碱性纤维母细胞生长因子对大鼠血管损伤修复的影响

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The Effects of Basic Fibroblast Growth Factor on Repairment of Balloon Denuded Injury to Rat Aorta

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ABSTRACT

Aim　To study the effects of basic fibroblast growth factor (bFGF) on the repairment of the injured intima by balloon denudation in rat aorta.

Methods　The male Wistar rats were divided into six groups as follows, two early treatment groups (a 7 day-group and a 21 day-group), one late treatment group, and three control groups including a simulated operation group. The intima of the aortae was denuded by using a balloon catheter. bFGF was injected into the tail veins of the animals on the 1st, 2nd and 3rd day (early treatment) and on 15th, 16th and 17th day after balloon denudation (late treatment), respectively. The denuded aorta rings were perfused in the Krebs-Henseleit's balance salt bath. The plasma NO_2^- levels were determined by the method described by Green and his colleagues, and the plasma endothelin levels and the cGMP content in the aortic tissue were measured by radioimmunoassay.

Results　Intravenous injection of bFGF, not only in the early treatment but also in the late treatment, could significantly promote the relaxation reaction induced by acetylcholine. The maximal relaxation magnitude of the denuded aorta rings in the 7-day-treatment group and 21-day-treatment group were 2.2 and 1.7 times that of both control groups, respectively, and the maximal relaxation magnitude in the late treatment group was 2.6 times as high as in control group.

After denudation, the plasma NO_2^- levels of the 7-day and 21-day control group were lower by 12% and 27%, and the cGMP contents of both control groups were lower by 57% and 48%, whereas the plasma endothelin levels of both control groups were higher by 70% and 40%, respectively, than that of the simulated operation group. Meanwhile, the plasma NO_2^- levels of both early treatment groups were higher by 50% and 115%, and the cGMP content of both early treatment groups were higher by 106% and 100%, respectively, than that of the control groups, whereas the plasma endothelin levels were significantly lower. The results of the late treatment group were simular to that of the early treatment groups.

Conclusion　Using bFGF might promote the repairment of denuded endothelium, inhibit the over-proliferation of denuded intima, and therefore might play a role in preventing restenosis of balloon denuded aorta.

KEY WORDS　Fibroblast growth factor, basic; Endothelium; Aorta; Rats

摘要　观察碱性纤维母细胞生长因子对大鼠主动脉内皮剥脱所致血管损伤修复的影响，以探讨碱性纤维母细胞生长因子在防治经皮腔内血管成形术后再狭窄中的应用前景。在制作动物模型后，用放射免疫法测定了内皮剥脱大鼠主动脉组织环一磷酸鸟苷和血浆内皮素的含量。结果发现，无论是早期或晚期给碱性纤维母细胞生长因子都能明显增强剥脱血管对不同浓度的乙酰胆碱的舒张反应，促进一氧化氮的合成。表现为血浆一氧化氮含量和主动脉组织环一磷酸鸟苷含量升高，

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血浆内皮素浓度和血管壁内膜/中膜面积比值下降。由此可见,适当应用碱性纤维母细胞生长因子可促进内皮功能的恢复,抑制内膜的过度增生而防止再狭窄。

关键词 碱性纤维母细胞生长因子; 再狭窄; 主动脉内皮剥脱

经皮腔内冠状动脉成形术(percutaneous transluminal coronary angioplasty, PTCA)是闭塞血管再通的首选方法。然而术后半年内约有30%～50%的病人发生血管再狭窄[1]。由于再狭窄的发生机理尚未完全阐明,因此临床无有效治疗措施,目前治疗致力于抑制平滑肌细胞(smooth muscle cell, SMC)增殖,但效果并不理想[2]。近年发现血管损伤后重塑(remodeling)、内皮功能紊乱、细胞外基质增生和血栓形成等因素都参与再狭窄的发生过程。碱性纤维母细胞生长因子(basic fibroblast growth factor, bFGF)是广泛存在的生长因子,可促进平滑肌细胞增殖和迁移。它还具有促进血管内皮修复,刺激内皮依赖性血管舒张功能等内皮营养作用[3]。本文在球囊剥脱大鼠主动脉内皮模型上,观察 bFGF 对损伤血管内皮功能恢复的影响,以探讨应用 bFGF 防治再狭窄的可行性。

