

Formation Of Nucleus Pulposus-Induced Disc Hernia-Like Nodules On The Disc Surface Is Not Induced By TNF.

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Citation

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Abstract

It was recently seen that puncture of an intervertebral disc in the rat induced disc hernia-like nodules over the puncture site and that the presence of nucleus pulposus was necessary to produce such nodules. Since TNF from the nucleus pulposus have been found to be involved in pain production the aim was to see if also TNF was involved in the formation of such nodules. Two TNF-inhibitors, infliximab and methotrexate, were administered to rats following disc puncture. Non-treated animals were used as controls. Macroscopic evaluation after one and two weeks did not demonstrate any notable differences between non-treated and treated animals. The data thus indicate that TNF is not involved in the formation of nucleus pulposus-induced disc hernia-like nodules on the disc surface. This may instead be induced by other bioactive substances derived from the nucleus pulposus, such as growth factors. However, further studies with direct administration of TNF-inhibitors must also be performed to understand the contribution of TNF in this regard.

CONFLICT OF INTEREST

The author is CEO and part owner of the company Sciaticon AB that holds patent on pharmacological treatment of low back pain and sciatica by TNF-inhibition.

INTRODUCTION

It was recently understood that puncture of an intervertebral disc in the rat produced disc hernia-like nodules over the puncture site(1). Since there were no nodules formed in connection with the puncture and that the nodules, including osteophytes on the adjacent vertebrae, seemed to appear some time later, it was suggested that the nodules were formed by the action of bioactive substances derived from the nucleus pulposus. A later study demonstrated that the nodules also appeared if nucleus pulposus was placed on the disc surface without simultaneous disc injury from a disc puncture, whereas nodules did not appear after superficial disc injury without nucleus pulposus leakage or the application of fat tissue(2). These data further supported the assumption that substances derived from the nucleus pulposus were involved in the formation of these nodules. In previous investigations on the pathophysiology of sciatica and low back pain it has been found that nucleus pulposus is intimately involved in producing pain, seen as changes by pain behaviour assessments(3-7). One key substance in this regard is TNF(8-13). To assess if TNF is also involved in the

formation of these nucleus pulposus-induced disc hernia-like nodules the effects of two TNF-inhibitors in reducing the formation of such nodules was studied in the same model as used previously.

MATERIAL AND METHODS

A total of 30 Sprague-Dawley rats weighing 200-250g were anaesthetized with isoflurane inhalation (Baxter Medical AB, Kista, Sweden). The left facet joint between the 4th and the 5th lumbar vertebra was removed and the 4th lumbar dorsal root ganglion and the 5th lumbar nerve root, including the intervertebral disc between the 4th and 5th lumbar vertebrae, were visualized. The L4-5 disc was punctured using a 0.4 mm diameter injection needle. After injecting some air into the disc space the puncture typically induced a leakage of nucleus pulposus out into the spinal canal. The spinal muscles were sutured and the skin closed by metal-clips.

Infliximab (n=10): Following disc puncture the rats received one intra peritoneal injection of (4mg/kg) infliximab (Remicade®, Centocor, Leiden, the Netherlands).

Methotrexate (n=10): The rats in this group received an intra peritoneal injection of (3mg/kg) methotrexate (Methotrexate Wyeth®, Wyeth AB, Sollentuna, Sweden) after puncture and one additional injection (3mg/kg) after one week for the

five rats there were supposed to be analysed after 2 weeks.

No treatment (n=10): The remaining rats did not receive any treatment and served as positive controls.

Five rats in each group were killed after 1 week and the remaining rats after 2 weeks. Changes in the spinal canal at the puncture site were evaluated macroscopically via the operation microscope and by probing regarding; fibrosis, inflammation, healing of the disc injury, height and consistency of any nodule over the site of disc puncture, and height of osteophytes on the vertebrae adjacent to the disc. The findings were graded according to a semi-quantitative scale. The evaluations were performed in a blinded fashion.

The study was approved by the local animal ethics committee.

