

· 基础研究 ·

椎间盘造影中碘海醇剂量对大鼠椎间盘退行性变的影响

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【摘要】目的 探讨椎间盘造影中造影剂碘海醇剂量对椎间盘退行性变的影响。**方法** 以10只成年雄性SD大鼠的40个尾椎椎间盘(每只4个)为实验对象,分为空白组(C₀₆/C₀₇)、对照组(C₀₇/C₀₈)、常规剂量组(C₀₈/C₀₉, 2 μL碘海醇)和高剂量组(C₀₉/C₁₀, 3 μL碘海醇)。分别于椎间盘造影术后2周和4周使用X线检测椎间高度指数(DHI),使用MRI评估椎间盘信号;术后4周检测椎间盘含水量,并采用HE染色检测组织学变化。**结果** 术后2周和4周时,与空白组相比,对照组DHI未见明显变化,常规剂量组和高剂量组DHI降低,且高剂量组比常规剂量组降低更为显著,差异均有统计学意义($P<0.05$);对照组、常规剂量组和高剂量组椎间盘信号均降低,且剂量越大信号越低,差异均有统计学意义($P<0.05$)。与空白组相比,对照组椎间盘含水量未见明显变化,常规剂量组和高剂量组椎间盘含水量降低,且高剂量组降低更为显著,差异均有统计学意义($P<0.05$)。与空白组相比,对照组椎间盘组织学评分无明显变化,常规剂量组和高剂量组椎间盘组织学评分均增高,且剂量越大评分越高,差异均有统计学意义($P<0.05$)。**结论** 椎间盘造影时注入碘海醇可促进椎间盘退行性变,且呈剂量依赖性。在造影操作时应尽量减少造影剂的使用,以降低对椎间盘的影响。

【关键词】 椎间盘退行性变;放射摄影术;造影剂;碘海醇;大鼠**【中图分类号】** R 681.5 **【文献标志码】** A **【文章编号】** 1672-2957(2020)05-0325-06**【DOI】** 10.3969/j.issn.1672-2957.2020.05.008

Effect of iohexol dosage on intervertebral disc degeneration during discography in rats

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【Abstract】Objective To investigate the effect of iohexol dose on disc degeneration in discography. **Methods** Forty coccygeal intervertebral discs of 10 adult male SD rats were divided into 4 groups: blank group (C₀₆/C₀₇), control group (C₀₇/C₀₈), conventional dose group (C₀₈/C₀₉, 2 μL iohexol), and high dose group (C₀₉/C₁₀, 3 μL iohexol). Roentgenography and MRI were used to detect disc height index (DHI) at 2 and 4 weeks after discography. The intervertebral disc tissue was obtained 4 weeks after discography, and the water content of the intervertebral disc was measured and the histological changes were detected by HE staining. **Results** At 2 and 4 weeks after discography, compared with the blank group, the DHI in the control group did not change significantly, the DHI in the conventional dose group and the high dose group decreased significantly, and the reduction in the high dose group was more significant, and the differences were statistically significant ($P<0.05$); the signal of intervertebral disc decreased in the control group, the conventional dose group and the high dose group, and the larger the dose, the lower the signal, and the difference was statistically significant ($P<0.05$). Compared with the blank group, there was no significant change

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in the water content of the intervertebral disc in the control group. The water content of the intervertebral disc in the conventional dose group and the high dose group decreased significantly, and the reduction in the high dose group was more significant, and the differences were statistically significant ($P < 0.05$). Compared with the blank group, there was no significant change in the histological score of intervertebral disc in the control group. The histological score in the conventional dose group and the high dose group decreased significantly, and the larger the dose, the higher the score, and the difference was statistically significant ($P < 0.05$). **Conclusion** During discography, the injection of iohexol can significantly increase disc degeneration, and it is positively correlated with the iohexol dose. During the discography, the use of iohexol should be minimized to reduce the disc degeneration.