1 材料与方法

1.1 材料

Wistar 大鼠由北京医科大学动物实验中心提供,环一磷酸鸟苷(cyclic guanosine monophosphate, cGMP)放射免疫药盒为上海中医药大学制备,内皮素放射免疫药盒为美国 Phoenix Pharmacy 产品。重组人 bFGF 由北京白鹭园生物技术公司提供。去甲肾上腺素和乙酰胆碱为 Sigma 公司产品。余为市售分析纯产品。

1.2 方法

1.2.1 大鼠主动脉内皮剥脱[4,5] 体重 300～380 g 雄性 Wistar 大鼠,40 mg/kg 戊巴比妥钠麻醉,经左侧颈总动脉插入自制的球囊导管至腹主动脉,注入 0.1 mL 生理盐水扩张球囊,反复抽拉 3 次,剥脱主动脉内皮。实验分 6 组(每组 $n=6$)。对照 I 组和 I 组:术后第 7 和 21 天处死动物取材。早期给药 I 组和 I 组:术后第 1、2 和 3 天尾静脉各注射 bFGF 10、5 和 2 μg(溶于 0.1 mL 生理盐水中),分别于术后第 7 和 21 天处死大鼠取材。晚期给药组:术后第 15、16 和 17 天于尾静脉各注射 bFGF 10、5 和 2 μg,第 21 天处死动物取材。假手术组:除不插入球囊导管外,其余操作同对照组。

1.2.2 血管反应性测定[6] 取大鼠胸主动脉,剪成约 3 mm 长血管环,置于 37℃、通 95%O_2-5%CO_2 的 Krebs-Henseleit(K-H)平衡液浴槽内,调节静止张力为 0.5 g,预激后,用不同浓度去甲肾上腺素收缩。分别加入各种浓度的乙酰胆碱(acetylcholine, ACh),经张力换能器在记录仪上记录其舒血管反应。

1.2.3 NO_2^-、内皮素和 cGMP 含量测定 取主动脉血分离血浆,按 Green 等[7]方法测定血浆 NO_2^- 浓度和放射免疫法测定血浆内皮素含量,取约 25 mg 主动脉放射免疫法测定组织 cGMP 含量。

1.2.4 病理组织学检查 取主动脉组织,10%甲醛固定,石蜡包埋,切片,HE 染色。在显微镜下观察血管形态变化,计算机图像分析(Sigma Scan 软件),计算内膜(intima)和中膜(medium)面积比率(I/M)。

1.3 统计学处理

实验结果以 $\bar{x}\pm s$ 表示,组间作 q 检验。

2 结果

2.1 碱性纤维母细胞生长因子对损伤血管舒张功能的影响

对照组在内皮剥脱后,血管对 ACh 的内皮依赖性舒张反应较假手术组明显减弱,甚至丧失,术后 21 日较术后 7 日血管舒张反应无显著恢复(见图 1 和图 2, Figure 1 and Figure 2)。

