

封闭负压引流联合局部给氧治疗兔耳缺血性创面的实验研究

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摘要 目的 :观察封闭负压引流联合局部给氧促进兔耳缺血性创面愈合的效果。方法 28 只大耳白兔 , 双耳背各造成直径 2.5cm 全层皮肤缺损 结扎中央血管神经束及后边缘动静脉 形成缺血创面。56 个创面随机分为七组 :A 组(-50mmHg 负压同时给氧 ,浓度为 40%±5% ,每日 4 小时)、B 组(持续 -50mmHg 负压 4 小时继之局部给氧 1 小时)、C 组(负压治疗 4min ,停止 1min ,每日 4 小时 ,之后给氧 1 小时) ,D 组(-50mmHg 负压治疗每日 4 小时)、E 组(-125 mmHg 负压治疗每日 4 小时)、F 组(单纯给 40%±5%氧 1 小时)和 G 组(空白对照)。在创面形成第 0、1、3、5、7、10、14、18 天拍照 ,测量创面面积 ,计算创面愈合率及创面愈合时间 ,在各时相点切取创面标本 组织学观察创面肉芽、上皮生长、水肿和炎细胞 ,Ki67 免疫组化标记增殖细胞并计算增殖指数 ,TUNEL 法计算凋亡指数。结果 :负压给氧组在相同时相点创面愈合率高于单纯负压、氧疗或空白组(P<0.05) ,创面肉芽生长快 ,水肿和炎症轻 ,细胞增殖指数高于对照组(P<0.05) ,而细胞凋亡指数显著低于对照组(P<0.05)。结论 :负压联合局部给氧能显著促进兔耳缺血创面的愈合。

关键词 封闭负压引流技术; 局部氧疗; 创面愈合; 增殖; 凋亡

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Block Negative Pressure Drainage Combined with Local Oxygen for Ischemic Wound Healing of Rabbit Ears

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ABSTRACT Objective: To investigate the effect of negative pressure therapy (NPT) in combination with Topical oxygen therapy (TOT) on the treatment of ischemic wound in rabbit dorsal ear. **Method:** There were 56 wounds on both ears of 28 rabbits, and the central neurovascular bundle and the posterior peripheral artery and vein were ligated. They were divided into seven groups: Group A were treated with ONPT for four hours every day; Group B was treated with continuous NPT (-50mmHg) for four hours and an hour TOT; Group C was treated with intermittent NPT (-50mmHg) for four hours and an hour TOT; Group D was treated with NPT (-50mmHg) for four hours; Group E were treated with NPT (-125mmHg) for four hours; Group F was treated with TOT for one hour and group G was treated with conventional dressing change every other day. Take photos of the wounds at the given time point (immediately the wounds were created and the 1st, 3rd, 5th, 7th, 10th, 14th, 18th day after the treatment), then calculate the areas and the rate of healing with the image analysis. Tissue specimens, collected from the wound, were embedded with paraffin and studied with HE staining to detect the formation of granulation, inflammatory cell infiltration, and the edema. Furthermore, immunohistochemistry for the related antigen of Ki67 was done to estimate the proliferating cells and the Cell Apoptosis Detection Kit was used to assess the apoptosis cells. Then the proliferation and the apoptotic index were calculated. **Results:** ①the healing rates at the given time point of group A, B and C were higher than that of other groups (P<0.05). ②Formation of the granulation in group A, B and C was faster than that of other groups, but the cell infiltration and the edema were less serious than that of group C, D, E and F. ③The proliferation index of group A, B and C is larger than that of other groups, but the apoptotic index in the first three groups is less than that in other ones. **Conclusion:** NPT in combination with TOT can significantly promote healing of ischemic wound in rabbit dorsal ear.

