论著

高血糖对大鼠局灶性脑缺血再灌注时小胶质细胞活化的影响

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【摘要】目的 观察高血糖对大鼠局灶性脑缺血再灌注时小胶质细胞活化的影响。方法 健康雄性SD大鼠80只。随机分为正常血糖假手术组(NG sham组, n=5)、高血糖假手术组(HG sham组, n=5)、正常血糖脑缺血再灌注组(NG手术组, n=35)和高血糖脑缺血再灌注组(HG手术组, n=35)。采用链脲佐菌素(STZ)腹腔注射法制备SD大鼠I型糖尿病模型, 大脑中动脉阻塞法(MCAO)建立大鼠脑缺血再灌注模型, 应用小胶质细胞特异性标志蛋白Iba-1免疫组化标记小胶质细胞, 观察NG组和HG组大脑中动脉阻塞30min。再灌注0.5、3、6h以及1、3、7、14d大鼠(每时间点5只)室周带和尾状核区小胶质细胞的变化, 采用Iba-1和内核增殖抗原(PCNA)免疫荧光双标法检测小胶质细胞的增殖变化。结果 脑缺血再灌注后, 可见大鼠脑组织明显水肿, 呈网格状, HE染色变淡, 神经元肿胀, 胞质空泡状, 胞核固缩, 并可见炎细胞浸润。HE染色结果显示HG手术组与NG手术组比较脑损伤更为明显。脑缺血再灌注后第3天, 免疫组化染色可见梗死周边区、裂纹状皮质和胶体样变性皮质的小胶质细胞明显活化, 于第7天达到峰值, 且其活化状态可持续到再灌注第14天。免疫荧光双标染色结果显示脑缺血再灌注后小胶质细胞数量增加与其增殖有关, 其增殖程度同样是在缺血再灌注后第3天增加, 第7天时达高峰。与NG手术组比较, HG手术组小胶质细胞的活化及增殖较弱(P < 0.05), 但均明显高于各自的假手术组(P < 0.05)。结论 高血糖导致的缺血后脑组织小胶质细胞活化、增殖抑制可能参与了高血糖加重缺血性脑损伤的过程。

【关键词】小胶质细胞; 高血糖; 脑; 再灌注损伤

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Effects of hyperglycemia on the activation of microglia in focal cerebral ischemia and reperfusion

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【Abstract】Objective To observe the changes of microglia in diabetic cerebral ischemia and reperfusion, and further explore the role of microglia in diabetic rats with cerebral ischemic injury. Methods Eighty healthy male SD rats were randomly divided into 4 groups: normal blood glucose sham operation group (NG sham group, n=5), diabetic hyperglycemia sham operation group (HG sham group, n=5), normal blood glucose with cerebral ischemia-reperfusion group (NG MCAO group, n=35) and diabetic hyperglycemia with cerebral ischemia-reperfusion group [HG middle cerebral arterial occlusion (MCAO) group, n=35]. The diabetic rats models were established by intraperitoneal injection of streptozotocin (STZ). The cerebral ischemia reperfusion models were made with MCAO, the specific marker protein Iba-1 was used to immunohistochemically label the microglia. The changes of microglia in the periventricular zone and caudate putamen region of the rats in HG MCAO group and NG MCAO group were observed at ischemia 30min and reperfusion 30min, 3h, 6h, 1d, 3d, 7d and 14d (each time point contains 5 rats). Iba-1 and proliferating cell nuclear antigen (PCNA) immunofluorescence double labeling method were performed to detect the proliferation of microglia. Results After ischemia-reperfusion, the brain tissue appeared as obvious edema, mesh-like, HE staining faded, neurons swollen, cytoplasm vacuolization, nuclear pyknosis, and inflammatory cell infiltration. All these symptoms of brain injury were more obvious in HG group than in NG group. On the 3rd day after ischemia reperfusion, microglial cells were markedly activated in the
infarct peripheral zone, piriform cortex and somatic sensory cortex, the activation reached the peak value at the 7th day, and the activated state continued to the 14th day of reperfusion. It was found with Iba-1 and PCNA immunofluorescence double labeling that, after cerebral ischemia-reperfusion, the increase of microglia number was related to its proliferation. The microglia proliferation also increased at the 3rd day after ischemia-reperfusion, and reached the peak value at the 7th day. The degree of microglia activation and proliferation was weaker in NG group than in HG group (P<0.05), but higher obviously when compared with their each sham group (P<0.05). Conclusion Hyperglycemia induced ischemia brain tissue microglia activation and proliferation inhibition may be involved in the hyperglycemia induced ischemic brain damage.