【Key Words】 Intervertebral disc degeneration; Radiography; Contrast media; Iohexol; Rats

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椎间盘造影术是将造影剂注入椎间盘,利用影像学手段观察椎间盘的形态,反映椎间盘的病理特征^[1]。作为一种侵入性的有创诊断技术,椎间盘造影术一直存在争议^[2]。然而,椎间盘造影术具有不可替代的优势,如典型的疼痛复制、直观地显示椎间盘损伤程度等^[3],其临床应用仍十分广泛。近年来,椎间盘造影对椎间盘退行性变的影响倍受重视。临床研究表明,椎间盘造影可加速腰椎椎间盘退行性变的进展^[4]。体内研究表明,椎间盘造影和椎间盘阻滞可显著增加椎间盘内细胞的凋亡^[5]。体外细胞实验表明,局部麻醉药和非离子型造影剂对纤维环细胞和髓核细胞均有不利影响^[6]。目前关于椎间盘造影术中注射造影剂剂量及注射速度仍没有明确的标准,且存在很大争议^[7-9],本研究通过观察椎间盘造影过程中造影剂剂量对大鼠尾椎椎间盘退行性变的影响,为评估椎间盘造影术的临床价值和风险提供实验依据。

1 材料和方法

1.1 实验动物

10只3月龄雄性SD大鼠(海军军医大学动物实验中心提供),体质量(350 ± 50)g。选择3月龄的雄性SD大鼠是因为此时大鼠骨骼趋于成熟,以尽量减少年龄增长引起的实验误差^[10]。每笼2只分笼饲养,环境温度(25 ± 1)℃,12 h/12 h昼夜循环,自由饮食,实验前适应环境至少1周。所有动物实验均通过海军军医大学实验动物伦理委员会审批备案。

1.2 模型建立

正常成人椎间盘内髓核容积约为3 mL,而临床中使用的常规椎间盘内注射剂量为2 mL。成年大鼠尾椎椎间盘的平均椎间盘高度为0.94 mm,髓核面积为3.30 mm²,总髓核体积为3.10 mm³^[11]。因此,本研究选择2 μ L模拟临床椎间盘造影中的常规注射剂量,选择3 μ L即大鼠尾椎椎间盘的极限容积作为高剂量。

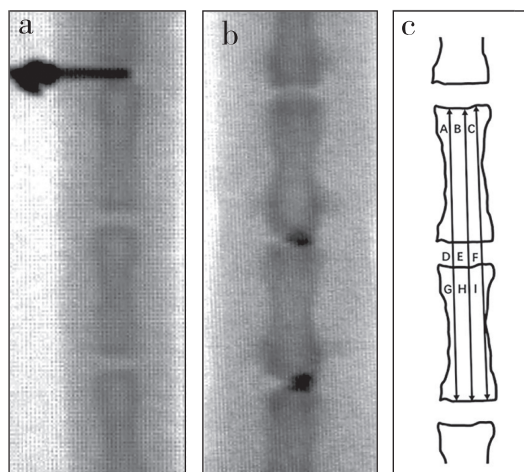
将实验大鼠用10%的水合氯醛以30 mL/kg的剂量麻醉。选取尾部4个椎间盘为研究对象:Co₆/Co₇为空白组,不行任何处理;Co₇/Co₈为对照组,以30号穿刺针刺入椎间盘但不进行注射;Co₈/Co₉为常规剂量组,以30号穿刺针注入2 μ L碘海醇(欧乃派克,含碘质量浓度300 mg I/mL,质量渗透摩尔量为780 mOsm/kg,上海通用电气药业有限公司);Co₉/Co₁₀为高剂量组,以30号穿刺针注入3 μ L碘海醇。所有穿刺操作均在X线引导下完成,穿刺针方向平行于所选椎间隙的上下终板,以医用止血钳控制进入椎间盘的导针长度为5 mm(图1a、b)。碘海醇的注入使用3 μ L微量注射器(Hamilton,瑞士),注射完毕后将导针滞留于椎间盘内2 min以防止液体渗漏。

