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二乙基二硫代氨基甲酸钠对沙土鼠脑缺血再灌注损伤的影响

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Effects of sodium diethyldithiocarbamate on ischemia-reperfusion-induced brain injury in Mongolian gerbil

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ABSTRACT Brain injury in Mongolian gerbil (*Merisones unguiculatus*) was induced by occluding bilateral common carotid arteries for 60 min followed by reperfusion for 5 or 30 min. Oxygen free radicals in brain tissue were measured by electron spin resonance (ESR) technique, malondialdehyde (MDA) was measured by fluorescence spectrometry, and superoxide dismutase (SOD) was measured by nitrite kit. Oxygen free radicals and MDA were not significantly increased, but activities of T-SOD and Mn-SOD were decreased after 60 min of cerebral ischemia. The free radicals were increased at 5-min reperfusion, and then reduced to the level of ischemia group after 30-min reperfusion. MDA was increased remarkably after reperfusion of 30 min, whereas the activity of SOD continued to decrease. Sodium diethyldithiocarbamate (DTC), iv 5—100 mg·kg^{-1} 15 min before occlusion, decreased the production of MDA and increased the activities of T-SOD and Mn-SOD. The formation of oxygen free radicals was depressed by iv DTC 50 mg·kg^{-1}. The result suggested that the protective effects of DTC on ischemia-reperfusion-induced brain injury might be induced by scavenging the oxygen free radicals, increasing the Mn-SOD activity and decreasing the production of MDA.

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摘要 沙土鼠脑缺血60 min 后，脑组织氧自由基和 MDA 含量无明显升高，而 Mn-SOD 的活性下降. 缺血60 min 再灌注5 min 时，氧自由基显著升高. 再灌注30 min 时，MDA 的生成显著增多，Mn-SOD 和 Cu、Zn-SOD 活性显著降低. 缺血前15 min，iv DTC 对缺血再灌注脑组织中氧自由基和 MDA 升高有显著抑制作用、对 Mn-SOD 活性有显著保护作用、且呈剂量依赖关系.

关键词 二乙基二硫代氨基甲酸；自由基清除剂；超氧化物歧化酶；丙二醛；电子自旋共振；波谱学

脑缺血再灌注损伤与氧自由基增多有关[1-5]. 活性氧自由基增加，可引发脂质过氧化，导致脑组织脂质膜的损伤[6]. 氧自由基清除剂对脑缺血再灌注损伤有一定保护作用[7]. 免疫增强剂二乙基二硫代氨基甲酸钠(DTC)在体外具有清除自由基的作用[8]，但未见其对缺血再灌注损伤保护作用的报道. 本研究的目的是用沙土鼠脑缺血再灌注模型，观察 DTC 在体内对氧自由基、超氧化物歧化酶(SOD)和丙二醛(MDA)的影响，以探讨 DTC 对脑缺血再灌注损伤的保护作用.

MATERIALS AND METHODS

DTC 本院中西药研究室合成(mp 94—96 ℃)，临用前溶解稀释；SOD 试剂盒海军总医院出品；四甲氧基丙烷(1,1,3,3-tetramethoxypropane, TMP)标准品及硫代巴比妥酸(thiobarbituric acid, TBA)美国 Sigma 公司产品；其余试剂全部国产 AR 级.

脑缺血再灌注模型 蒙古沙土鼠106只，♀♂不拘，体重53±*s* 6 g. 乙醚轻度麻醉，再 ip 10 %乌拉坦 1.25 g·kg^{-1}麻醉. 分离双侧颈总动脉，同时气管插管. 用无损伤动脉夹夹闭两侧总动脉，造成脑缺血60 min. 打开动脉夹再灌注5 min 和30 min. 鼠分为假手术组，缺血组，缺血再灌注组和给药组，给药组在缺血前15 min iv 给药.

自由基的测定 运用冷冻成形法[9]. 取脑组织 300 mg 迅速装入内径3 mm，长30 mm 的塑料吸管中，并立即置液 N_2中保存待测. 测定时、取样品管立即放入装有液 N_2的石英 dewar 里，用 Bruker ER 200D-SRC 型电子自旋共振仪记录样品77K 温度下的 ESR 谱. 微波功率为10 mW；微波频率为9.30 Hz；磁场扫描50G；磁场调制5G；时间放大8×10^5；时间常数 200 ms.

SOD 活性及 MDA 的测定 SOD 活性用亚硝酸法[10]测定；MDA 用 TBA 法[11]测定.