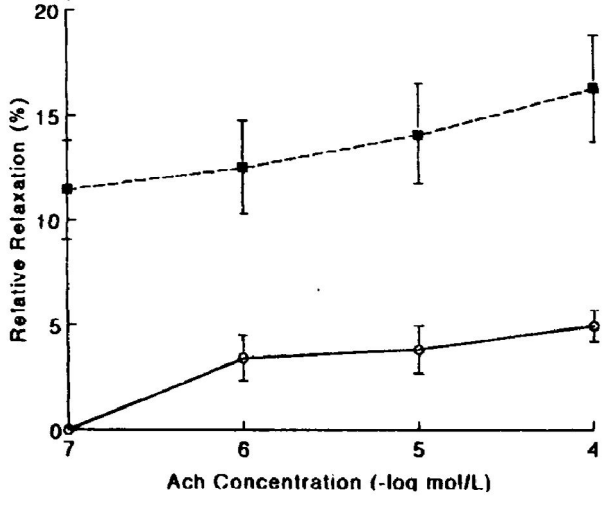

Figure 1. The relaxation reaction induced by acetylcholine in the denuded aortic ring. 0—0: 7-day control group; ●—●: 7-day-early treatment group.

早期给 bFGF 后 7 日和 21 日处死的动物,

血管对 ACh 舒张反应较对照组强烈，最大舒张反应分别增加 2.2 和 1.7 倍($P<0.01$)；晚期给药组亦具有类似作用，血管对 ACh 的最大舒张反应比对照组增加 2.6 倍($P<0.01$)。

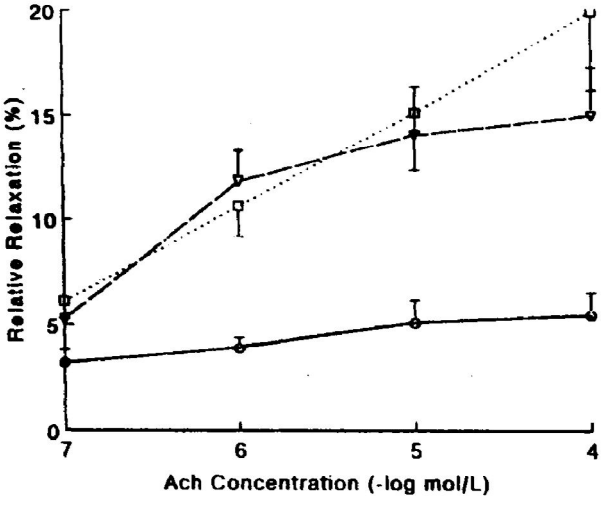

Figure 2. The relaxation reaction induced by acetylcholine in the denuded aorta ring. ○—○: 21-day-control group; ▽—▽: 21-day early treatment group; □—□: Late treatment group.

2.2 血浆 NO_2^-、内皮素和动脉壁 cGMP 含量的改变

内皮剥脱的对照 I 组和 II 组动物血浆 NO_2^- 含量分别较假手术组低 12%($P>0.05$)和 27%($P<0.01$)，血管 cGMP 含量分别少 57%和 48%，血浆内皮素水平分别高 70%和 40%(附表，Table)。

早期给 bFGF 治疗的 I 组和 II 组，血浆 NO_2^- 含量分别较相应对照组高 50%($P<0.05$)和 115%($P<0.01$)，动脉壁 cGMP 含量分别较相应对照组高 106%和 100%($P<0.01$)，而且明显降低了内皮素水平($P<0.01$)。晚期给药组和相应对照组相比亦发生类似变化，其变化幅度与早期给药 II 组相近(附表，Table)。

2.3 内膜增生的形态学改变

40 倍光镜下观察，内皮剥脱术后 7 天，血管壁增厚，新生内膜形成。21 天时内膜增厚更严重，其 I/M 比值增加，静脉注射 bFGF 可明显降低 I/M，早期给 bFGF I 组和 II 组 I/M 比值较相应对照组分别低 36%和 66%($P<0.01$)，晚期给药组较相应对照组低 69%($P<0.01$，附表，Table)。

Table. The effect of bFGF on plasma NO_2^-, endothelin concentrations and on aoetic cGMP level, rate of I/M in balloon denuded rats ($\bar{x}\pm s$, $n=6$).