Key words: Negative pressure; Topical oxygen therapy; Wound healing; Proliferation; Apoptosis

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前言

慢性创面治疗仍是当前的棘手问题^[1]。外科医生常借助清创换药 ,去除坏死组织 ,进行皮片或皮瓣移植的办法 ,使创面达到

愈合。但很多慢性创面是其它疾病的并发症 ,或是严重创伤后未得到妥当治疗的结果 ,患者往往体制欠佳^[2]。形成慢性创面的病因多种多样 ,病理机制复杂 ,当今尚不能模拟出真正符合人慢性创面特点的动物模型 ,而缺血创面可能是最具人慢性创面特点模型 ,具有代表意义^[3]。研究缺血创面的治疗可以对揭示慢性创面的病理机制和治疗提供一定的理论依据。局部给氧治疗创面是根据高压氧疗法机理和其缺点提出的 ,多数病例报告肯定其治疗效果 ,但缺乏大样本随机对照研究 ,故其机理有待

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进一步研究^[4]。本实验利用兔耳背缺血创面模型,观察封闭负压引流联合局部氧疗的治疗作用,对其作用机理进行初步探索。

1 材料与方法

1.1 材料与设备

①仪器设备:以诺负压仪(ILSino RNPT-I,山西以诺医疗科技有限公司),氧浓度检测仪(Mini 3000, Mine Safety Appliances Company Pittsburgh Pennsylvania),数码相机(NikonDx-40, 尼康映像仪器销售(中国)有限公司),显微镜(Nikon ECLIPSE 55i, 尼康映像仪器销售(中国)有限公司),医用氧气瓶(护生 HS 系列便携式供氧器,北京健生新世纪科技发展公司),恒温孵箱(上海力申 BB16 μ v 上海力申科学仪器有限公司),微波炉(美的 EG823EA6-NS 佛山市顺德区美的微波电器制造有限公司),电冰箱(海尔 BCD-170H 青岛海尔集团)。②试剂材料:聚氨酯负压敷料(带贴膜),细胞凋亡检测试剂盒(武汉博士德生物工程有限公司, MK1020),鼠抗 Ki67 抗体(北京中山公司),HRP 标记的羊抗鼠抗体(北京中山公司),DAB 显色剂(北京中山公司),阿拉伯树胶(西安化学试剂厂),硫化钠(西安化学试剂厂),戊巴比妥钠(DH0602 西安舟鼎国生物技术有限责任公司)。

1.2 动物分组及动物模型

28 只大白兔共 56 只耳,随机化分为七组,每组 8 只。实验前 3 天,兔耳背用 10%硫化钠脱毛。实验日动物用 1%戊巴比妥钠耳缘静脉注射麻醉(30mg/kg 体重)后,俯卧位固定。75%乙醇消毒双侧耳背皮肤。距耳根 3-3.5cm 处分离结扎兔耳中央血管神经束及后边缘动静脉,结扎兔耳背侧远端 2-3 条较大的血管交通支,在耳背中央形成直径 2.5cm 圆形全层皮肤缺损创面,保留软骨膜及部分皮下组织,创面压迫及电凝止血。将动物放入特制木盒中,头部外露。

1.3 分组治疗

实验共分为七组(A-G 组,前 3 组为实验组,后 4 组为对照组),每组 8 只兔耳。A 组为负压同时局部给氧治疗组:实验创面形成后将单层油纱布和无菌多孔聚氨酯海绵敷料按照创面大小剪裁(厚 0.8cm),依次覆盖于皮肤缺损区。取 A、B 两根内径 0.3 cm 的塑料引流管,各在其一端形成 3 个侧孔后分别插入海绵,最后用透明贴膜及阿拉伯树胶将创面及敷料完全封闭。A 管由创缘皮肤表面引出经过氧浓度监测仪传感器连接至负压引流瓶,B 管在创面另一端引出连接氧气瓶。-50mmHg 持续负压同时给与氧浓度 40% \pm 5%的局部氧疗每天 4 小时,B 组为持续负压给氧组:连接同 A 组,每日给与 -50mmHg 负压治疗 4 小时之后给予氧浓度 40% \pm 5%的局部氧疗 1 小时,C 组为间歇负压给氧组:连接同 A 组,每日给予 -50mmHg 间歇负压治疗(仪器运行 4 分钟,停止 1 分钟,如此反复)4 小时之后给与氧浓度 40% \pm 5%的局部氧疗 1 小时,D 组为单纯负压组:仅从创面引出一根引流管连接负压仪,每日 -50mmHg 负压 4 小时,E 组为单纯负压组:连接同 D 组,每日 -125 mmHg 负压 4 小时,F 组为单纯给氧组:两根引流管一跟接氧气瓶,一跟接氧浓度检测仪,每日给予氧浓度 40% \pm 5%局部氧疗 1 小时,G 组为空白对照组:用单层油纱和纱布覆盖。以上各组,均隔日更换敷料。

