

# Inhibition Of Tumor Necrosis Factor May Improve Wound Healing And Reduce Scar Formation Following Laminectomy. A Pilot Study In Pigs.

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## Abstract

In rats treated with a selective TNF-inhibitor it was accidentally found that wound healing was improved and scar/adhesion formation was reduced. The present pilot study was performed to evaluate if this could be repeated in a controlled setting using a laminectomy model in the pig. Following anesthesia a laminectomy of all sacral vertebrae was performed in 6 pigs. Three pigs were treated with infliximab (a selective TNF inhibitor) and three pigs received saline and served as control. After 7 days the laminectomy site was evaluated by a macroscopical analysis and specimens of skin and muscle tissue over the laminectomy were processed for histology. Skin, fascia, muscle and bone healing was remarkably better in the pigs treated with infliximab. In pigs treated with infliximab there were signs of a partial healing of the laminectomy defect and in the centre of the defect there was a gel-like tissue that allowed for observation of the underlying nervous tissues. The laminectomy defect in saline treated animals had less bone formation and a soft scar in the centre of the defect. The present pilot investigation may thus indicate that an inhibition of pro-inflammatory cytokines may increase wound-healing rate and reduce scar formation/adhesion. However, further studies that evaluate exact mechanisms, when and how long to use anti-inflammatory treatment, long term effects, wound quality, risks for infection, if this may be translated to humans, must be undertaken before any conclusions regarding a clinical use may be drawn. The study nevertheless implies that it might be possible to develop a biological agent that alone or in combination with a mechanical barrier may reduce epidural scarring following spinal surgery.

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## CONFLICT OF INTEREST

The author is part owner and part employee of Pharmasurgics AB, Gothenburg, Sweden, a company that is developing pharmacological compounds for improving post-surgical wound healing.

## INTRODUCTION

It is common that there will be various degrees of scar tissue formation and adhesions following spinal surgery. This may induce problems if repeated surgery is needed and the scar tissue per se has even been suggested to be able to induce compression of the intraspinal nervous tissues per se (1-6).

There have been many recent attempts to control and reduce scar tissue formation. Most common is the intra operative placement of certain barriers, either by other tissues such as fat grafts or by applying specially developed compounds or even using irradiation (7-19). The outcomes have, however, not been entirely satisfactory. When harvesting nervous tissues 3 weeks after performing animal surgery with simultaneous treatment with a selective inhibitor of Tumor Necrosis Factor (TNF) in the rat, it was surprisingly noticed that epidural scar tissue formation and adhesions were remarkably reduced compared to previous experimental experiences. It even seemed that the wound-healing rate had been increased. TNF is a pro-inflammatory cytokine that mediates various inflammatory events. Inflammation is generally considered to be an integral part of the wound healing but this accidental finding thus seemed to imply that inhibition of the inflammation might be beneficial for the healing process. The present pilot study was undertaken in order to study if inhibition of the pro-inflammatory cytokine TNF would induce a reproducible reduction of scar tissue

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and adhesion formation as well as to study the effects on wound healing, in a controlled experimental model of laminectomy in the pig in a limited number of animals.

## MATERIAL AND METHODS

Six pigs (body weight app. 25 kg) received an intra muscular injection of 20 mg/kg body weight of Ketalar (ketamine 50mg/ml; Parke-Davis, Morris Plains, New Jersey), an intravenous injection of 20 mg/kg body weight of Hypnodil (methomidate chloride 50 mg/ml; AB Leo, Helsingborg, Sweden), and 0.1 mg/kg body weight of Stresnil (azaperon 2 mg/ml; Janssen Pharmaceutica, Beerse, Belgium).

Anesthesia was maintained by additional intravenous injections of 2mg/kg body weight of Hypnodil and 0.05mg/kg body weight of Stresnil. The pigs also received an intravenous injection of 0.1mg/kg of Stesolid Novum (diazepam, Dumex, Helsingborg, Sweden) after surgery.

