

# Effects of Morphine Dependency on Intervertebral Disc in the Rat

H. Reihani Kermani, M. Posti,  
R. Malekpour Afshar, N. Nakhaee

## Abstract

**Background:** A large proportion of patients presenting with lumbar disc disease are opium addicts in our region. It was, therefore, hypothesized that morphine might affect the intervertebral disc. We investigated the histological findings of morphine dependency on intervertebral discs in rat.

**Methods:** Forty NMRI adult male rats (230-250 g) placed on ordinary diet and received aqueous solution of morphine. The solution contained 0.1mg/ml on the first day which gradually increased to 0.3 mg/ml during the first week, then continued at 0.4 mg/ml for four, seven and 11 weeks. Morphine dependency was confirmed by the presence of withdrawal syndromes using intraperitoneal naloxone at the end of each period. The H&E stained tissues were used to study the structure of disc and evaluation of degenerative changes.

**Results:** Except for vascular proliferation of nucleus pulposus, an increase in the rate of matrix fibrillation, fibrosis, hyalination, and dehiscence of nucleus pulposus was seen in morphine dependent rats compared with control group although it was not statistically significant possibly owing to relatively low sample size which limited the power to precisely reveal the difference. The duration of dependency did not affect the pathologic markers.

**Conclusion:** The results of this study suggest that morphine dependency induces some pathologic changes in the intervertebral disc of the rat. Further investigation into degenerative markers with larger sample size is required.

**Iran J Med Sci 2006; 31(3): 147-150.**

**Keywords** • Addiction • intervertebral disc • degeneration • morphine

## Introduction

Low back pain disorders are significant health problems in human societies. Its prevalence is approximately 20%, indicating that at any time, one fifth of adult population may complain about low back pain. Of various causes of low back pain, the most intractable treatments is neural compression syndrome by degenerated or herniated intervertebral disc (ID). ID is composed of various proteoglycans, glycoprotein, noncollagenous proteins and interstitial water.<sup>1</sup> The disc continues to undergo synthesis and degradation of these macromolecules.<sup>1</sup> In adults, the nucleus pulposus is not directly supplied by blood vessels, thence its metabolites

Department of Neurosurgery  
and Neuroscience Research center,  
Kerman University of Medical Sciences,  
Kerman, Iran.

## Correspondence:

Hamed Reihani Kermani MD,  
Department of Neurosurgery,  
Bahonar Hospital,  
Kerman University of Medical Sciences,  
Kerman, Iran.  
**Tel/Fax:** +98 341 2450215  
**E-mail:** hreihani@kmu.ac.ir

and minerals are transported through vascular channels of the subchondral cancellous bone of the vertebral bodies.<sup>2</sup> Diffusion occurs through the cartilaginous endplates and into nucleus pulposus.<sup>2</sup> Conversely, the annulus has segmental vessels for blood supply and transport. Naylor found that the normal aging process results in decreased permeability between the annulus and the nucleus.<sup>1</sup>

In our daily practice we noticed that many patients who required lumbar disc surgery were opium addicts. Some had started using opium before and others after the onset of low back pain. The degenerative effects of nicotine on ID has previously been studied.<sup>3-6</sup> Numerous studies have also showed the effects of morphine, an important component of opium, on kidney and brain but no study has been performed on biological effect(s) of morphine on ID.<sup>7-9</sup> Therefore, in the present investigation we tried to study the histological aspects of morphine dependency on ID in the rat.

## Materials and Methods

### Experimental protocol

Experiments were performed on 40 NMRI species of adult male rats (230-250 g). They were randomly divided into four groups of equal size. All animals were kept at a regular diet and 12-hr light-dark cycle with room temperature of 22±2°C. Each group, except control, received aqueous solution of morphine as drinking water for four, seven and 11 weeks. Having completed the scheduled morphine treatment, all animals were tested for morphine addiction using intraperitoneal Naloxone test, and then killed under deep anesthesia using anesthetic ether.

### Addiction Protocol

Addiction to morphine was performed by oral route.<sup>10-12</sup> At the start of the experiment the drinking water contained morphine at concentrations of 0.1mg/ml, which subsequently increased to 0.2 and 0.3mg/ml every two days and finally reaching to 0.4 mg/ml on the seventh day. After this initial adaptation to morphine groups I, II and III were subsequently treated for four, seven and 11 weeks. One rat died in the control and one rat in group III. At the end of the experiment withdrawal syndrome

was tested by intraperitoneal injection of 2mg/kg of Naloxone hydrochloride as described.<sup>13</sup> Animals then were killed under deep anesthesia with anesthetic ether to obtain their lumbar vertebral column.

### Histological studies

By posterior approach, and after dissecting paravertebral muscles, the lumbar vertebral column was removed *en bloc* and fixed in 10% formalin. The intervertebral discs were removed from at least three levels, embedded in paraffin wax and cut into sections. The sections were stained using H&E and Toluidine blue methods to evaluate the structure of the disc and degenerative changes that may have occurred, including necrosis, fibrosis, hyalinization, dissociation of multilayer structure, vascular proliferation and loss of normal fibrillation of cartilage. The results were reported as absence or presence of each parameter.