1.4 检测指标

1.4.1 创面愈合率及愈合时间 创面形成的当天及治疗后第 1、3、5、7、10、14、18 天观察各组创面大小、肉芽生成和表皮覆盖

情况。用 NikonDx-40 数码相机自动对焦曝光,镜头与创面垂直 20cm 拍照。利用图像分析软件测量照片上的创面面积,按以下公式计算创面愈合率:创面愈合率=(1-现有创面面积/原始创面面积) \times 100%。当愈合率大于 90%时判定为创面愈合。

1.4.2 组织学检查 创面形成当天及治疗后第 1、3、5、7、10、14、18 天切取创面床和创缘 1mm \times 2mm 全层组织,生理盐水冲洗,置于 4%多聚甲醛液,4 $^{\circ}$ C 冰箱内固定 1 周,石蜡包埋做 4 μ m 切片。HE 染色,光镜观察各时间点创面床和创缘组织表皮增殖、移行,肉芽生成,组织水肿和炎性细胞浸润情况

1.4.3 增殖指数检测 采用免疫组织化学的方法检测进入细胞增殖周期的细胞,一抗为鼠抗 Ki67,二抗为 HRP 标记的羊抗鼠抗体,DAB 显色,细胞核呈棕黄色者为处于增殖期的细胞;参照 Marcus 等^[5]的方法计算增殖指数(Proliferative Index),显微镜 400 倍视野下,对同一标本的 8 张连续切片的同一位置的阳性细胞进行计数,计算平均数,增殖指数即 8 个 400 倍视野的平均增殖细胞数。

1.4.4 细胞凋亡检测 采用 TUNEL 法,按试剂盒说明书对石蜡切片染色,细胞核呈棕色者为凋亡细胞。参照 Marcus 等^[5]的方法计算凋亡指数(Apoptotic Index):凋亡细胞计数方法同增殖细胞计数方法,凋亡指数即 8 个 400 倍视野的平均凋亡细胞数。

1.5 数据统计分析

采用 SPSS12.0 统计软件包处理,实验数据以均数 \pm 标准差表示,采用重复测量资料的方差分析比较各组之间差异。

2 结果

2.1 创面愈合率

(图表 1-3):实验组(A-C)愈合率高于对照组(D-G),且 A 组愈合最快,E 组愈合最慢($P<0.05$);D 组和 F 组愈合快于 G 组($P<0.05$)。实验第 18 天实验组创面基本上皮化,达愈合标准,对照组创面大部分已经上皮化,仍未愈合。

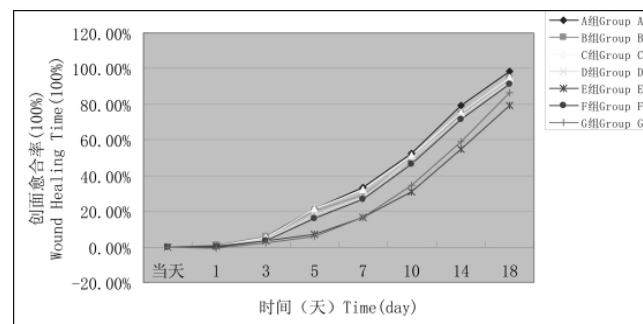


图 1 创面愈合率 愈合率随时间而增加,各组曲线上升趋势一致,3-7 天 E 组和 G 组曲线上升慢于其他组。7 天后各组曲线上升速率基本相同

Fig.1 Wound healing rate The wound healing rates increased with the time, the tendency of all the curves were same. The curves of group E and G began to rise more slowly than that of other groups in the third day.

