

doi: 10.13241/j.cnki.pmb.2015.05.004

大鼠二次脑损伤后脑组织 SOD 及 MDA 的变化研究*

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摘要 目的:观察大鼠二次脑损伤后脑组织 SOD 及 MDA 的变化,进一步探讨过氧化反应在二次脑损伤机制中的作用。**方法:**90 只雄性 SD 大鼠随机分为正常对照组(A 组)、弥漫性脑损伤组(B 组)和二次脑损伤组(C 组),建模成功后,分别于伤后 1 h、3 h、6 h、12 h、24 h、48 h 处死动物,取额叶组织匀浆,分别测定大鼠脑内 SOD 及 MDA 的含量。**结果:**伤后 1 h 开始,B、C 组中 SOD 含量呈现先上升后下降的趋势,并随时间呈持续下降趋势直至伤后 24 h,C 组较 B 组下降程度更加明显($P<0.05$);伤后 3 h,B、C 组中 SOD 含量均增高,B 组增高程度和速率均高于 C 组($P<0.05$)。伤后 1 h 开始,B、C 组 MDA 含量呈升高趋势,并于 24 小时达峰值,C 组升高趋势较 B 组更显著,伤后 6 h、24 h、48 h,C 组与 B 组之间相比统计学差异显著($P<0.05$)。**结论:**创伤性脑损伤及二次脑损伤后,脑组织内过氧化反应明显加重,SOD 及 MDA 均出现明显变化,二次脑损伤的过氧化反应较脑损伤组更加严重且持续时间更长,且 MDA 较 SOD 的变化具有滞后性。

关键词:弥漫性脑损伤;二次脑损伤;超氧化物歧化酶;丙二醛

中图分类号:Q95-3;R651.1 **文献标识码:**A **文章编号:**1673-6273(2015)05-812-03

Research on Changes of Brain SOD and MDA after Secondary Brain Insults in Rats*

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ABSTRACT Objective: Testing the changes of superoxide dismutase(SOD) and malondialdehyde (MDA) in rats with diffuse brain injury (DBI) or secondary brain insult (SBI), to analyze the effects and significance of peroxidation in development of rats SBI. **Methods:** 90 male SD rats were randomly divided into normal control group (group A), DBI group (group B) and SBI group (group C). After the success of the modeling, the rats were put to death, and the frontal tissue homogenate were taken after injury 1 h, 3 h, 6 h, 12 h, 24 h, and 48 h respectively, to determine the content of SOD and MDA. **Results:** From the first 1 h after injury, SOD content in B and C groups presents the downward trend after rising first, and the declining trend over time until 24 h after injury, the degree of decline in group C is more obvious compared with that in group B ($P<0.05$); 3 h after injury, SOD content in B and C groups were higher, the higher degree and the rate of B group were higher than in group C ($P<0.05$). From the first 1 h after injury, MDA content in groups B and showed a trend of increase, and in 24 hours to spike, group C increased more significantly than group B, at 6 h, 24 h, and 48 h after injury, the differences between Group B and Group C were statistically significant ($P<0.05$). **Conclusion:** After DBI or SBI in rats, peroxidation increase obviously, SOD and MDA change apparently, peroxidation in SBI is more serious than that in DBI and last longer, and the changes of MDA are later than those of SOD.

Key words: Diffuse brain injury; Secondary brain insult; Superoxide dismutase; Malondialdehyde

Chinese Library Classification(CLC): Q95-3; R651.1 **Document code:** A

Article ID:1673-6273(2015)05-812-03

前言

二次脑损伤(Secondary brain insult, SBI)是指在已有原发性脑损伤的基础上,合并缺氧、高热、低血压等因素时,进一步加重的脑损害。创伤性颅脑损伤及缺血性脑损伤后的过氧化反应已有较多研究,且已明确过氧化反应参与脑损伤进程^[1,2],但过氧化反应参与二次脑损伤的机制及时效变化,研究不多。本研究拟通过观察弥漫性脑损伤(Diffused brain injury, DBI)及二次脑损伤后脑组织内 SOD 和 MDA 的变化,探讨过氧化反应在二次脑损伤中的作用机制及时间效应关系。

1 材料与方法

1.1 动物

选取 SD 雄性大鼠(3 月龄)90 只,体重 $250\text{g} \pm 30\text{g}$,由南京军区福州总医院比较医学科提供。

1.2 方法

1.2.1 大鼠 DBI 及 SBI 模型制作 制作大鼠 DBI 及低血压性 SBI 动物模型,按文献^[3]进行。

1.2.2 实验动物分组及处理 按照完全随机实验设计分组,90 只 SD 大鼠称重编号后分为 3 组:1、对照组(A 组,30 只);2、

* 基金项目:南京军区医药卫生基金(12M015A);福建省自然科学基金项目(2014J01425)

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(收稿日期:2014-08-17 接受日期:2014-09-14)

DBI组(B组,30只);3、DBI合并SBI组(C组,30只)。B、C组按伤后时间不同分为1h、3h、6h、12h、24h、48h 6个亚组,每亚组5只。建模过程中若有大鼠死亡,予以补齐数量。

