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二磷酸盐促进假体周围骨溶解后假体翻修骨整合的实验研究 *

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摘要 目的:评价阿仑膦酸钠对新西兰大白兔假体周围发生骨溶解后,再进行对新西兰大白兔假体翻修骨整合的影响。**方法:**选取雄性新西兰大白兔 30 只,随机分成 3 组(正常组,实验组,对照组,每组 10 只)。正常组在胫骨松质骨区域植入钛合金假体,实验组和对照组分别植入钛合金假体和钛颗粒,饲养 8 周后,三组统一进行假体翻修。实验组用阿仑磷酸钠治疗 8 周后取材,对照组和正常翻修组也分别进行取材。通过假体推出力学实验、硬组织切片观察,评价阿仑磷酸钠对假体周围翻修后骨整合的影响。**结果:**假体推出力学实验结果显示,实验组假体最大推出载荷明显大于对照组(P<0.01)。硬组织学切片通过苦味酸 -- 品红染色,利用图像分析仪器统计假体周围骨整合的面积实验组假体周围骨量明显优于对照组假体周围骨量(P<0.05)。**结论:**二磷酸盐 - 阿仑磷酸钠可以提高假体翻修后假体周围骨整合。

关键词:二磷酸盐;骨溶解;假体翻修;骨整合

中图分类号:Q95-3,R68 **文献标识码:**A **文章编号:**1673-6273(2014)07-1243-04

Promote Implant Osteointegration after Wear Particles Induced Osteolysis Revision Operation with Biphosnnate*

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ABSTRSCT Objective: To evaluate the alendronate New Zealand white rabbits of periprosthetic osteolysis, renovated New Zealand white rabbits prosthesis osseointegration. **Methods:** 30 selected male New Zealand white rabbits were randomly divided into three groups (normal control group, the experimental group, the control group, n = 10).Area of the normal group tibia cancellous bone implanted titanium prosthesis, the experimental and control groups were implanted with a titanium prosthesis and titanium particles 8 weeks, three groups of reunification of the renovation of the prosthesis. Sacrificed after 8 weeks of treatment in the experimental group with alendronate control group and normal group were drawn renovated. Through the the prosthesis launch mechanics experimental hard tissue slice observation, evaluation of the effects of alendronate on periprosthetic renovated osseointegration. **Resluts:** Prosthesis launched mechanics experimental.Results: Show that the experimental group (alendronate treatment group) prosthesis launched load was significantly greater than the control group (P<0.01). Hard histological sections through of picric - Magenta staining using image analysis instrument Statistics periprosthetic osseointegration periprosthetic bone area of the experimental group was significantly better than the control group periprosthetic bone mass (P<0.05). **Conclusion:** Bisphosphonates-alendronate the periprosthetic bone integration can improve the prosthesis renovated.

Key words: Bisphostates; Osteolysis; Revision; Osteointegration

Chinese Library Classification(CLC): Q95-3, R68 Document code: A

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前言

人工关节置换术现代人工关节置换术开始于 20 世纪 50 年代末,是 20 世纪骨科手术最伟大的突破之一[1]。人工关节置换术明显改善了患者的焦虑、抑郁状况和疼痛程度以及生活质量,目前全世界每年有约 150 万的患者接受关节置换术治疗[2]。虽然人工关节置换术已被广泛开展,但术后远期可能发生骨溶解和无菌性松动等并发症也不断增多[3]。因此,如何研究人工关节假体无菌性松动的发生机制及采取预防和治疗对策,是迫在眉睫的重要课题。

导致人工关节发生假体无菌性松动的主要因素是人工关节磨损颗粒诱导的骨溶解,有些学者提出以药物来预防人工关节假体周围骨溶解,更有些学者提出在对骨溶解处理时行髋臼杯翻修术。二膦酸盐 (bisphostates,BPs)是近 30 年来发现的一种可治疗与骨吸收增加有关的疾病的新药,其作用机制是通过对破骨细胞中骨吸收必需的底物中蛋白去磷酸化如 C-Src 抑制,

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从而抑制体内异位钙化和各种药物引起的骨吸收[4-6]。阿仑膦酸钠 (alendronatesodium)属第三代二膦酸盐类骨吸收抑制剂，它具有抑制骨吸收、降低骨转换率来治疗骨质疏松和降低骨折发生率的作用。本研究给予阿仑膦酸钠液肌注以观察其促进假体周围成骨，来观察假体周围骨整合的情况。

1 材料和方法

1.1 试剂和设备

阿仑膦酸钠高纯度药粉（alendronate，ALEN，批号R140-108060801-07110601，石家庄制药集团华盛制药有限公司生产）。钛颗粒（由 Dr. John Cuckler, University of Alabama, Birmingham, AL 赠送）。钛合金假体(直径 3.0 cmm 和 4.0 mm)由 Dr. John Cuckler, University of Alabama, Birmingham, AL 赠送)。生物力学测试仪，(型号：SHIMADZU，由昆明理工大学实验室提供)硬组织切片机器（型号:LEICA SP1600 由上海九院骨科实验室提供)光学显微镜(型号：lei ca DM4000B 由上海九院骨科实验室提供)苦味酸 -- 品红染色(由上海九院骨科实验室提供)。

