

二磷酸盐对关节磨屑刺激单核细胞分泌 IL-1 β 的抑制作用

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摘要 目的: 研究二磷酸盐对关节磨屑刺激单核细胞分泌 IL-1 β 的影响。方法: 分离培养人外周血单核细胞, 加入关节磨屑及不同浓度的阿伦膦酸钠, ELISA 检测细胞上清中 IL-1 β 的含量, 原位杂交检测 IL-1 β mRNA 的表达。结果: 关节磨屑组 IL-1 β 及基因的表达显著高于对照组 ($P < 0.01$), 不同浓度的阿伦膦酸钠组 IL-1 β 及基因的表达明显低于关节磨屑组 ($P < 0.01$)。结论: 阿伦膦酸钠能通过下调人外周血单核细胞 IL-1 β 及 mRNA 的表达, 对人工关节松动可能起防治作用。

关键词 二磷酸盐; 关节磨屑; 白细胞介素-1

Inhibitory effect of bisphosphonates on IL-1 β expression of human monocytes induced by wear debris

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Abstract Objective: To study the effect of bisphosphonates on IL-1 β expression of human monocytes with wear particles stimulation. **Methods:** In vitro human peripheral blood mononuclear cell (PBMC) were cultured with wear particles and bisphosphonates of different concentration, then productions of IL-1 β and IL-1 β mRNA were determined by ELISA and in situ hybridization techniques. **Results:** The production of IL-1 β were increased significantly when wear particles was added, compared with control group ($P < 0.01$). And bisphosphonates reduced the level of IL-1 β . Hybridization demonstrated bisphosphonates might down regulate IL-1 β mRNA expression. **Conclusion:** Bisphosphonates can inhibit IL-1 β expression induced by wear debris and may be used in the prevention and treatment of aseptic loosening of prosthesis.

Key words Bisphosphonates; Wear debris; Interleukin-1

当前, 人工关节置换术已日趋成熟, 愈来愈多的关节疾患患者从中受益。与此同时, 随着人工关节置换术后长期病例增多, 使我们也面临国外学者所面临的同样挑战: 人工关节后期无菌性松动。现已公认: 关节磨屑诱导关节假体周围骨吸收是引起人工关节松动的重要原因。本研究通过观察二磷酸盐对关节磨屑刺激单核细胞分泌 IL-1 β 的影响, 探讨该药物能否抑制关节磨屑诱导的骨吸收。

1 材料与方 法

1.1 主要材料与试剂 人外周血取自同济医院骨科股骨颈骨折拟行关节置换的患者(无其他慢性疾患)。IL-1 β ELISA 试剂盒(深圳晶美生物工程有限公司); 超高分子量的聚乙烯颗粒(ultrahigh molecular weight polyethylene, UHMWPE)(Zimmer 公司),

90% 颗粒大小在 2 μ m 左右; 阿伦膦酸钠(意大利默沙东公司, 注册证号: x20000448)。淋巴细胞分离液(上海试剂二厂)。IL-1 β 原位杂交试剂盒(武汉博士德生物工程公司, 产品编号: MK1198); 胎牛血清、RPMI 1640 培养基(Gibco 公司)。

1.2 人外周血单核细胞分离培养及分组 无菌采集股骨颈骨折拟行关节置换患者的外周血, 肝素抗凝, 用 0.1 mol/L 的 PBS 稀释 1 倍, 将稀释血轻铺在淋巴细胞分离液上, 水平离心(2 000 rpm/min, 20 min), 用毛细吸管小心吸出单核细胞, PBS 洗涤 3 次, 将细胞用含 10% 胎牛血清的 1640 培养基混悬, 调整细胞浓度为 5×10^6 /ml, 每孔 1.5 ml 细胞悬液加入 6 孔板, 6 孔板内预先放入多聚赖氨酸处理过的盖玻片。培养过夜后, 分为 5 组, 分别加入等体积的空白、关节磨屑(终浓度 10^9 /ml)、磨屑和不同浓度

(10^4 mol/L、 10^{-5} mol/L、 10^6 mol/L)阿伦磷酸钠的培养基(即 A、B、C、D、E 组), 37°C 、5% CO_2 细胞培养箱内培养。

1.3 细胞上清液中 IL-1 β 的检测 细胞干预培养 24 h 后,取上清液,离心(2 000 rpm, 20 min),按 IL-1 β ELISA 试剂盒说明书检测 IL-1 β 含量。

1.4 原位杂交 细胞上清液取出后,将盖玻片取出,用冷的 0.1 mol/L 的 PBS 洗涤 3 次,4%的多聚甲醛(含 0.1% DEPC)固定 25 min, PBS 洗涤后吹干,滴加新鲜配制的 5%过氧化氢-甲醇液灭活内源性过氧化物酶,经蛋白酶处理,每片滴加 25 μl 预杂交液,2~4 h 后,滴加含地高辛标记的 IL-1 β 探针杂交液,用原位杂交专用盖玻片覆盖, 42°C 湿盒孵育过夜。系列 SSC 洗涤,封闭液封闭,滴加生物素化地高辛抗体, 37°C 孵育 1 h, DAB 显色。光学显微镜下,胞浆内棕黄色颗粒为核酸杂交阳性信号,随机选 50 个细胞用全自动图像分析仪测定细胞内杂交产物平均吸光光度值(A 值)。