There was no difference after the seventh day

2.2 组织学检查

对比各时间点的 HE 染色切片,1-3 天各组均以炎症反应为主,实验组切片组织水肿状况轻于对照组,第 5 天实验组炎症细胞浸润开始减轻,对照组 7-10 天才可观察到相同情况。10 天后各组无明显差异。

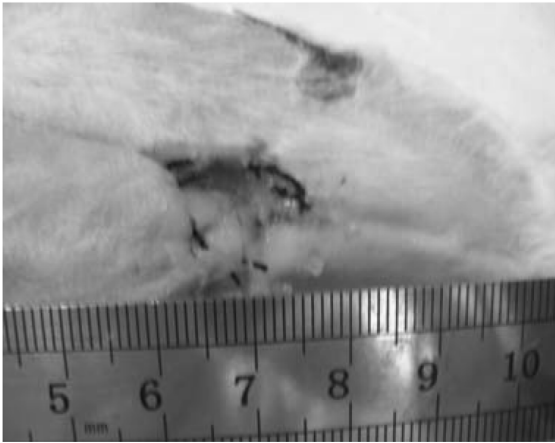


图2 创面照片(A组第18天)

Fig.2 Photo of wound (the eighteenth day in group A)

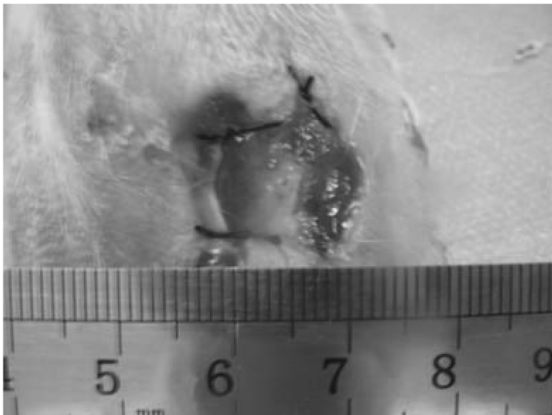


图3 创面照片(G组第18天)

Fig.3 Photo of wound (the eighteenth day in group G)

2.3 增殖指数

(图表 4-6) :各组增殖指数曲线均呈现先增高后降低的趋势,峰值出现在5天左右,实验组曲线上升快、峰值高、下降慢;实验组增殖指数高于对照组,A组最高,E组最低($P<0.05$);D组和F组增殖指数高于G组($P<0.05$)。以鼠抗 Ki67 为一抗进行免疫组化染色,细胞核被染成棕黄色的为进入增殖期的细胞。相同时相点上实验组细胞增殖指数高于对照组。

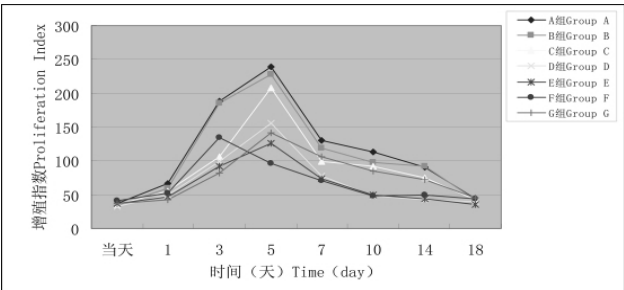


图4 增殖指数 各组增殖指数曲线变化趋势大致相同,5天前逐渐增高,5天后随之下降,峰值出现在5天左右。实验组增殖指数曲线上快、峰值高、下降慢,在相同时间点高于对照组。

Fig.4 Proliferation index All the curves had the same tendency, the curves rose gradually before the 5th day and reached peak at the fifth day, then decreased gradually. The curves in experimental groups rose faster, decreased slower and had the higher peak. Meanwhile, the proliferation index in experimental groups were higher than that in control groups at the same time points.