1.3 X线检查

椎间盘造影后2周及4周,使用X线检测椎间盘高度指数(DHI)的变化。将大鼠用浓度10%的水合氯醛以30 mL/kg的剂量经腹部注射麻醉,使大鼠尾部肌肉松弛以便观察。使用X线拍摄大鼠尾椎椎间盘高度。采用Han等^[12]提出的方法测量DHI,即 $DHI(\%) = 2 \times (D+E+F) / (A+B+C+G+H+I) \times 100\%$,其中A、B、C分别为上位椎体前缘、中点和后缘的高度,D、E、F分别为椎间隙前缘、中点和后缘的高度,G、H、I分别为下位椎体前缘、中点和后缘的高度(图1c)。所有图像均由2名对样本不知情的独立观察者测量。通过Sante DICOM Viewer FREE图像软件分析DHI的变化。

1.4 MRI检查

椎间盘造影后2周及4周使用MRI对大鼠尾椎椎间盘信号进行检测。将大鼠用10%水合氯醛以30 mL/kg的剂量麻醉,使尾部肌肉松弛以便观察。采用3.0 T小动物MRI机(飞利浦,荷兰)对椎间盘进行薄层扫描,以T2加权像评估椎间盘信号改变情况^[13]。所有测量均由2名医师各自独立进行,不同时间段椎间盘信号强度变化应用NIH Image软件进行分析。



注: A~C, 上位椎体前缘、中点和后缘高度; D~F, 椎间隙前缘、中点和后缘高度; G~I, 下位椎体前缘、中点和后缘高度。

Note: A-C, height of anterior, middle and posterior edge of superior vertebra; D-F, height of anterior, middle and posterior edge of intervertebral space; G-I, height of anterior, middle and posterior edge of inferior vertebra.

图1 X线引导下椎间盘造影及DHI测量

Fig. 1 Discography guided by roentgenography and DHI measurement

a: X线引导下进行椎间盘造影 b: 椎间盘造影后的X线影像 c: DHI测量示意图

a: Discography guided by roentgenography b: Roentgenographic image after discography c: Schematic diagram of DHI measurement

1.5 椎间盘含水量的测定

椎间盘造影4周后, 随机取5只大鼠, 将大鼠腹腔注射高浓度水合氯醛麻醉处死, 分离椎间盘与周围软组织, 然后迅速置于 -20°C 液氮中冷冻。10 min后取出冷冻的椎间盘, 使用精度为0.01 mg的电子秤称得其湿质量(WW)。再将椎间盘置于 65°C 烘箱中脱水24 h, 用同一台电子秤称得其干质量(DW)。每次称量均重复3次, 取平均值。椎间盘含水量($\%$)= $(\text{WW}-\text{DW})/\text{WW} \times 100\%$ 。

1.6 组织学检查

影像学数据采集完毕后, 将另外5只大鼠腹腔注射高浓度水合氯醛麻醉处死, 迅速取出C₆/C₇/C₈/C₉/C₁₀椎间盘组织, 置于4%多聚甲醛溶液中固定24 h, 用无菌蒸馏水冲洗后放入EDTA中脱钙48 h, 经不同梯度乙醇脱水, 二甲苯透明, 石蜡包埋, 沿平行于椎间盘软骨排列的方向进行5 μm 连续切片, 进行HE染色。使用HE组织学评分评估椎间盘退行性变的程度^[14]。椎间盘退行性变组织学评分为0~6分。纤维环评分: 0分, 纤维环结构完整、无破损; 1分, 纤维环结构有破损, 但小于整体纤维环的30%; 2分, 纤维环结构有破损, 且大于整

体纤维环的30%; 3分, 纤维环完全破损。髓核评分: 0分, 有典型的含有大空泡状结构的髓核细胞散在分布; 1分, 髓核细胞数量下降低于总细胞数的50%, 存在或不存在细胞集聚; 2分, 髓核细胞数量中度至严重($>50\%$)下降, 且增殖性结缔组织占据小于50%总面积; 3分, 髓核中增殖性结缔组织占据大于50%总面积。

