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Early effects of nucleus pulposus application on spinal nerve root morphology and function

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Abstract It is known that 24 h or more after epidural application of autologous nucleus pulposus, functional and structural changes are established in adjacent nerve roots. It is, however, not known how soon after the application these changes appear. The aim of this study was to reduce the exposure duration to 3 h and to evaluate nerve function and histological changes in the nerve tissue during this time period. A total of 12 pigs was used. In ten pigs, autologous nucleus pulposus (NP) was applied epidurally on the cauda equina. Nerve function was then monitored for 3 h by measurements of muscle action potentials (MAP) in the tail muscles, following nerve root stimulation cranial to the exposed zone. In five of the ten pigs with NP application, nerve root compression to 50 mm Hg was added by means of an inflatable balloon. In two control animals, neither NP nor compression was applied. At the end point, nerve root specimens were harvested for histological assessment. No reduction of MAP amplitude was detected in any of the series. However, there was an epidural accumulation of leucocytes and mast cells, as well as minor axonal and Schwann cell changes in both the NP and NP+ compression series, as compared to the control series. Morphological changes in terms of an epidural inflammatory reaction and minor axonal and Schwann cell damage may thus be demonstrated within 3 h of NP application, with or without compression. However, there is no functional deterioration of the nerve roots detectable within this time period.

Key words Nerve roots · Nucleus pulposus · Nerve function · Inflammation · Mast cells

Introduction

It has been demonstrated experimentally that spinal nerve roots will undergo both functional changes and histological injury after epidural exposure to autologous nucleus pulposus (NP) [16–24]. Functional impairment, seen as a reduction in nerve conduction velocity, has been found to be present as early as 24 h after application [24]. After 3 and 7 days, this reduction is even more pronounced. These effects are completely blocked if high-dose methylprednisolone is given intravenously within 24 h after NP application [20]. Thus, there are reasons to believe that the pathophysiologic mechanisms are initiated relatively soon after the application. The aim of the present study was, therefore, to assess the structural and functional changes in spinal nerve roots during the first 3 h after local epidural application of NP. The study protocol comprised the effects of NP alone, as well as in combination with controlled nerved root compression, just below the pressure level known to induce functional changes during the chosen time period [25, 27].

Materials and methods

A total of 12 pigs, body weight 25–30 kg, were anaesthetized by an intramuscular injection of 20 mg/kg body weight of Ketalar (Ketamine 50 mg/ml, Parke-Davis, Morris Plains, N. J.) and an in-

travenous injection of 35 mg/kg bodyweight of Pentothal (thiopental sodium, Abbott Lab, Chicago, Ill). The pigs were ventilated on a respirator. Anaesthesia was maintained by an intravenous bolus injection of 100 mg/kg body weight Chloralose (a-D(+)-glucochloralose, Merck, Darmstadt) and by a continuous supply of 30 mg/kg/h Chloralose. The pigs were placed on their side and NP from the L3-L4 intervertebral disc was harvested through a retroperitoneal approach. The wound was sutured and the pigs were placed prone. Laminectomies of the second and third coccygeal vertebrae were performed, thus exposing the cauda equina nerve roots with their dural sleeves. Local tissue temperature was continuously monitored with a thermistor probe and maintained at 38–39.0° C by means of a heating lamp.

Nucleus pulposus application (n = 5)

The intact NP which was in a semiliquid state, was gently placed epidurally over and around the dural sleeves of the caude equina nerve roots in five pigs.

Nucleus pulposus + compression (n = 5)

In this series, a compression pressure at 50 mm Hg was applied to the nerve roots for 3 h in addition to the NP. This was done by means of an inflatable plastic balloon (10 mm in diameter) which was placed across the opened spinal canal with the nerve roots. The balloon was fixed to the spine by two L-shaped pins and a plexiglass plate (Fig. 1). When the balloon was inflated by a graded, compressed-nitrogen system (Stille-Werner, Stockholm), the nerve roots of the cauda equina were compressed towards the anterior aspect of the spinal canal. This compression model has earlier been shown to have a high accuracy in pressure transmission from the balloon to the nerve roots [21].

Control (n = 2)

In two pigs neither nucleus pulposus nor compression was applied.

