inhibition of the inflammatory cytokines might therefore result in a relative increase in activity of certain growth factors. This shift in balance could thus theoretically result in increased regeneration. The increased rate of wound healing and bone formation could thus relate to increased activity of growth factors on the expense of the inflammatory cytokines thus being pharmacologically inhibited. However, further experiments must be conducted to fully understand the mechanisms present in the present experimental set-up.

In conclusion, the present pilot investigation may indicate that an inhibition of pro-inflammatory cytokines may increase wound-healing rate and reduce scar formation/adhesion. However, the mechanisms present requires further studies to be understood. It nevertheless seems possible to be in the future design of potent pharmacological treatment modalities to reduce post-surgical adhesion formation and scarring, maybe in combination with physical barriers.

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Conflict of interest statement None.

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