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Effect of ovariectomy on contrast agent diffusion into lumbar intervertebral disc: a dynamic contrast-enhanced MRI study in female rats☆,☆☆

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Abstract

Purpose: The purpose was to study the effect of estrogen deficiency on contrast agent diffusion into intervertebral disc in a rat model.
Materials and Methods: Seven-month-old female Sprague–Dawley rats were used. Fourteen rats had ovariectomy, and nine rats had sham surgery. Magnetic resonance imaging (MRI) of sagittal midsection of lumbar spine was performed with a 1.5-T magnet. Dynamic MRI was performed after a bolus injection of Gd-DOTA (0.3 mmol/kg) through tail vein. Eight hundred images were acquired at 0.6 s per acquisition. Regions of interests were drawn over three discs per rat. Maximum enhancement (E_{max}) and enhancement slope (E_{slope}) were evaluated. MRI was carried out at baseline and 8 weeks postsurgery.
Result: All disc enhancements demonstrated an initial fast wash-in phase followed by a second slower wash-in phase. For initial wash-in phase, E^1_{max} and E^1_{slope} of all rats remained unchanged at the two time points. For second wash-in phase, E^2_{max} and E^2_{slope} of control rats remained unchanged, while with ovariectomized rats, E^2_{max} showed reduction at 8 weeks (4.5%±5.6%) compared to baseline (10.3%±6.3%, P=.037), and E^2_{slope} was lower at 8 weeks (0.015±0.017) than the baseline (0.029±0.022), although it was not statistically significant (P=.101).
Conclusion: Ovariectomy induced detectable decrease in second wash-in phase of contrast agent into lumbar disc.

Keywords: Ovariectomy; Estrogen deficiency; Lumbar; Intervertebral disc; Contrast agent; Dynamic MRI; Wash-in

1. Introduction

Lumbar spine disc degeneration is a common musculoskeletal condition. Disc degeneration can progress to disc herniation, spinal canal stenosis and, in conjunction with facet joint arthrosis, degenerative spondylolisthesis. Although intervertebral disc degeneration has a multifactorial etiology involving age and mechanical, genetic, systemic and toxic factors [1], a final common pathway of decreased nutrition has been proposed [2]. The intervertebral disc in adulthood is the largest avascular tissue in the body and renders cells of the disc, particularly in the central nucleus, a long way from a source of nutrition or metabolite clearance. The diffusion from marginal capillaries in the outermost annulus and the endplate is the only source of nutrition to the avascular inner annulus layers and nucleus pulposus [3]. Such deficiencies in metabolite transport limit both the density and metabolic activity of disc cells. As a result, discs have only a limited ability to recover from mechanical injury. Vascular pathologies, such as atherosclerosis of the abdominal aorta and lumbar arteries, or disorders such as sickle cell disease have also been shown to be associated with more severe disc degeneration [4–6]. Low

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oxygen tension in the central parts of the disc leads to an acidic microenvironment due to lactic acid accumulation, and oxidative stress leading to cell nuclear quiescence and hypoglycemic-induced cell apoptosis [7,8].

Increasing evidence suggests that sex hormones also influence the severity of disc degeneration [9]. Young and middle-aged males are more likely to have lumbar disc degeneration than females. However, this trend is reversed in elderly subjects, with females tending to have more severe lumbar disc degeneration than males [10,11]. Relative estrogen deficiency likely contributes to the accelerated disc degeneration seen in postmenopausal females [9]. One animal study also demonstrated that disc degeneration was found in female rats 6 months after ovariectomy, though the mechanism has not being fully explored [12].

Magnetic resonance imaging (MRI) is a noninvasive method to assess the pathophysiologic status of intervertebral discs [13], with paramagnetic contrast agents being used as an MRI tracer to track diffusion (i.e., transport) into the disc in vivo [14,15]. Reduced contrast agent diffusion has been found in degenerate lumbar discs [13,15]. Immature discs have greater disc diffusion than mature discs [16], with impaired contrast agent diffusion serving as a marker of early disc degeneration [17]. In this study, we applied dynamic contrast-enhanced (DCE) MRI to study the effect of estrogen deficiency on contrast agent diffusion into lumbar intervertebral discs in a female rat ovariectomy model.