1.2 方法

1.2.1 动物分组及手术 健康成年雄性新西兰大白兔 30 只(由昆明医科大学动物实验中心提供)，体重约(2.2-2.5) kg。以全价颗粒鼠饲料进行饲养，摄食、饮水不受干预和控制，12 h 日光交替照射，动物室内温度常温保持在 20 ℃ 左右。喂养 1 周后按随机数字表法分为正常组，实验组，对照组 3 组，每组 10 只。其中正常组将 3.0 mm 钛合金假体单纯植入双侧胫骨，饲养 8 周后，再植入 4.0 mm 喷砂钛合金假体进行翻修手术；对照组于双侧胫骨植入 3.0 mm 钛合金假体 +30 mg 钛颗粒，饲养 8 周后进行翻修：翻修手术植入 4.0 mm 喷砂钛合金假体；实验组于双侧胫骨植入 3.0 mm 钛合金假体 +30 mg 钛颗粒，饲养 8 周后进行翻修：翻修手术植入 4.0 mm 钛合金假体肌注阿仑磷酸钠每周一次，连续 8 周。

1.2.2 手术方法 用 2.5%的戊巴比妥按照 2.5 ml/kg 耳缘静脉注射，麻醉生效后，双下肢剃毛。用碘伏消毒皮肤，于双腿胫骨区域开刀以暴露胫骨松质骨区域，直径 3.0 mm 电钻于双侧胫骨近端钻孔，用 0.9%的生理盐水清理骨隧道。正常组左右胫骨区域分别单纯植入直径 3.0 mm 钛合金假体，实验组和对照组分别将悬浮在 100 μL 中 10 mg 的钛颗粒用移液枪注入隧道内再植入 3.0 mm 钛合金假体（见图 1)。术后肌注头孢唑林 0.1 mg/kg 抗炎治疗 1 周。饲养 8 周后正常组、实验组和对照组统一进行假体翻修手术，植入直径 4.0 mm 钛合金假体。具体给药途径与剂量参考 Koçer 等人的文献[7]。

1.2.3 大体观察 肌注后 8 周取新西兰大白兔右胫骨近端，剔除软组织后观察拍照。左侧每组统一用 0.9%生理盐水纱布包裹起来，放在 -20℃ 冰箱冻存，准备做假体推出力学实验用。右侧每组统一用中性福尔马林溶液固定标本，放在 4℃ 冰箱存放备用。

1.2.4 生物力学测试 将冻存好的标本统一取出来，室温解冻，用医用牙托粉固定标本胫骨两端，放入生物力学测试仪上，以 1 mm/s 速度将假体推出得出最大假体推出载荷，电脑记录数据(见图 2-4)。

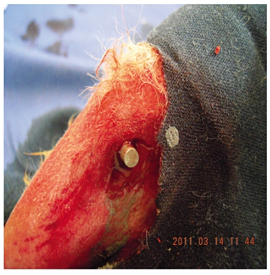

图 1 假体植入

Fig.1 Prosthesis implantation

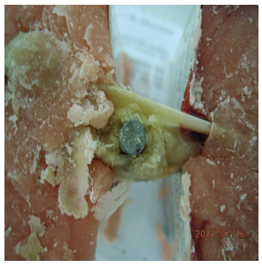

图 2 固定标本

Fig.2 Sample preparation

图 3 推出力学试验

Fig.3 Mechanical test

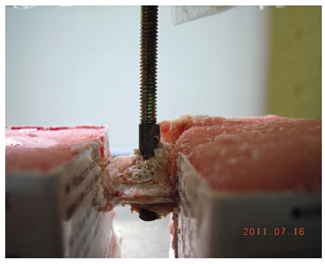

图 4 推出力学实验示意图

Fig.4 Experimental schematic

1.2.5 硬组织切片及苦味酸 - 品红染色 VAN GIESON 将固定好的标本统一取出来，流水冲洗 24 h 后，每组标本分别用 70%、80%、90%、100%酒精脱水 48 小时。然后把放入玻璃容器中进行标本包埋，标本透明使用纯二甲苯透明，总时间不超过 4 小时。切片及磨片具体方法和苦味酸 - 品红染色 VAN GIESON 参考王楠的文献[8]。组织形态计量学参考刘丰的文献[9]。

1.3 统计学处理

将数据录入 SPSS17.0 软件并进行统计分析，统计方法有 t 检验、单因素方差分析和 LSD-t 检验，用($\bar{x}\pm s$)表示计量资料，检验水准取 α=0.05。

2 结果

2.1 药物对新西兰大白兔假体推出最大载荷的影响

三组最大推出载荷差异有统计学意义(F=53.684，P<0.001)；正常组假体最大推出载荷高于实验组，差异有统计学意义(LSD-t=7.645，P<0.001)；正常组假体最大推出载荷高于对照组，差异有统计学意义(LSD-t=2.234，P=0.036)；实验组假体最大推出载荷高于对照组，差异有统计学意义(LSD-t=9.880，P<0.001)，见表 1。