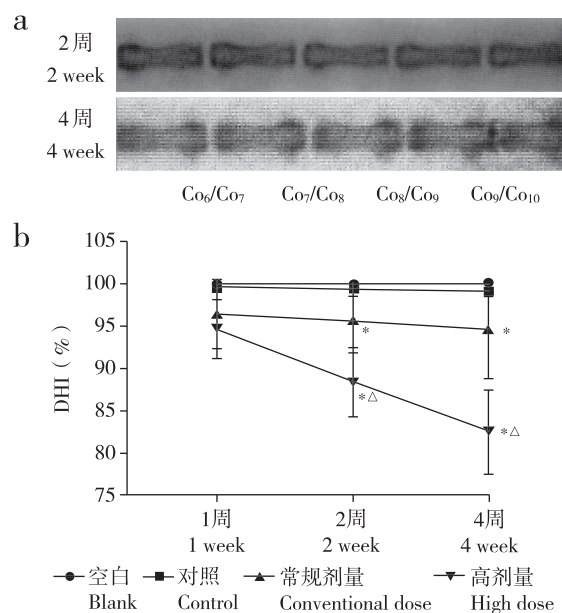
1.7 统计学处理

使用SPSS 21.0软件对数据进行统计学分析, 并使用Graphpad Prism 5软件作图。数据采用 $\bar{x} \pm s$ 表示, 使用单因素方差分析比较组间差异, 多重比较采用Student-Newman-Keuls检验; 以 $P < 0.05$ 为差异有统计学意义。

2 结 果

2.1 造影剂剂量对椎间盘DHI的影响

术后2周和4周时, 与空白组相比, 对照组DHI未见明显变化, 常规剂量组和高剂量组DHI降低, 且高剂量组比常规剂量组降低更为显著, 差异均有统计学意义($P < 0.05$, 图2)。



注: *与空白组相比, $P < 0.05$; Δ与常规剂量组相比, $P < 0.05$ 。
Note: * $P < 0.05$, compared with blank group; Δ $P < 0.05$, compared with conventional dose group.

图2 X线检测造影剂剂量对椎间盘DHI的影响

Fig. 2 Effect of contrast agent dose on DHI of intervertebral disc detected by roentgenography

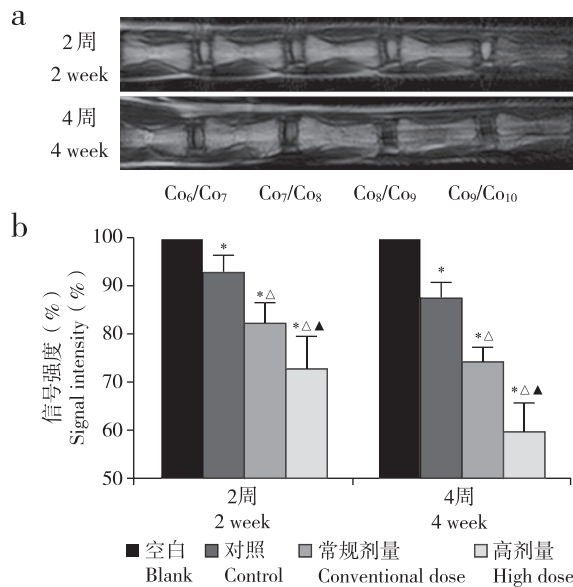
a: 椎间盘X线片 b: 各组DHI变化

a: Roentgenographic image of intervertebral disc b: Change of DHI in each group

2.2 造影剂剂量对椎间盘信号改变的影响

术后2周和4周时MRI检测示, 与空白组相

比, 对照组、常规剂量组和高剂量组椎间盘信号均降低, 且剂量越大信号越低, 差异均有统计学意义 ($P<0.05$, 图3)。



注: *与空白组相比, $P<0.05$; △与对照组相比, $P<0.05$; ▲与常规剂量组相比, $P<0.05$ 。

Note: * $P<0.05$, compared with blank group; △ $P<0.05$, compared with control group; ▲ $P<0.05$, compared with conventional dose group.