1.5 统计学处理 实验数据均以均数 \pm 标准差($\bar{x}\pm s$)表示,采用方差分析 q 检验,检测各组之间 IL-1 β 及其 mRNA 的表达有无显著性差异。

2 结果

2.1 阿伦磷酸钠对关节磨屑刺激单核细胞分泌 IL-1 β 的影响 细胞上清液中 IL-1 β 含量如下:A 组(125.08 ± 14.60) pg/ml, B 组(1347.87 ± 69.47) pg/ml, C 组(138.92 ± 15.59) pg/ml, D 组(145.09 ± 15.20) pg/ml, E 组(153.94 ± 19.17) pg/ml。方差分析表明各组上清液中的 IL-1 β 含量差异有显著性。磨屑组 IL-1 β 含量明显高于空白组($P<0.01$), 3 种浓度药物组 IL-1 β 含量均低于磨屑组($P<0.01$)。且随着药物浓度增加,IL-1 β 含量逐渐减少。

2.2 阿伦磷酸钠对关节磨屑刺激单核细胞分泌 IL-1 β mRNA 的影响 各组单核细胞内均可见棕黄色杂交颗粒,主要位于胞浆中。空白组 IL-1 β mRNA 表达呈弱阳性,关节磨屑组呈强阳性表达,不同浓度的药物使 IL-1 β mRNA 表达减弱。杂交反应光密度测量显示,磨屑组杂交信号平均光密度值 0.78 ± 0.04 较空白组 0.15 ± 0.02 高($P<0.01$),而加入阿伦磷酸钠后,随着药物浓度的增高,光密度值下降。3 组光密度值分别为 0.16 ± 0.02 、 0.21 ± 0.01 、 0.22 ± 0.03 ,均低于关节磨屑组($P<0.01$)。作用呈剂量效应关系。

3 讨论

关节磨屑启动并导致关节假体周围骨量丢失最终引起假体无菌性松动,是人工关节置换术后失败的重要原因。目前,关节松动的治疗仅靠翻修术。但翻修手术操作复杂、费用高昂,且远期效果远不如初次手术^[1],因而,如能找到非手术方法防治人工关节松动,对于诸多人工关节置换术后患者,尤其是不能耐受二次手术的患者,无疑是一个福音。

事实上关节磨屑产生不可避免,因而针对关节磨屑诱导骨吸收的中间环节进行干预,是阻止人工关节松动的一个可能途径。现已知道,关节磨屑在人工关节假体周围组织的积聚引发严重的异物反应。单核/巨噬细胞被激活,分泌前列腺素 E_2 (PGE_2)、白细胞介素-1(IL-1)、白细胞介素-6(IL-6)、肿瘤坏死因子- α (TNF- α) 等^[2-4],这些因子通过直接或间接途径激活破骨细胞性骨吸收。其中,IL-1 在假体松动中发挥着重要作用:激活破骨细胞破骨,刺激破骨细胞增生,促进破骨细胞与多核细胞融合,诱导破骨细胞分泌胶原酶和前列腺素 E_2 ; 阻止骨钙素合成,影响新骨合成;趋化巨噬细胞,并刺激其合成、分泌 IL-1;促进成纤维细胞分裂,刺激其产生更多的胶原。Jiranek 等^[5]发现,松动假体纤维膜中的巨噬细胞有 IL-1 β mRNA 表达,而且 IL-1 β mRNA 信号的强弱与关节磨屑的多少呈正比。二磷酸盐是以 P-C-P 基团为特征的磷酸盐复合物,其磷酸钙成分能和骨矿盐特异结合,抑制破骨性骨吸收。以往主要用于治疗骨质疏松、Paget 病及肿瘤性骨吸收^[6]。近年来,国外学者发现^[7],二磷酸盐能抑制巨噬细胞 IL-1、IL-6、TNF- α 等细胞因子的分泌。本研究发现,关节磨屑能刺激人外周血单核细胞分泌 IL-1 β 增加,而阿伦磷酸钠能抑制这种刺激作用,实验中未见药物对细胞有毒性作用,说明抑制作用不是通过药物引起细胞凋亡来实现的。既然阿伦磷酸钠能显著抑制单核细胞分泌骨吸收因子 IL-1 β ,那么它就有可能阻断关节磨屑诱导骨吸收的中间环节,从而防治人工关节松动。

当然,关节磨屑诱导的骨吸收因子众多,且各因子之间相互作用。本实验研究了阿伦磷酸钠对关节磨屑刺激单核细胞分泌 IL-1 β 的影响,至于此类药物对其他骨吸收因子的影响究竟如何,以及体内情况下如何作用,都有待进一步研究。

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