1.2.3 组织标本的获取与制备 大鼠伤后预定时间,以生理盐水100 mL及4%甲醛磷酸盐缓冲液250 mL依次从心脏进行灌注,然后快速断头取脑,取完整右额叶组织(80±20)g,称重后以预冷的NS冲洗,置于盛有预冷生理盐水的匀浆器中,在冰水浴中匀浆100 min,制成100 g/L的脑组织匀浆。匀浆液3500 r/min低温离心15 min,取适量上清分别进行蛋白定量、MDA和SOD测定。蛋白定量所需蛋白标准、双缩脲试剂、MDA和SOD测定试剂盒均由南京建成生物工程研究所提供,具体操作按说明书进行。

作按说明书进行。

1.2.4 统计学处理 测定结果以Excel 2003录入,以SAS9.1统计软件进行统计,计量资料采用两因素重复测量方差分析, $P<0.05$ 认为差异有显著性意义。

2 结果

2.1 不同时间点各组 SOD 含量的变化

伤后1h、6h、12h、24h、48h,B组SOD含量均较A组明显下降,统计学有显著差异($P<0.05$),而3h时反呈轻度上升趋势;伤后各时间点C组SOD含量均较A、B组显著下降,与两组相比,均有统计学差异($P<0.05$)。(见表1、图1)。

表1 各组大鼠脑组织 SOD 的变化 U/mgprot, ($\bar{x} \pm s$)
Table 1 SOD change(U/mgprot) of rat brain tissues in each group($\bar{x} \pm s$)

时间 Time	A组 Group A	B组 Group B	C组 Group C
1 h	138.67± 20.43	124.90± 21.47*	125.23± 22.25*
3 h	139.47± 20.45	145.57± 21.43	127.20± 17.68*△
6 h	139.27± 20.68	112.37± 18.12*	101.20± 15.26*△
12 h	139.17± 20.92	91.77± 18.92*	80.37± 18.76*△
24 h	138.17± 20.78	83.67± 19.27*	72.30± 17.25*△
48 h	138.07± 20.77	86.50± 19.43*	75.20± 20.16*△

注:与A组对比 $P<0.05$,△与B组对比 $P<0.05$ 。

Note: Compared with group A, $P<0.05$; △: Compared with group B, $P<0.05$ 。

2.2 各组 MDA 含量的变化

伤后各时间点,B组MDA含量均较A组明显增高,统计学有显著差异($P<0.05$);伤后各时间点C组MDA含量均较

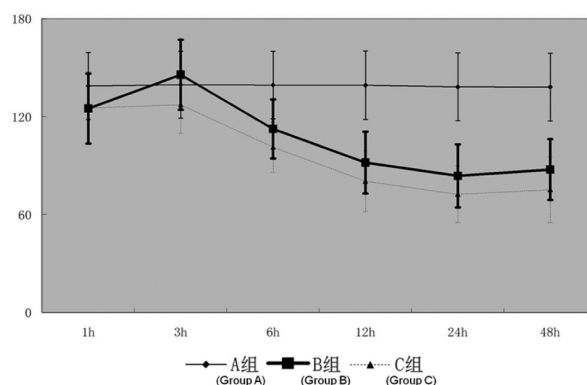


图1 各组不同时间点 SOD 变化曲线图

Fig. 1 Curve graph of SOD at different time points in each group

A组显著升高,伤后6h、12h、48hC组MDA含量较B组显著升高,统计学有显著差异($P<0.05$)。但在伤后1h、3h、12h时,C组与B组之间相比无统计学差异。(见表2、图2)。

表2 各组大鼠脑组织 MDA 含量变化 nmol/mg($\bar{x} \pm s$)
Table 2 MDA change(nmol/mg) of rat brain tissues in each group ($\bar{x} \pm s$)

Time	Group A	Group B	Group C
1 h	2.61± 0.56	2.98± 0.47*	3.03± 0.68*
3 h	2.63± 0.55	3.16± 0.34*	3.30± 0.31*
6 h	2.63± 0.54	3.47± 0.49*	3.73± 0.44*△
12 h	2.62± 0.55	3.81± 0.46*	4.16± 0.58*△
24 h	2.66± 0.54	4.29± 0.57*	4.55± 0.53*
48 h	2.65± 0.52	4.35± 0.65*	4.72± 0.55*△

注:与A组对比 $P<0.05$,△与B组对比 $P<0.05$ 。

Note: Compared with group A, $P<0.05$; △: Compared with group B, $P<0.05$ 。

2.3 各组 SOD 与 MDA 重复测量方差分析

对B、C组组内不同时间点SOD、MDA含量进行重复测量方差分析,显示各组别与实践之间均有交叉效应,认为SOD与MDA值在不同组别、时间点不全相同,且变化率也不全相同。其差异具有统计学差异(<0.001)。(见表3)。