RESULTS

Immediately after disc puncture there was usually nucleus pulposus material leaking out into the epidural space. However, within 1 minute the material was liquefied and no nodule in connection with the puncture was formed. The data from the macroscopic evaluation are displayed in table 1. Fibrosis was not seen after one week in any of the three groups. After two weeks there was mild fibrosis that was slightly more pronounced in the infliximab group as compared to the two other groups. Inflammation was seen only in one rat in the no treatment group after one week. After two weeks inflammation was slightly more pronounced but similar between the groups.

Figure 1

Table 1. Macroscopic findings

1 week:		Disc		Bone
	F	I	h. c.	h
No treatment	0	0	0	0
	0	0	(+)	(+)
	0	0	0	0
	0	0	++	(+)
	0	+	++	(+)
Methotrexate	0	0	(+)	(+)
	0	0	0	0
	0	0	0	0
	0	0	(+)	(+)
	0	0	+	(+)
Infliximab	0	0	+	+
	0	0	(+)	(+)
	0	0	0	0
	0	0	+	(+)
	0	0	+	(+)
2 weeks:		Disc		Bone
	F	I	h. c.	h
No treatment	(+)	0	(+)	(+)
	+	+	++	(+)
	0	0	0	0
	0	0	(+)	(+)
	(+)	0	0	0
Methotrexate	(+)	0	+	(+)
	(+)	++	0	0
	0	0	0	0
	(+)	0	+	+
	(+)	0	(+)	(+)
Infliximab	(+)	(+)	++	(+)
	(+)	0	+	(+)
	+	0	0	0
	+	0	+	(+)
	(+)	0	(+)	(+)

F = Fibrosis, I = Inflammation, Disc h. = Disc height, Disc c. = Disc consistency, Bone h. = Height of formed osteophytes on adjacent vertebrae

F, I, Disc h., Bone h.
 0 = No changes
 (+) = Slight changes
 + = Clear changes
 ++ = Pronounced changes

Disc c.
 0 = Nothing
 (+) = Gel-like
 + = Elastic
 ++ = Hard

Disc height was slightly less in the methotrexate group after one week and slightly more in the infliximab group after two weeks. Disc consistency was similar between the groups although slightly higher in the infliximab group at one week. Bone height (osteophyte formation) was similar between the groups although the bone height was slightly less in the no treatment group and slightly more pronounced in the infliximab group at two weeks.

DISCUSSION

Puncture of an intervertebral disc in the rat produced disc hernia-like nodules over the puncture site similar to the findings in previous studies(1, 2). Treatment with two different TNF-inhibitors did not reduce the formation of such nodules.

It was previously noted that puncture of an intervertebral disc in the rat induced a disc-hernia-like nodule over the puncture site and that the presence of nucleus pulposus was necessary to induce such nodule formation(1, 2). The suggestion was that bioactive substances present in the nucleus pulposus induced the nodule formation. Since TNF derived from the nucleus pulposus has shown to mediate

pain, as observed by pain behaviour recordings in the rat(8-13), the question arose if TNF was involved also in the formation of these disc nodules.

Two different TNF-inhibitors were selected to be used in the study. Methotrexate has been shown to reduce TNF release both in vivo and in vitro(14-16). Infliximab is an antibody to human TNF but has shown to inhibit also TNF in rats(17-20). The doses of the two inhibitors were chosen to correspond to the clinically used doses in humans. Both inhibitors were administered by intra peritoneal injection.

The data from the study demonstrated that the formation of nodules was not reduced by any of the two used inhibitors. This would imply that TNF is not involved in the formation of these nodules and a potential clinical use of a TNF-inhibitor to limit the formation of disc hernia formation is not an option. However, it is not clear if the intra peritoneal administration might have been less suitable and if direct administration into the epidural space would produce different data. Previous studies on pain using the same model utilized intra peritoneal injection as route of administration of infliximab and produced a reduction in pain behaviour(9). However, the effects may have been exerted in vascularized tissues such as dorsal root ganglion and nerve roots. Since the epidural space in the rat is less vascularized one may suspect that the inhibitors had less chance to penetrate into the area of nodule formation.

In conclusion, the data from the study indicate that TNF is not involved in the formation of nucleus pulposus-induced disc hernia-like nodules on the disc surface. This may instead suggest that other bioactive substances derived from the nucleus pulposus, such as growth factors, may be involved in this process. However, further studies with direct administration of TNF-inhibitors must also be performed to possibly rule out the role of TNF in this regard.

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