RESULTS

脑缺血60 min 后，缺血脑组织中的氧自由基含量比正常组织有上升趋势、但无显著差异. 再灌注5 min 后，氧自由基含量显著增加($P<0.01$)，近正常组织的二倍. 再灌注30 min 时，氧自由基含量又降至接近缺血时的水平. 给予 DTC 50 mg·kg^{-1}组中，再灌注5 min 时氧自由基含量与对照再灌注组比较，显著降低($P<0.01$)、而 DTC 25 mg·kg^{-1}对氧自由基含量无明显影响(Tab 1).

Tab 1. Effects of DTC on oxygen free radicals in Mongolian gerbil brain of ischemia reperfusion. $\bar{x}\pm s$. $^cP<0.01$ *vs* **ischemia group.** $^fP<0.01$ *vs* **reperfusion group.**

Group	*n*	ESR signal intensity/ cm·g^{-1}
Control	7	7.2±1.4
Ischemia	8	8.4±1.3
Reperfusion		
5 min	7	12.9±1.9^c
30 min	7	8.4±1.8
DTC/mg·kg^{-1}		
25 (5 min)	7	11.4±2.4
50 (5 min)	6	5.6±0.8^f

缺血脑组织中的 MDA 在缺血60 min 时没有显著增加，再灌注30 min 后有明显的增加（$P<0.01$）。不同剂量的 DTC 都可使再灌注后的 MDA 含量明显低于对照组（$P<0.05$—0.01），并呈剂量依赖关系（Tab 2）。

Tab 2. Effects of DTC on malondialdehyde in Mongolian gerbil brain of ischemia reperfusion. $\bar{x}\pm s$. $^cP<0.01$ *vs* **ischemia group.** $^fP<0.01$, $^eP<0.05$ *vs* **reperfusion group.**

Group	n	MDA/nmol·g^{-1}
Control	9	239±58
Ischemia	8	252±52
Reperfusion 30 min	9	472±56^c
DTC/mg·kg^{-1}		
5	6	408±79^e
10	9	370±38^f
25	7	266±66^f
50	8	211±74^f
100	8	236±77^f

缺血60 min 后，脑组织中的 T-SOD，Mn-SOD 活性明显下降（$P<0.01$），但 Cu，Zn-SOD 的活性无明显改变。再灌注30 min 后，T-SOD，Mn-SOD 的活性进一步下降，Cu，Zn-SOD 活性也较缺血时有显著下降（$P<0.01$）。给予不同剂量的 DTC 可对 T-SOD，Mn-SOD 活性有一定的保护作用（$P<0.05$—0.01），并呈剂量依赖关系，但对 Cu，Zn-SOD 无明显的影响（Tab 3）。

DISCUSSION

本研究证明，再灌注过程中脑组织氧自由基产生增加的同时，内源性自由基清除剂 Mn-SOD 和 Cu Zn-SOD 显著降低，而脂质过氧化产物 MDA 则显著增加。这些结果支持了脑缺血再灌注损伤的氧自由基学说。

有关脑缺血再灌注损伤的药理研究[12,13]多通过测定 SOD 和 MDA 等指标来间接证明氧自由基的变化，本研究则采用 ESR 技术直接测定沙土鼠脑缺血再灌注过程中脑组织的氧自由基变化。结果发现：再灌注5 min 时，氧自由基含量明显升高；而再灌注30 min 以后，又显著下降。说明大量自由基的产生主要发生在缺血再灌注的早期。

本研究还发现，DTC 在体内能清除脑缺血再灌注所产生的氧自由基，保护内源性 Mn-SOD 的活性，减少脂质过氧化产物 MDA 的产生，提示对脑缺血再灌注损伤可能有一定的保护作用。

Tab 3. Effects of DTC on SOD activity in Mongolian gerbil brain of ischemia reperfusion. $\bar{x}\pm s$. $^cP<0.01$ *vs* **control group.** $^fP<0.01$ *vs* **ischemia group.** $^iP<0.01$, $^hP<0.05$ *vs* **reperfusion group.**

Group	n	T-SOD/NU·g^{-1}	Mn-SOD/NU·g^{-1}	Cu, Zn-SOD/NU·g^{-1}
Control	8	6 376±152	5 573±208	804±66
Ischemia	8	5 962±183^c	5 162±225^c	800±67
Reperfusion 30 min	8	5 090±134^i	4 472±230^f	618±129^f
DTC/mg·kg^{-1}				
5	8	5 238±348	4 578±329	659±109
10	8	5 371±107^i	4 720±80^i	651±87
25	8	5 575±107^i	4 898±158^i	677±112
50	7	5 679±411^i	4 992±551^h	686±155
100	7	5 679±454^i	5 060±490^i	618±122

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