Following a midline incision, a laminectomy of the sacral vertebrae was performed. The laminectomy was standardized in the sense that it comprised all sacral vertebrae and was extended laterally to the pedicles. The muscles and skin were sutured. Three pigs received infliximab (Remicade®, Schering-Plough, Stockholm, Sweden; 4mg/kg) intravenously and three pigs received saline. After 7 days the pigs were reanaesthetized and killed 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. Tissue samples of skin and spinal muscles at the incision site were collected and processed for light microscopy.

The experimental protocol was approved by the local animal ethics committee.

## RESULTS

The data from the macroscopic evaluation are shown in table 1. The data clearly demonstrate that skin, fascia, muscle and bone healing was remarkably better in the pigs treated with infliximab. The scar in the laminectomy defect was less hard and less attached to the underlying nerves in the infliximab treated than in the saline treated animals (Table 1).

**Figure 1**

Table 1

	Skin	Fascia	Muscle	Bone h.	Consist.	Scar adh.
Control	++	+	+	++	+	+
	++	++	++	++	++	++
	(+)	++	(+)	+	(+)	0
Infliximab	(+)	(+)	0	+	(+)	0
	(+)	0	0	+	(+)	0
	(+)	(+)	0	(+)	(+)	0

Skin (healing of the skin incision):

0 = good healing, (+) = slight diastasis, + = pronounced diastasis, ++ = pronounced opening and/or infection

Fascia (healing of fascia) and Muscle (healing of muscle):

0 = good healing, (+) = slight diastasis, + = pronounced diastasis, ++ = hematoma or infection with loss of contact

Bone h. (bone healing):

0 = laminectomy healed, (+) = more than 50% of the laminectomy healed, + = 25-50% of the laminectomy healed, ++ = less than 25% healing

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

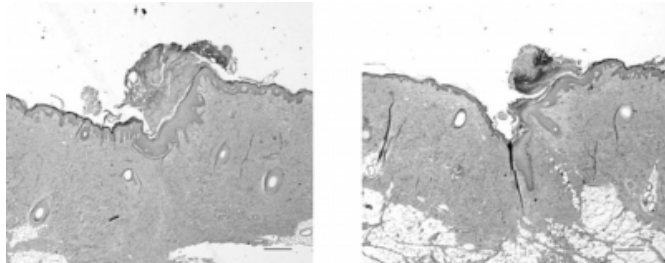
Scar adh. (adhesion of the scar to the spinal dura):

0 = no adhesion, (+) = gel-like adhesion, + = firm adhesion that is easily breakable, ++ = firm adhesion

Light microscopical analysis demonstrated that skin healing was similar between the two groups in the part of the scar where there was no diastasis (Figure 1).

**Figure 2**

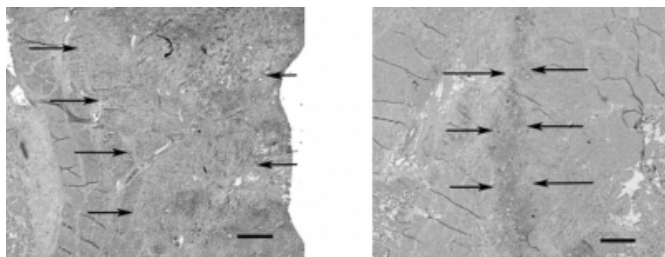
Figure 1 - skin healing: Both displayed specimens were taken where there was no diastasis of the wound. There are no remarkable differences between animals treated with saline (left) and infliximab (right) at this location of the wound. (Bar = 100Åµm, Htx-eosin).



The scar tissue in the underlying muscle was much wider in saline treated than in infliximab treated pigs (Figure 2). In the biopsies obtained from saline treated pigs there was seldom any normal muscle tissue encountered. Instead the biopsies mainly contained scar tissue. However, in infliximab treated pigs the scar was narrower and normal muscle tissue could be found outside the scar within the biopsies.

**Figure 3**

Figure 2 - muscle healing: The scar is much wider in the saline (left) treated animals than in the infliximab treated (right) animals, as indicated by the arrows. Normal muscle tissue is found just lateral to the scar in the infliximab treated animals. (Bar = 100Åµm, Htx-eosin).