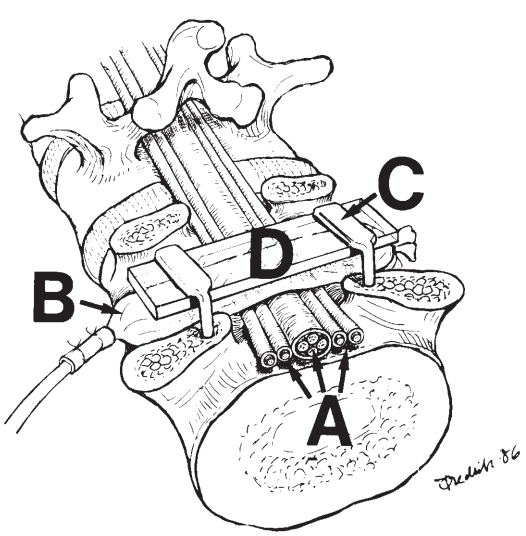

Fig. 1 Schematic drawing of the experimental model with compression device attached. The part of the cauda equina *(A)* that is exposed to nucleus pulposus, is compressed by an inflatable balloon *(B)*, which is fixed to the spine by two L-shaped pins *(C)* and a plexiglass plate *(D)*. (Reproduced with permission from *Spine*, Olmarker et al. 1989 [25])

Neurophysiologic assessment

The cauda equina was stimulated by two E2 platinum needle electrodes (Grass Instruments Quincy, Mass.) that were connected to a Grass SD9 stimulator (Grass Instrument) and gently placed intermittently, cranially and caudally to the exposed zone of the cauda equina. Muscle action potentials (MAPs) were recorded by two E2 platinum needle electrodes (Grass Instrument), which were placed into the tail muscles approximately 10 mm apart. The function of the motor fibres of the nerve roots was measured as the amplitude of the first peak of the MAP, which reflects the fastest conducting motor fibres. To ensure that any change in MAP had its origin in the exposure zone, each cranial stimulation was compared with a stimulation caudal to the exposure zone. The EMG was visualized using a MacIntosh IIci computer provided with Superscope software and MacADIOS II A/D converter (GW Instruments, Sommerville, Mass.), together with a Grass P18 preamplifier (Grass Instrument). To ensure that only impulses from NP-exposed nerve fibres were recorded, all nerves that left the spinal canal between the cranial stimulating electrodes and the exposure site were cut. A confirmation of the outcome of this procedure was obtained after the experiment by studying the EMG after cutting the cauda equina at the exposure site, which in all experiments resulted in a flat EMG curve. After completion of the operative procedures, MAP measurements were recorded every 5 min until four consecutive, stable, recordings were obtained. The mean of these recordings was regarded as a baseline value, and every MAP recording during the experiments was expressed as a percentage of this value. The NP, with or without compression at 50 mm Hg, was then applied, and MAPs were recorded every 15 min over 3 h.

Histologic assessment

After 3 h the cauda equina was tied to a wooden stick in order to avoid shrinkage artefacts during fixation. It was then removed and fixed by immersion in Karnovsky's mixture of formaldehyde and glutaraldehyde [13]. At the end of the experiments, the pigs were killed with an intravenous overdose of potassium chloride.

Specimens for light microscopy were obtained from the exposed segments of the cauda equina. The specimens were dehydrated and embedded in Epon 812. Sections 1 μm thick were prepared and stained according to Richardson [26]. All sections were coded and were subsequently analysed using light microscopy by a neuropathologist, who thus was unaware of the test protocol for the different specimens. The collected data were organized in tables following code breakage.

Nerve fibre damage was graded according to a seven-degree scale: 0 = no nerve fibre damage, (+) = single scattered fibres damaged, + = ≤ 10% of fibres damaged, ++ = 11–25% of fibres damaged, +++ = 26–50% of fibres damaged, ++++ = 51–75% of fibres damaged, +++++ = 76–100% of fibres damaged.

Other histological findings were recorded as follows: 0 = no changes, (+) = minute changes, + = mild changes, ++ = moderate changes, +++ = prominent changes.