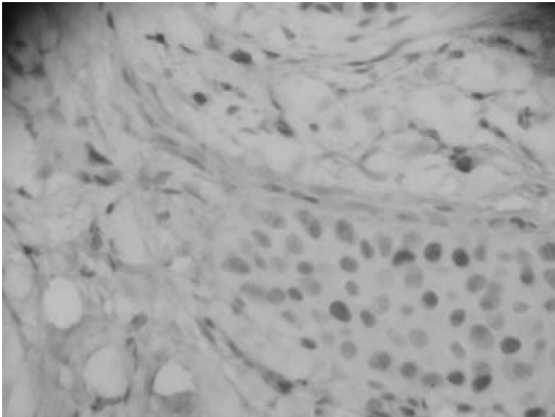


图5 增殖细胞(A组第5天)×400

Fig.5 Proliferating cells (the fifth day in group A)×400

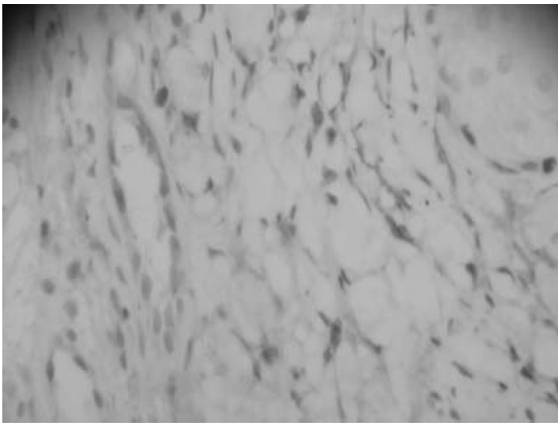


图6 增殖细胞(G组第5天)×400

Fig.6 Proliferating cells (the fifth day in group G) ×400

2.4 凋亡指数

(图表 7-9) :各组凋亡指数曲线在3天和10天出现两次高峰;实验组凋亡指数低于对照组,A组、B组最低,G组最高($P<0.05$);D组和F组凋亡指数高于G组($P<0.05$)。TUNEL 试剂盒检测细胞凋亡,细胞核被染成棕黄色的为凋亡细胞。相同时相点实验组的凋亡细胞少于对照组。

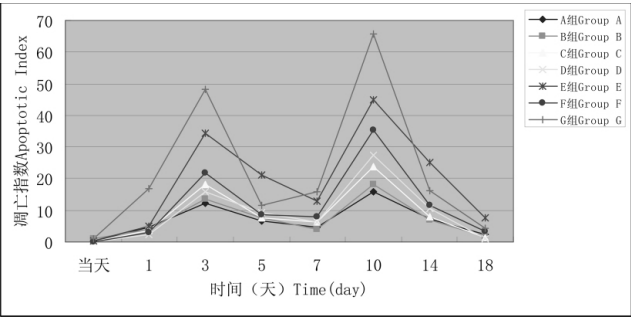


图7 凋亡指数 凋亡指数随时间变化而起伏,出现两个峰值分别在3天和10天左右,且第二峰值高于第一峰值,各组趋势大致相同。E组和G组曲线上快、峰值高、下降慢,E组和G组增殖指数高于其他组($P<0.05$)

Fig. 7 Apoptotic index The cell apoptotic curve had two peaks; one was nearly on the third day and the other was nearly on the tenth day and all curves had the same tendency. The curve in group E and G rose faster, decreased slower and had the higher peak. The apoptotic index in group E and G were larger than that in other groups at the same time points