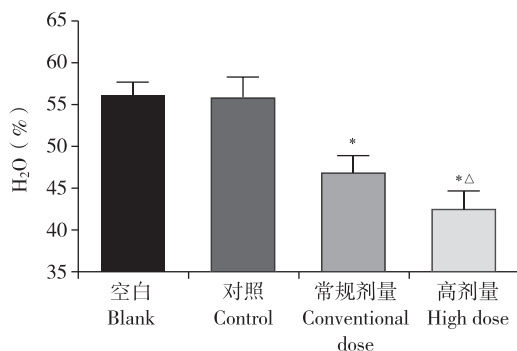
图3 MRI检测造影剂剂量对椎间盘信号强度的影响

Fig. 3 Effect of contrast agent dose on signal intensity of intervertebral disc detected by MRI

a: 椎间盘MRI T2加权像 b: 椎间盘信号强度变化
a: MRI T2-weighted image of intervertebral disc b: Signal intensity change of intervertebral disc

2.3 造影剂剂量对椎间盘含水量的影响

椎间盘造影术后4周, 与空白组相比, 对照组椎间盘含水量未见明显变化, 常规剂量组和高剂量组椎间盘含水量降低, 且高剂量组降低更为显著, 差异均有统计学意义 ($P<0.05$, 图4)。



注: *与空白组相比, $P<0.05$; △与常规剂量组相比, $P<0.05$ 。

Note: * $P<0.05$, compared with blank group; △ $P<0.05$, compared with conventional dose group.

图4 造影剂剂量对椎间盘含水量的影响

Fig. 4 Effect of contrast agent dose on water content of intervertebral disc

2.4 造影剂剂量对椎间盘组织学评分的影响

椎间盘造影术后4周, 通过HE染色检测椎间盘组织学变化(图5)。结果显示, 空白组和对照组未见明显组织病理学改变, 而常规剂量组和高剂量组椎间盘组织病理学改变明显, 纤维环结构有破损, 髓核细胞数量减少。病理组织学评分结果显示, 常规剂量组和高剂量组椎间盘病理组织学评分高于空白组, 且高剂量组病理组织学评分高于常规剂量组, 差异均有统计学意义 ($P<0.05$, 图6)。

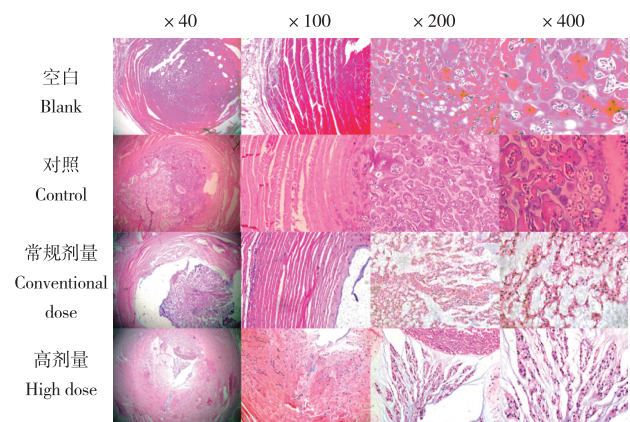
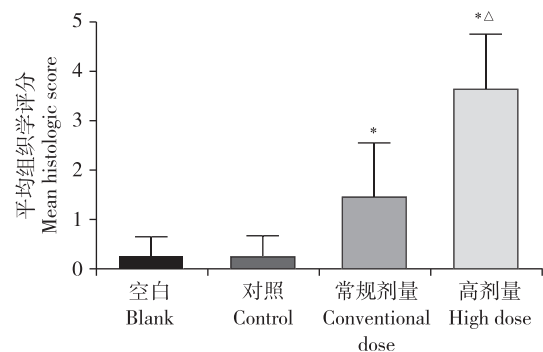


图5 HE染色

Fig. 5 HE staining



注: *与空白组相比, $P<0.05$; △与常规剂量组相比, $P<0.05$ 。

Note: * $P<0.05$, compared with blank group; △ $P<0.05$, compared with conventional dose group.