The macroscopical appearance of the laminectomy site in pigs treated with saline revealed a laminectomy defect with less bone formation and a soft scar in the centre of the defect (Figure 3). Contrary, in pigs treated with infliximab there were signs of a partial healing of the laminectomy defect and in the centre of the defect there was a gel-like tissue that allowed for observation of the underlying nervous tissue.

**Figure 4**

Figure 3 - laminectomy defect: In saline treated animals (left) there is not so much bone growth and the laminectomy is almost intact. The borders of the bone growth into the laminectomy defect is indicated by white arrows. Contrary, infliximab treated animals (right) there is a clear indication of bone growth and that the former laminectomy defect is partly being overgrown. In the center of the defect there is a gel-like tissue and the nervous tissue of the spinal canal could be seen. (Left is cranial in the pictures).



## **DISCUSSION**

Data from the present study indicate that a reproducible reduction in scar formation/adhesion and improved wound healing may be obtained in a laminectomy model in the pig after treatment by infliximab, a selective inhibitor of TNF.

In general, wound healing is a positive physiological reaction that may restore anatomy and function of various tissues after trauma. The trauma may be accidental or the result of surgical intervention. The ideal end result of wound healing should 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 the newly formed connective tissues (scar tissue) may interfere negatively with the normal function of the healing tissues. Wound healing with formation of new connective tissues may also induce adhesions that may induce pathological conditions per se. Adhesions and scarring may also reduce the possibilities of later surgical intervention of the injured tissue if needed. Examples of adhesions and scarring may be found virtually in any organ or tissue undergoing wound healing after trauma or surgery. Following abdominal surgery and following gynaecological 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 and infertility (20-24). In spinal surgery it is common that surgery per se induces a dense scar formation called epidural fibrosis. This may in certain cases induce significant difficulties for repeated

surgery and has even been suggested to induce compression of the adjacent nerve tissue in certain cases (1-6). A method for controlling the wound healing, particularly the formation of scar tissue and adhesions, would be of a great value in most cases of posttraumatic or post surgical wound healing.

The role of inflammation in wound healing is acknowledged but not fully understood. In general it has been assumed that the inflammatory reaction is beneficial for the healing process since it increases blood flow to the injured site and also the permeability of the blood vessels, thus allowing various cells to invade the injured area. The fact that inflammation is present per se strongly suggests that this is a crucial part of the wound healing and one may suspect that interference could possibly reduce the wound healing rate and also the quality of the healed wound. However, there has been some early attempts to improve wound healing by reducing the inflammation (25, 26) and there is also recent work indicating that monitoring inflammation at the molecular level may be beneficial in certain cases of wound healing (27-29).

The present study is a preliminary pilot study performed in order to see if the accidental observation that wound healing seemed to be improved and that scar formation seemed to be reduced following simultaneous treatment with a selective inhibitor of a pro-inflammatory cytokine, Tumor Necrosis Factor (TNF), could be repeated in a controlled setting. A similar study was recently performed in rats with a non-specific TNF-inhibitor (doxycycline) and showed that 1 week following laminectomy there was less scar formation and adhesions in the doxycycline treated rats than in the control rats (30). In the present pilot study, a selective TNF-inhibitor was selected (infliximab) and administered to a larger animal. The data from the study further support that selective inhibition of the inflammatory response during wound healing may reduce adhesion formation and possibly enhance wound healing. However, the number of animals is too small to allow for any general conclusions. Another limitation is that the study only evaluates the effects after one week. It is therefore questionable if the observed scar tissue is readily transferable to a clinically manifest epidural scar. The wound healing processes is much longer and there are other mechanisms contributing to wound strength and scar formation that may occur later that could not be studied with the duration used in this study. Later studies may also employ various functional tests such as biomechanical testing of wound strength.

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, further studies that evaluate exact mechanisms, when and how long to use anti-inflammatory treatment, long term effects, wound quality, risks for infection, if this may be translated to humans, must be undertaken before any conclusions regarding a clinical use may be drawn. The study nevertheless implies that it might be possible to develop a biological agent that alone or in combination with a mechanical barrier may reduce epidural scarring following spinal surgery.

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