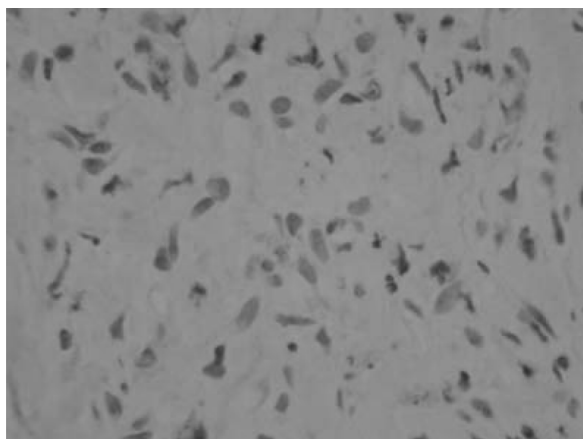


图 8 凋亡细胞(A 组第 10 天)×400

Fig. 8 Apoptotic cells (the tenth day in group A) ×400

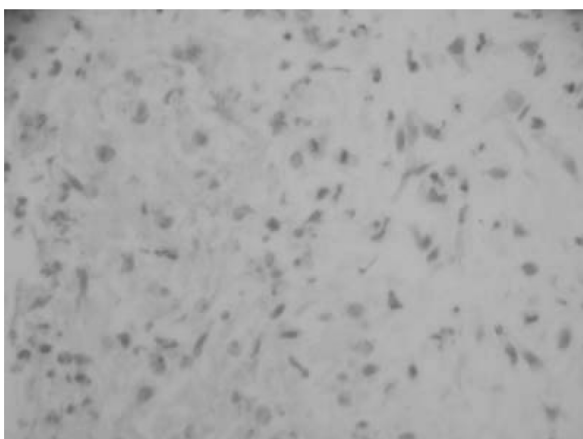


图 9 凋亡细胞 G 组第 10 天×400

Fig. 9 Apoptotic cells (the tenth day in group G) ×400

3 讨论

创面愈合率是评定创面愈合的最直接的指标。实验组(A-C 组)愈合率高于对照组(D-G 组)是两种技术联合促进创面愈合最有力的证据。病理检查结果显示,3 天前创面以炎症反应为主,但实验组水肿情况较对照组轻,5 天时实验组炎症反应较对照组明显减轻,至第 10 天各组间无明显差异,说明两种治疗方法联合的促进愈合的作用主要体现在愈合过程的早期。血管内皮细胞增殖是血管新生的基础,成纤维细胞的增殖是基质形成的基础,两者构成了肉芽组织形成的条件,加上上皮细胞爬行覆盖,导致创面愈合。本实验应用的一抗为 Ki-67,对于所有进入增殖期的细胞都予以标记,因而是修复细胞活动的综合表现。第 5 天左右在增殖曲线的高峰期,体现了早期炎症反应消退,而增殖期的到来,是细胞增殖为主的表现。凋亡的曲线表现为双峰的形式,第一峰出现在 3 天左右,第二高峰在 10 天左右。这与增殖指数检测结果是一致的,前者代表炎症反应期^[6],后者代表细胞凋亡为主期,这是各种修复细胞在创面愈合过程中的正常规律。细胞凋亡的机制并不十分明确,正常组织中也有凋亡发生,但很少^[7]。创面中凋亡过度增多必然不利于愈合^[8],这与本实验结果一致。

负压治疗能够减轻组织水肿^[9,10],减少创面感染^[11-13],减轻炎症反应^[14],充足的氧供是创面愈合所必需的^[15,16],负压封闭引流与局部氧疗结合能够促进创面愈合与其作用互补有关。研究

发现,给予负压治疗的创面中氧分压会降低,且随着负压值的增大而加重^[17]。很多学者也发现,缺氧在创面修复早期有着诱导血管生成的作用,但血管生成的过程和组织的修复过程都要消耗大量的氧,并依赖充足的氧供^[18,19],局部的氧疗可以弥补负压治疗对于局部造成的缺氧状况。局部给氧主要靠氧气的弥散发挥作用,影响氧弥散的因素主要是氧分压与弥散距离^[20],负压治疗能够清除的坏死组织和减轻水肿,这些都有利于氧气的弥散。两种疗法互相弥补了缺点又扩大了优点,可能是两种技术联合应用的理论基础所在。

本实验中值得注意的是 D 组(-50mmHg 负压)的愈合情况好于 E 组(-125mmHg 负压)。这表明负压值的过大会对创面造成损害,特别是缺血性的创面应当更加注重负压值的选择。

至于负压值与局部给氧浓度的最优的组合尚有待深入探索。氧气的输入可能需要略增大负压,氧浓度的适当控制也很必要。

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水平或其他未知因素相关,需进一步研究^[20]。在所检测样本中,经拉米夫定、阿德福韦酯联合用药组患者其 DNA 拷贝数、ALT 在正常数值范围内,有良好的治疗效果。

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