### Statistical analysis

Data are present as Mean±SD. Comparison of opium treated animals with the control group was performed by using two-tailed Fisher's exact test. The level of statistical significance was corrected from the convention of 0.05 to 0.017 (0.05 divided by the number of comparisons) following the Bonferroni adjustment.

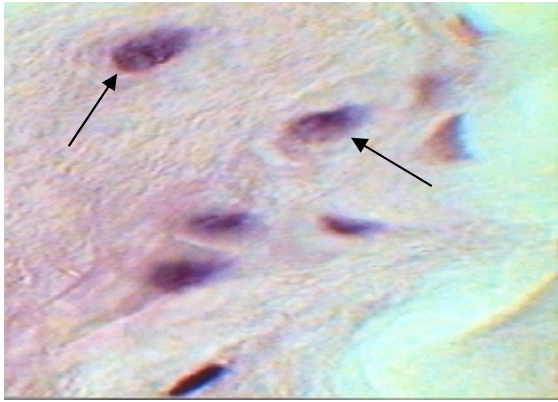
## Results

Quantitative results of pathologic findings of control and three morphine treated groups I, II and III are presented in Table 1. Except for the vascular proliferation, other pathologic markers were more frequent in morphine treated groups. Microscopic examination of the control group did not show any tissue necrosis (Fig.1). Whereas, necrosis was present in 10-20% of the samples obtained from morphine treated groups (Fig.2). The normal appearance of matrix, as delicate fibrillation, was obvious in all rats of control group (Fig 1), but in morphine treated groups degeneration of matrix was observed in 10 to 20 percent of specimens (Fig. 3).The rate of hyalinization was reported 40% (in group I) 30% (in group II), and 67% (in group III) in morphine-treated rats, whereas, hyalinization rate insignificant the control group. Vascular proliferation also absent in all groups.

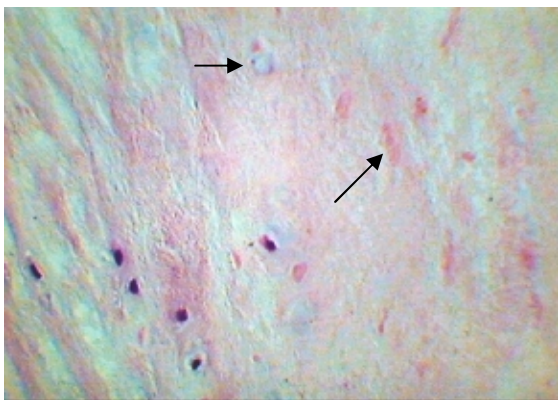
**Table 1:** Comparison of pathological findings of control and morphine- treated rats of groups I, II, and III.

Groups	NC	FB	H	D	VP	MF
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CNT(n=9)	0 (0)	1(11)	1(11)	1(11)	0(0)	0(0)
I (n=10)	2 (20)	2(20)	4(40)	5(50)	0(0)	3(30)
II (n=10)	1(10)	4(40)	3(30)	4(40)	0(0)	4(40)
III (n=9)	1(11)	0(0)	6(67)	4(44)	0(0)	4(44)

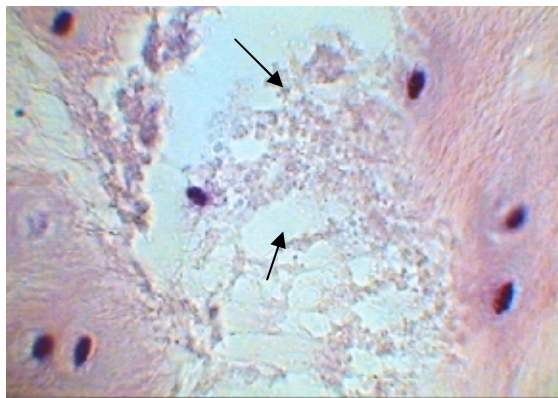
NC= Necrosis; FB= Fibrosis; H=Hyalinization; DS= Dehiscence; VP= Vascular proliferation; MF= Matrix fibrillation



**Fig. 1:** Intervertebral disc, normal chondrocytes (arrows) set in a delicate homogenous stain matrix (H& E, x400)



**Fig. 2:** Necrosis of chondrocytes as shadow of cells (arrows) and their nuclei after 7 weeks morphine treatment (H& E, x400).



**Fig. 3:** Marked degeneration of matrix (arrows) as disintegration and coars fragmentation after 11 weeks morphine treatment (H& E, x400).

## Discussion

The most frequent risk factors for ID herniation in man are Job, pregnancy and cigarette smoking etc.<sup>15-17</sup> In our daily practice we found that a large proportion of patients presenting with lumbar disc disease were also addicted to opium. So we think opium addiction might affect the integrity of ID. Therefore, we conducted a study to evaluate

the degenerative effects of morphine, the major component of opium; on the ID of the rat.

The results of this study indicated that the possibility that morphine may contribute to the process of disc degeneration. Johnson and colleagues showed that kidney degeneration associated with opioid abuse reflected the effects of opioid per se.<sup>9</sup> The presence of opioid receptors outside the central nervous system is well recognized.<sup>18</sup> Morphine may have a direct role in pathogenesis of disc degeneration by peripheral opioid receptors. Another possible mechanism may be through opioids mediating immunosuppression.