图6 造影剂剂量对椎间盘组织学评分的影响

Fig. 6 Effect of contrast agent dose on histological score of intervertebral disc

3 讨论

根据分子结构分类, 造影剂可分为离子型和非离子型。离子型造影剂因其不良反应发生率高、机体耐受性差而逐渐被非离子型造影剂所取代。非离子型造影剂也有一定的毒性, 不同种类的非离子造影剂的细胞毒性不尽相同, 可能与造影剂的渗透压和黏度相关^[6, 15-17]。碘海醇属于第二代非离子型造影剂, 目前在临床上应用最为广泛, 也是临床椎间

盘造影常用的造影剂^[18]。体外细胞研究表明, 碘海醇具有一定的细胞毒性^[19]。本研究在大鼠体内就造影剂剂量对椎间盘退行性变的影响进行研究, 于造影术后2周和4周分别对椎间盘的退行性变程度进行评估, 评价指标包括DHI、MRI信号强度、椎间盘含水量和椎间盘组织学改变, 证明了椎间盘造影中造影剂剂量对椎间盘退行性变的影响, 为评估临床椎间盘造影技术的价值和风险提供了实验依据。

本研究证明, 在动物体内碘海醇可以引起椎间盘退行性变, 并且注射剂量越大, 椎间盘退行性变越明显。椎间盘是人体最大的无血管组织, 通过被动扩散完成营养和物质更新, 椎间盘内存在特殊的高渗环境(430~496 mOsm/kg)^[20-21]。本研究使用的碘海醇为上海通用电气药业有限公司产品, 规格为300 mg I/mL, 渗透压为780 mOsm/kg, 明显高于椎间盘的渗透压。且由于椎间盘是无血管器官, 注射的碘海醇可以较长时间残存在椎间盘内, 随着碘海醇注入量的增加, 其对椎间盘的影响也较大。椎间盘退行性变的程度还和椎间盘的静水压有关。体内外研究已经清楚地表明, 静水压可以显著影响细胞外基质成分、细胞合成代谢蛋白的活力和基因的表达^[22-25]。碘海醇剂量对椎间盘退行性变的影响可能包括两方面原因: ①在密闭的椎间盘内注射碘海醇体积越大, 密闭椎间盘的静水压越高, 导致椎间盘退行性变的程度越高; ②碘海醇为高渗液体且具有细胞毒性, 注射体积越大, 高渗且有细胞毒性的溶液的量越大, 椎间盘的退行性变程度也越大。此外, 本研究还发现, 仅进行穿刺针刺入而不进行注射的对照组MRI椎间盘信号也较未进行任何处理的空白组降低, 说明针刺本身也可导致椎间盘退行性变。

本研究的不足之处包括以下几点。①仅从造影剂的剂量角度探讨椎间盘造影对椎间盘退行性变的影响, 还有其他造成椎间盘退行性变的因素需要考虑, 如穿刺针的直径、椎间盘尺寸(纤维环的厚度)、操作者的技术熟练程度、实验动物的营养状态及其他未知因素^[26-27]。②研究对象为正常大鼠尾椎椎间盘, 未研究椎间盘造影术对已经发生退行性变的椎间盘的影响, 这和临床中椎间盘造影术的应用有所差异。主要基于两方面考虑: 一方面, 临床标准的椎间盘造影诊断技术需要1~2个邻近节段的椎间盘造影作为阴性对照, 研究椎间盘造影术对正常椎间盘的影响可有效评估阴性对照的价值和风险; 另一方面, 考虑到椎间盘退行性变造模时, 为避免模

型的退行性变基线不尽相同造成系统误差, 故使用正常椎间盘。③以大鼠椎间盘作为研究对象, 大鼠尾椎椎间盘退行性变造模技术成熟, 操作简便, 安全性高, 可重复性强, 但大鼠椎间盘结构及髓核细胞形态、生理功能和人体有差异, 且大鼠椎间盘内的渗透压和人体不尽相同, 因此, 碘海醇造成的大鼠椎间盘退行性变和人体实际情况存在差异。但因伦理等实际操作问题, 本研究未将人体作为研究对象。造影剂剂量对人椎间盘退行性变的影响有待进一步深入研究。

综上所述, 本研究发现, 造影剂可引发椎间盘退行性变, 且剂量越大, 椎间盘退行性变的程度越高。因此, 在进行椎间盘造影时, 应当充分考虑造影剂剂量对椎间盘的影响, 尽量减少造影剂的使用量, 以使其对椎间盘的影响最小化。

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