Groups	NO_2^-(μmol/L)	cGMP(nmol/g)	endothelin(ng/L)	I/M
7-day-control	5.4±0.94	1.90±0.19	14.0±1.2	0.14±0.01
21-day-control	4.5±0.8	2.28±0.36	11.5±1.5	0.29±0.04
7-day-early treatment	8.1±0.8^{b}	3.91±0.33^{b}	12.2±1.5	0.09±0.01^{c}
21-day-early treatment	11.6±3.3^{bd}	3.80±0.44^{a}	8.5±1.0^{d}	0.10±0.02^{a}
Late treatment	9.0±2.2^{a}	3.68±0.32^{a}	9.6±0.8^{bd}	0.09±0.03^{a}
Simulated operation	6.1±0.9	4.41±0.51^{b}	8.3±1.2^{b}	0.004±0.007^{b}

a: $P<0.05$, compared with 21-day-control group; b: $P<0.01$, compared with 7-day-control group; c: $P<0.05$, compared with 7-day-control group; d: $P<0.001$, compared with 21-day-control group.

3 讨论

目前防治再狭窄多以抑制平滑肌细胞增殖和内膜增厚为目标。血管损伤后平滑肌细胞增殖和内皮修复本质上都是抗损伤性反应，反应不足与过度均可加重血管病变，因此，寻找合适的干预因素促进损伤血管修复无疑对再狭窄的治疗具有重要意义。早期工作发现 bFGF 可促进平滑肌细胞增殖，因而认为 bFGF 是再狭窄产生的危险因子[8]。也有资料表明，人血管再狭窄病变处细胞成分仅占内膜体积的 11%，其余

均为细胞外基质,说明细胞外基质是造成内膜增厚的关键因素,而平滑肌细胞增殖不起主要作用[9]。近年发现 bFGF 能够:①促进内皮细胞修复,有利于内皮下平滑肌细胞与血液隔离开,并能减轻其增殖程度[10];②刺激自发性高血压大鼠内皮细胞 NOS 产生,改善内皮依赖性的血管反应[11];③bFGF 可逆转动脉粥样硬化病变,而 VEGF 则无效[12];④可增强胶原酶的活性,抑制胶原的产生[13]。bFGF 的上述生物学效应提示对形成再狭窄的具有潜在的拮抗作用。

本工作观察到球囊拉伤后,血管对 ACh 的内皮依赖性舒张反应明显减弱,甚至丧失,而且术后 21 日与 7 日比较无明显恢复,血浆 NO_2^- 和动脉壁 cGMP 含量下降。NO_2^- 为内皮舒张因子 NO 的终末产物,NO 是强大的内皮舒张因子,以旁分泌方式作用于血管 SMC,增加其下游产物第二信使 cGMP 的产生。本实验结果还表明,球囊拉伤后,内皮素水平明显增高,后者是内皮细胞分泌的一种缩血管物质,血管拉伤后,SMC 也可产生大量内皮素,内皮素通过促进 SMC 的增殖参与再狭窄的形成。应用 bFGF 显著地改善内皮的舒张反应,早期给药 I 组、Ⅱ 组较对照组最大舒张反应增加 2.2 倍和 1.7 倍,血浆 NO_2^- 的含量和动脉壁 cGMP 含量增加。相反,内皮素的产生却明显下降。组织形态学观察到内皮剥脱后 7 天,管壁增厚,新生内膜形成,21 天后主动脉内膜增厚更严重,应用 bFGF 不仅没有造成新生内膜增厚,反而使 I/M 比值显著降低,因此推测 bFGF 不仅促进内皮细胞产生内皮舒张因子,还可促进内皮修复,从而抑制新生内膜的增厚。本实验还观察到晚期给 bFGF 和早期应用有类似的效果,根据以往的资料,大鼠主动脉球囊剥脱血管,2 周后内皮的修复达高峰[13]。但 bFGF 在术后第 15 天开始应用仍有效。其作用机制有待进一步研究。总之,bFGF 通过促进内皮结构和功能的修复,可能有助于防止再狭窄的形成。

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(1997-05-14 收到, 1997-09-06 修回)