Acute and chronic opioid administration was known to have inhibitory effects on humoral and cellular immune responses including antibody production, natural killer cell activity, cytokine expression, and phagocytic activity. Opiates behave like cytokines, modulating the immune response by interaction with their receptors in the central nervous system and in the periphery.

The potential mechanisms by which opiates modulate peripheral immune functions may involve both the hypothalamic-pituitary-adrenal axis and the autonomic nervous system.<sup>18</sup> The most widely accepted explanations for the association between smoking and disc degeneration is malnutrition of spinal disc cells by carboxy-hemoglobin-induced anoxia or vascular disease.<sup>3</sup> Whether the same mechanism could be applicable to Morphine remains to be determined.

## Conclusion

The results of present study suggest that morphine dependency induced some pathologic changes in intervertebral disc. It may have important clinical implications for those patients receiving long-term opioid therapy for malignant and nonmalignant and also for Morphine –addicted individuals. Further investigation into degenerative markers with large sample size is required to improve information regarding the effect of Morphine dependency on intervertebral disc.

## Acknowledgments

*This work is financially supported by a grant of provided by Neuroscience Research Center of Kerman University of Medical Sciences. The authors appreciate the constructive suggestions of Dr. Manzoumeh Shamsi Meymandi.*

## References

- 1 Naylor A. Intervertebral disc prolaps and degeneration. The biochemical and biophysical approach. *Spine* 1976; 1: 108-14.
- 2 Brown M, Tsaltas T. Studies on the perme-

- ability of the intervertebral disc during skeletal maturation. *Spine* 1976; 1: 240-44.
- 3 Akmal M, Kesani A, Anand B, et al. Effect of nicotine on spinal disc cells: a cellular mechanism for disc degeneration. *Spine* 2004; 29: 568-75.
  - 4 Oda H, Matsuzaki H, Tokohashi Y, et al. Degeneration of intervertebral discs due to smoking: experimental assessment in a rat-smoking model. *J Orthop Sci* 2004; 9:135-41.
  - 5 Kim KS, Yoon ST, Park JS, et al. Inhibition of proteoglycan and type II collagen synthesis of disc nucleus cells by nicotine. *J Neurosurg* 2003; 99: 291-7.
  - 6 Iwahashi M, Matsuzaki H, Tokuhashi Y, et al. Mechanism of intervertebral disc degeneration caused by nicotine in rabbits to elucidate intervertebral disc disorders caused by smoking. *Spine* 2002; 27: 1396-401.
  - 7 Uematsu Y, Matuzaki H, Iwahashi M. Effects of nicotine on the intervertebral disc: an experimental study in rabbits. *J Orthop Sci* 2001; 6: 177-182.
  - 8 Kofke WA, Garman RH, Stiller RL, et al. Opioid neurotoxicity, fentanyl dose response in rats. *Anesth Analg* 1996; 83: 1298-306.
  - 9 Johnson JE, White JJ, Walovitch RC, London ED. Effect of morphine on rat kidney glomerular podocyte a scanning electron microscopy study. *Drug Alcohol Depend* 1987; 19: 249-57.
  - 10 Badawy AA, Evans CM. Production of tolerance and physical dependency in the rat by single administration of morphine in drinking water. *Br J Pharmacol* 1982; 75: 485-91.
  - 11 Chu LF, Clark DJ, Angst MS. Opioid tolerance and hyperalgesia in chronic pain patients after one month of oral morphine therapy: a preliminary prospective study. *J Pain*. 2006; 7: 43-8.
  - 12 Nasiraei-Moghadam S, Sahraei H, Bahadoran H, et al. Effects of maternal oral morphine consumption on neural tube development in Wistar rats. *Brain Res Dev Brain Res* 2005; 159: 12-7.
  - 13 Wakasa Y, Kawaguchi T, Yanagita T. Withdrawal characteristics following frequent intravenous administration of several opioids in rats. *Arukuru Kenkyuto Yakubutsu Ison* 1994; 29: 40-51.
  - 14 Frymoyer JW. Lumbar disc disease: Epidemiology. In: Frymoye JW. *The Adult Spine: Principle and Practice*. New York; Raven Press; 1991. p. 217-23.
  - 15 Stephens SE, Bell GR. Natural history and epidemiology of lumbar disc degeneration. In: Hardy RW. *Lumbar disc disease* 2<sup>nd</sup> ed. New York Raven Press; 1991. p. 13-15.
  - 16 Kelsev JL, Githens PB, O' Conner T, et al. Acute prolapsed lumbar intervertebral disc. An epidemiologic study with special reference to driving automobiles and cigarette smoking. *Spine* 1984; 9: 608-13.
  - 17 Leboeuf-Yde C, Kyvik KO, Bruun NH. Low back pain and life style Part 1: smoking information from population-based sample of 29424 twins. *Spine* 1998; 23: 2207-13.
  - 18 Vallejo R, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. *Am J Ther* 2004; 11: 354-65.