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

Results

There were no endoneural changes in the control series except for minor hyperaemia (Table 1). In the NP and the NP+compression series, there were endoneural changes affecting approximately 8–10% of the fibres. They comprised minor axonal and Schwann cell injury (Fig. 2) as well as some hyperaemia and bleeding. There were no apparent differences between the NP and the NP+compres-

Table 1 Endoneural changes (*NP* nucleus pulposus)

	Other parameters[b]				
	Nerve fibre damage[a]	Leucocytes	Bleeding	Hyperaemia	Schwann cell swelling
Control (n = 2)	0	0	0	+	0
	0	0	0	+	0
NP (n = 5)	0	0	0	+	0
	0	0	+	+	(+)
	+	0	+	+	+
	+	0	+	++	+
	0	0	0	0	0
Np + 50 mm Hg compression (n = 5)	0	0	0	+	(+)
	0	0	+	+	0
	+	0	0	++	+
	+	0	+	0	0
	0	0	0	+	0

[a] Nerve fibre damage: 0 = no nerve fibre damage, (+) = single scattered fibres damaged, + = ≤ 10% of fibres damaged, ++ = 11–25% of fibres, +++ = 26–50% of fibres, ++++ = 51–75% of fibres, +++++ = 76–100% of fibres

[b] Other parameters: 0 = no changes, (+) minute changes, + = mild changes, ++ = moderate changes, +++ = prominent changes

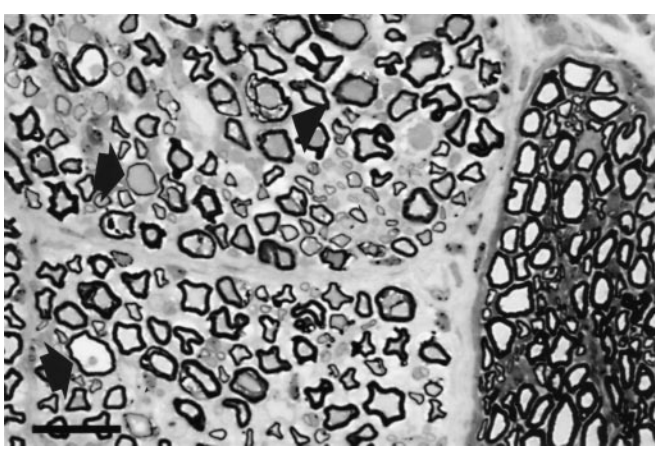

Fig. 2 Three nearby fascicles in a nerve root exposed to nucleus pulposus. No compression added. A majority of the myelinated axons in the two left fascicles are condensed and several nerve fibres show attenuation *(arrows)* and splitting of myelin sheaths *(arrowhead)*. The endoneurium in the two left fascicles is oedematous (Richardson staining, bar: 25 μm)

sion series. There were no inflammatory cells detected in the endoneural space in any of the three groups.

Regarding the epidural changes, there were no obvious differences between the NP and the NP+compression series. However, the presence of inflammatory cells was more pronounced in these two series than in the control series (Table 2). The inflammatory cells in the control series comprised only leucocytes, while in the other two series there were also a number of mast cells present in nine of the ten animals.

There were no detectable reductions of MAP amplitudes during the 3 h of exposure in any of the series (Table 3). Instead, there were stable conditions throughout the entire experiment.

Discussion

The results of the present study did not demonstrate any reductions in motor nerve fibre function during 3 h of epidural exposure to NP, with or without the addition of

Table 2 Epidural changes (0 = no changes, (+) minute changes, + = mild changes, ++ = moderate changes, +++ = prominent changes

	Hyperaemia	Bleeding	Fibroblasts	Mast cells	Leucocytes
Control (n = 2)	+++	++	0	0	+
	+++	++	0	0	+
NP (n = 5)	++	+	0	0	++
	+++	++	0	+	++
	+++	++	0	+	++
	+++	++	0	+	++
	+++	+++	0	+	+
NP + 50 mm Hg compression (n = 5)	+++	++	0	++	++
	+++	+	0	+	+
	+++	+++	0	+	++
	+++	+++	0	+	++
	+++	+++	0	+	++

Table 3 Average muscle action potential (MAP) amplitude after 3 h expressed as a percentage of pre-experimental MAP (% ± SD)

Control (n = 2)	103 ± 7
NP (n = 5)	105 ± 5
NP + 50 mm Hg (n = 5)	104 ± 8

50 mm Hg compression over the exposure zone. However, these 3 h seemed to be enough to initiate an accumulation of inflammatory cells, including mast cells, in the epidural space, as well as endoneural changes in the nerve roots, such as minor injury to axons and Schwann cells.