3 讨论

二次脑损伤的实质是原发性脑损伤后,由于血压、体温、颅

内压、脑灌注压、脑血流量等的异常而引起的二次脑损害,各种二次脑损伤因素可发生于脑损伤后的各个阶段^[46]。颅脑损伤后,对二次脑损伤的基础研究多集中在兴奋性氨基酸毒性、钙超载、炎症损伤和细胞凋亡机制等方面^[7-9]。近年研究表明,过氧化反应可在多个方面影响脑损伤后的细胞功能,可通过加重细胞膜损伤、影响ATP酶活性、抑制钙流出、参与介导炎症反应等,进一步加重细胞损害,成为二次脑损伤发生机制中的最重要环节之一^[10-15]。

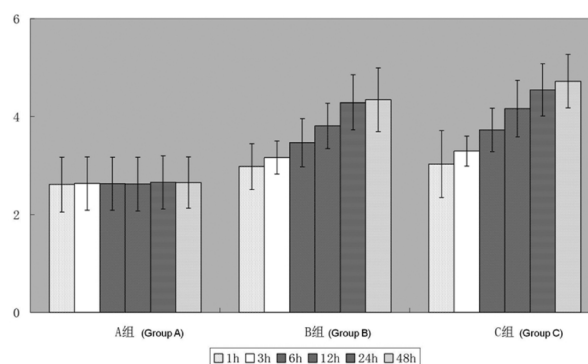


图2 各组不同时间点 MDA 含量柱状图

Fig. 2 Column graph of MDA at different time points in each group

表3 各组 SOD 与 MDA 重复测量方差分析

Table 3 Square variance ananlysis for SOD and MDA of repeating measuring

Object	Project	F value	P
SOD	Group	42.23	<0.001
	Time	392.59	<0.001
	group*time	98.14	<0.001
MDA	Group	67.54	<0.001
	Time	186.47	<0.001
	group*time	44.84	<0.001

本实验中,伤后 1 h、6 h、12 h、24 h、48 h, B 组 SOD 含量均较 A 组明显下降,统计学有显著差异($P < 0.05$),而 3 h 时反呈轻度上升趋势;伤后各时间点 C 组 SOD 含量均较 A、B 组显著下降,与两组相比,均有统计学差异($P < 0.05$)。提示:脑损伤及二次脑损伤后 1h,即出现 SOD 的大量消耗,且大量消耗直至伤后 24h 达最低点。伤后 3h 时,B、C 组 SOD 水平不降反升,随后出现单边下降,提示机体在出现过氧化反应早期,可快速大量释放 SOD 随着过氧化反应的持续,所释放的 SOD 仍不足以维持,因此呈现单边下降。同时,我们发现,在伤后 3h 时,B 组上升速率较 C 组更为明显,也提示二次脑损伤后,早期 SOD 的消耗量较单纯脑损伤更为严重,以致伤后早期 SOD 的反升并不明显。

MDA 作为脂质过氧化反应的产物,其含量越高提示过氧化反应越严重,细胞损伤越严重。Hsiang 等^[16]研究了大鼠弥漫性脑损伤后不同区域脑组织 MDA 水平的变化,发现弥漫性脑损伤后额叶、顶叶、脑干 MDA 水平明显增加,且于伤后 4 h 达高峰,以后逐渐下降。本研究中,在脑损伤及二次脑损伤后各时间点,B、C 组 MDA 均较对照组明显升高($P < 0.05$),提示过氧化反应的持续存在。C 组较 B 组 MDA 水平在伤后 6 h、24 h、48 h 显著升高($P < 0.05$),但在伤后 1 h、3 h、12 h,C 组与 B 组之间相比无统计学差异。一方面提示伤后早期二次脑损伤较单纯脑损伤的过氧化程度更严重,另一方面,与 SOD 结果对比,也反映出了 MDA 水平较 SOD 水平变化的滞后性,特别是在伤后 24~48 h 时,SOD 水平已趋于稳定,而 MDA 水平仍呈下降趋势。

我们在前期的研究中发现^[17,18]:(1)大鼠 DBI 合并 SBI 后,脑内 c-fos 基因表达在伤后 3 h、24 h 分别升高,呈双峰特征;(2)血浆 β -EP 在 SBI 后早期含量较高;(3)SBI 后 24 h 脑水肿即达高峰期。这些结果与本实验结果在时间节点上有一致性。因此我们认为,二次脑损伤后 3 h 是机体各项负面反应的时间节点,24 小时是二次脑损伤相对于单纯脑损伤的第二次恶化时间点。由此提出,针对二次脑损伤的治疗最佳时间窗应在伤后 3 小时内,第二治疗时间窗在伤后 24 小时内。

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能是抑制交感神经活性的结果。研究显示交感神经可调控白色脂肪组织中一些脂肪因子的释放^[1]。另有文献报道,循环 ghrelin 可通透血脑屏障^[21], 辣椒素阻断迷走神经可消除外周注射 nesfatin-1 的厌食作用^[22], 因为外周 nesfatin-1 可激活大脑交感神经兴奋性^[20], 外周 nesfatin-1/NUCB2 表达增强可能恢复脑 VMH 损毁所致的交感神经活性降低。

本实验结果表明, VMH 损毁大鼠皮下和内脏脂肪组织中 nesfatin-1/NUCB2 表达增加, 可能通过抑制交感神经活动介导。

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