2.2 组织学观察结果

结果显示，正常组的假体周围骨整合接触良好(图 5-6)。实验组的假体周围骨整合接触稍差(图 7-8)。对照组的假体周围骨整合接触最差(图 9-10)。实验组钛合金假体周围新生骨的厚度和面积统计数值明显大于对照组，差异有统计学意义(P<0.01)，见表 2。

3 讨论

瑞典诺贝尔实验室的骨科专家 Branemark 于 1960 年提出

表 1 三组标本假体最大推出载荷

Table 1 The prosthesis maximum launch load of three groups of specimens

	分组 Groups	标本数量 Numbers	Mean	SD	标准误 Standard error
假体最大推出载荷 Prosthesis maximum launch load	正常组 Normal group	8 只	375.64375	42.246070	14.936241
	实验组 Experimental group	8 只	255.16750	20.504603	7.249472
	对照组 Control group	8 只	219.95750	27.83343	9.840619

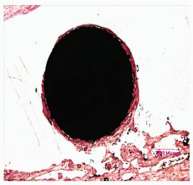

图 5 正常组 (× 10)

Fig.5 Normal group (× 10)

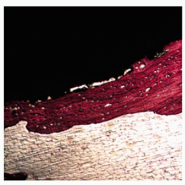

图 6 正常组 (× 40)

Fig.6 Normal group (× 40)

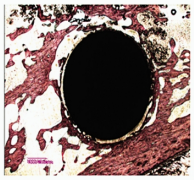

图 7 实验组 (× 10)

Fig.7 Experimental group (× 10)

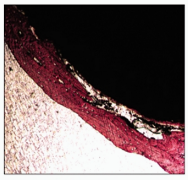

图 8 实验组 (× 40)

Fig.8 Experimental group (× 40)

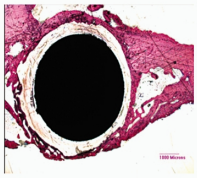

图 9 对照组 (× 10)

Fig.9 Control group (× 10)

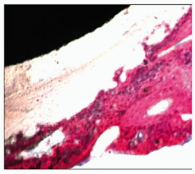

图 10 对照组 (× 40)

Fig.10 Control group (× 40)

表 2 实验组和对照组钛棒合金假体周围新生骨的厚度和面积

Table 2 Thickness and area of titanium alloy periprosthetic bone between the experimental group and control group

项目 Projects	组别 Groups	例数 Cases	$\bar{x}\pm s$	t	P
厚度 Thickness	实验组 Experimental group	8	2.841± 0.817	2.665	0.018
	对照组 Control group	8	1.625± 0.999		
面积 Area	实验组 Experimental group	8	242.915± 69.529	5.728	<0.001
	对照组 Control group	8	62.065 ± 56.040		

了骨结合(osteointegration,也称骨整合)的理论。随后种植在医学临床上迅猛发展,广泛应用于因创伤或肿瘤造成的颌面缺损的整复。骨整合的定义是:在光学显微镜下,种植体与周围骨组织直接接触,其间无纤维结缔组织界面层的直接接触,目的就是将假肢与患者的骨骼完美结合在一起[10]。

近年来,骨整合相关研究多集中于注射骨生长因子(如 BMP, TGF-B, FGF 等)[11-14]、新型种植体材料[15-17]及纳米化和假体表面改性[18,19]对假体骨整合的影响。虽然假体表面镀以 HA 涂层及新型纳米材料的应用能可提高假体 - 骨界面结合的强度,促进假体骨整合,提高假体固定的稳定性,同时也能提高新型材料的强度和韧性。但笔者认为其大范围推广的可能性很低,新型材料的花费昂贵姑且不论,就 IL-1、IL-6、TGF、BMP 等局部生长因子能否长期有效稳定复合的问题,目前学术界尚无定论。因此把另辟蹊径把目光转向研究能够控制假体周围骨丢失及发生假体无菌性松动的药物未尝不是一个新的选择。

二膦酸盐是一种可治疗与骨吸收增加有关的疾病的新药[20]。本次研究发现,正常组假体周围骨整合接触良好,假体周围的新生骨较多,骨密度明显高于其余两组,包绕的十分牢固。实验组的假体周围骨整合接触稍差,界面处可以见到一薄层灰白色骨质包绕在假体周围,而钛棒假体周围的纤维界膜变得稀少且较薄,新生骨多是直接与假体界面接触,钛合金假体周围新生骨的厚度和面积统计数值明显大于对照组。对照组的假体周围骨整合接触最差,钛棒假体与周围骨质结合处有一层可见的较为明显的纤维界膜,二者结合紧密度较差,容易分离;假体周围的纤维界膜较厚,但细胞成分少,且多为整齐的线性排列的长梭形成纤维细胞,与新生骨之间产生了一条清晰界限,但却未发现多核巨细胞、单核 / 巨噬细胞及破骨细胞。

综上所述,如果发生骨溶解且进行假体翻修,阿仑膦酸钠治疗能很大程度上预防假体周围早期骨量减少,假体周围的骨生长速度得以促进,能够促使假体周围骨量和骨密度的提高,同时促进假体周围骨的骨整合,提高其假体的长期固着力。

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