It is today well established that autologous NP alone, without mechanical compression, may initiate pathophysiologic processes leading to functional and structural injuries in exposed nerve roots [15, 22, 24]. The specific properties of the NP tissue that initiate these changes are largely unknown, despite strenuous efforts through both clinical and experimental studies to elucidate the mechanisms behind sciatica [18].

From experimental studies, there is evidence indicating that NP may possess inflammatogenic properties. McCarron and co-workers demonstrated in a dog model that epidural injection of NP resulted in local epidural inflammation and invasion by leucocytes [16]. In a model using small, perforated, subcutaneously placed titanium chambers filled with either NP or fat, Olmarker and co-workers recently demonstrated that, within 7 days, an inflammatory reaction was initiated in the chambers filled with nucleus pulposus [19]. There has also been speculation regarding an immunological aetiology to the demonstrated changes [1, 6, 32].

As a part of an inflammatory reaction after NP application one would expect vascular reactions in the affected nerve roots. Such reactions, including increased permeability to albumin and reduction in intraneural blood flow, have been demonstrated in a recent study by Byröd and co-workers [3]. Both the increase in permeability and the reduction in blood flow were established within 3 h after NP application. A majority of previously published studies assessing the effects of disc tissue on epidural space and spinal nerve roots are based on analyses made 24 h or more after the application (Otani et al. 1997, unpublished data, and [4, 5, 9, 10, 14, 15, 17, 20, 24, 34]). At that stage, the early pathophysiology will most probably be accompanied by changes of a more chronic, inflammatory nature.

In order to gain further insight into these issues, the present study was designed to assess the very early effects on spinal nerve roots after epidural application of nucleus pulposus. According to the obtained results, it seems plausible that exposure to nucleus pulposus may induce epidural as well as endoneural changes within 3 h.

In the compression series, both the demonstrated axonal and the Schwann cell injuries may have been induced by the compression, rather than by exposure to NP. However, regarding the nerve fibre damage, there were no differences between the two study groups. Moreover, the compression pressure level used has in earlier studies not resulted in any histological changes, even after compression at 50 mm Hg for up to 4 h [25, 28]. It is thus likely that NP per se may induce very early nerve fibre damage.

The present study revealed epidural, inflammatory changes. These were seen as an accumulation of inflammatory cells, including mast cells, at the location of the NP application. The presence of mast cells is particularly interesting, since they produce and excrete a number of inflammatory mediators, such as proteases, heparin, VIP and histamine, which may induce vasodilation, increased vascular permeability and chemotaxis of inflammatory cells [7, 29]. The mast cells are also highly interesting in the pathophysiology of sciatica, since they are known to excrete cytokines such as TNF-alpha, which has been considered to play a key role in neuropathic pain [8, 31, 33]. However, the exact role of the mast cells in the demonstrated epidural inflammation is still unclear, and their origin is not known. They may be basophils recruited from the circulation or tissue-bound mast cells already present before the start of the experiment. The latter seems less probable, since no mast cells were found in the epidural space of the control nerve roots. Formerly, mast cells were thought of as non-migrating cells, but this hypothesis has been revised during the current decade [2, 11, 12, 30]. Published evidence indicates the possibility that mast cells may be recruited to an area of inflammation, even in the central nervous system [2, 11, 12, 30].

The results of the present study indicate a relatively short time course for the initiation of inflammatory events. This speaks in favour of the hypothesis that inflammatogenic substances are released from the NP tissue in the epidural space. They may then initiate inflammatory reactions, which in turn will affect the adjacent spinal nerve roots. Recent studies demonstrating that the NP-induced changes may be efficiently blocked by steroids, indomethacin or cyclosporin A, further support the pathophysiologic importance of inflammatory mechanisms (Arai et al. 1996, unpublished data; Konno et al. 1996, unpublished data; and [20]).

In conclusion, the present paper shows that, although there were no functional changes seen during 3 h of epidural exposure to nucleus pulposus, an inflammatory process was initiated in the epidural space. There were also early signs of axonal and Schwann cell injury. The study thus suggests that the NP-induced changes develop rapidly and initiate histologic changes already within 3 h of application. This may indicate that one component of lumbar radiculopathy is an acute inflammatory reaction, triggered by inflammatogenic substances already present in the NP tissue at the moment of herniation.

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