2. Materials and methods

The experimental protocol was approved by the local University Animal Experiment Ethics Committee. A total of 27 female Sprague–Dawley rats were studied. All rats were 7 months old at baseline. All animals were bred at the Laboratory Animal Services Centre of our University. Animals were housed two to three animals per stainless steel cage at 22°C temperature with a 12-h light and 12-h dark cycle and received a standard rat chow (Prolab RMH 2500, PMI Nutrition International LLC, Brentwood, MO, USA) and water ad libitum. For MRI examinations and surgery, the rats were anesthetized using a combination of xylazine (10 mg/kg) and ketamine (90 mg/kg).

DCE MRI study of the rat lumbar spine was performed according to the descriptions of Wang et al. [17]. In brief, MRI was carried out on a 1.5-T clinical whole body imaging system (Intera NT, Philips Medical Systems, Best, the Netherlands) with a maximum gradient strength of 30 mT/m. A commercially available surface coil with a diameter of 4.7 cm (Micro 4.7, Philips Medical Systems) was used as the RF signal receiver. A custom-made plastic cradle was used to hold the surface coil. Rats were anesthetized and placed in the cradle supine, and the tail vein was cannulated with a 24-ga heparinized catheter (Introcan Safety, B. Braun Medical Inc., Bethlehem, PA, USA). Using the pelvic crest as the anatomical reference, the rat lumbar spine region was positioned on the surface coil so that the L3 and L4 vertebrae were located central to the surface coil. A coronal scout scan confirmed positioning of rat lumbar vertebrae in relation to the surface coil with repositioning if centering was not optimal. Thereafter, sagittal T1-weighted fast spin echo images of the lumbar spine were obtained using the following parameters: slice thickness 2 mm, repetition time (TR) 425 ms, echo time (TE) 24 ms, echo train length 3, in-plane resolution 0.25×0.25 mm, matrix 304×243, average 5.

DCE MRI was then obtained in the sagittal midsection using the following parameters: short T1-weighted gradient echo sequence in three-dimensional mode, TR 4 ms, TE 1.4 ms, flip angle 15°, slice thickness 5 mm, matrix 128×51, in-plane resolution 0.625×0.625 mm, average 1. The temporal resolution was 0.6 s per image acquisition. MRI contrast agent was Gd-DOTA (Guerbet Group, Roissy CDG Cedex, France). A quick bolus dose of 0.3 mmol/kg (0.24 ml for a 400-g rat) was injected manually following acquisition of 60 baseline acquisitions (i.e., 36 s) followed by a flush of 0.5 ml normal saline. Bolus injection and normal saline flush were carried out by the same operator skilled in in vivo small animal procedures. Time for dynamic MRI was approximately 8 min, with 800 images being acquired.

Dynamic MRI images were processed on a radiological workstation (Viewforum, Philips Medical System). Regions of interest (ROIs) were drawn on the lumbar intervertebral discs on sagittal images prior to generation of a dynamic MRI enhancement curve (signal intensity in arbitrary units vs. time in seconds). Particular care was taken to ensure that each ROI did not include any of the endplate. For each examination, three intervertebral discs closest to the center of the receiver coil were selected for DCE MRI measurement (Fig. 1A, C). The investigator undertaking analyses of enhancement curve data was blinded to the animal status (control group or ovariectomy group).

Semiquantitative evaluation of intervertebral disc contrast enhancement was performed [17, 18]. Maximum enhancement (E_{max}), defined as the maximum percentage increase in signal intensity from baseline, was derived from the following equation.

$$E_{max} = [(SI_{max} - SI_{base}) / SI_{base}] \times 100,$$

where SI_{max} denoted maximum signal intensity postenhancement and SI_{base} denoted signal intensity pre-enhancement.

Enhancement slope (E_{slope}), defined as the constant to describe the rate of wash-in of enhancement signal in tissues, was derived from the following equation:

$$E_{slope} = E_{max} / (T_{max} - T_{base}),$$

where T_{max} denoted the time point of maximum signal intensity and T_{base} denoted the time point of the end of baseline intensity.

The intervertebral disc contrast enhancement curve comprised a fast first wash-in phase followed by a slower second wash-in phase (Fig. 1B, D). Therefore, four DCE MRI