[Key words] microglia; hyperglycemia; brain; reperfusion injury

1.2.2 局灶性脑缺血再灌注模型制备 采用大脑中动脉阻塞法(middle cerebral artery occlusion, MCAO)法制备大鼠右侧大脑中动脉局灶性脑缺血模型。NG手术组和HG手术组大鼠用3%戊巴比妥钠(30mg/kg)腹腔注射麻醉，常规备皮、消毒，沿颈正中切口逐层钝性分离出两侧颈动脉、颈外动脉及迷走神经，翻外动脉远端端双钳夹闭，牵拉外动 脉使与颈外动脉成直线，自颈外动脉切口插入线栓，当感到有阻力时停止插入，固定线栓。缺血30min后拔出线栓，缝合皮肤。术中术后通过灯泡照射给予保温。

在脑缺血30min，再灌注0.5、3、6及1、3、7、14d时间点分别麻醉处死大鼠，4%甲醛溶液灌注后取脑，于距前脑约0.3mm水平冠状切面自脑组织，于甲醛溶液中固定48h，石蜡包埋，之后5μm连续切片。

1.2.3 HE染色 石蜡包埋组织切片，常规脱蜡至水，苏木素染色10min，流水冲洗至水清无紫色，75%酒精乙醇分化3次(30s)后，流水冲洗，镜下观察细胞核变蓝，伊红染色5min，切片透明，中性树胶封片。

1.2.4 Iba-1免疫组织化学染色观察小胶质细胞活化情况 石蜡包埋组织切片，常规脱蜡至水；间歇高压热修复法进行抗原修复。0.3%过氧化氢孵育15min，10%山羊血清37℃孵育30min，加入1：500稀释(1% BSA)的一抗Iba-1后4℃过夜，温浴30min，PBS冲洗，滴加辣根过氧化酶标记的山羊抗小鼠IgG二抗、PBS冲洗，DAB显色，苏木素染色。以PBS代替一抗作为阴性对照。

1.2.5 Iba-1与PCNA免疫荧光双标染色观察小胶质细胞活化与增殖情况 切片脱蜡，间歇高压热修复，0.3%过氧化氢孵育15min，10%山羊血清37℃孵育30min，滴加Iba-1(1:500，1% BSA)和PCNA(1:200，1% BSA)一抗混合液，4℃过夜。PBS冲洗后，滴加荧光标记(Alexa Fluor 488)的抗小鼠二抗(1:300，Invitrogen公司)和荧光标记(Alexa Fluor 561)的抗兔二抗(1:300，Invitrogen公司)混合液，37℃孵育1h。DAPI复染、封片，激光共聚焦显微镜下观察。
1.2.6 结果判定 Iba-1免疫组织化染色及Iba-1与PCNA免疫荧光双标染色后，高倍镜下观察并分别计数缺血侧大脑皮质Iba-1及荧光标记(Iba-1、
PCNA)阳性表达的细胞数，每张切片取脑缺血侧5个不同视野采集图像，分别计数每个视野下的阳性
细胞个数，计算5个不同视野阳性细胞的平均数作为该标本的阳性小胶质细胞数。
1.3 统计学处理 采用SPSS 22.0软件进行统计分析。计量资料以±s表示，组间比较采用单因素
方差分析，进一步两两比较采用Student’s-t检验。P<0.05为差异有统计学意义。

2 结 果
2.1 脑缺血再灌注1d内脑缺血区形态学改变 HE
染色光镜下观察显示，NG sham组大鼠脑组织结构
清楚，细胞形态正常，排列整齐，神经元、胶质细
胞形态正常。NG手术组缺血再灌注6h脑组织明显
水肿，主要位于室周带和尾状壳区，血管管壁和神
经细胞周围出现明显裂隙，部分神经元出现核固缩、
胞质染色，细胞呈三角形，毛细血管扩张。组织水
肿程度和神经元核固缩以缺血侧更为明显。NG手
术组脑水肿和神经元损伤在再灌注1d时最重，可见
脑组织结构破坏，细胞排列紊乱，部分神经细胞消
失，少量胶质细胞、炎细胞浸润。HG sham组大鼠
可见脑组织水肿，HG手术组缺血再灌注后6h，脑
缺血导致的水肿较同期的NG手术组加重，神经
元损伤程度也明显加重，可见神经元肿胀明显，胞
质空泡状，细胞核固缩；缺血再灌注1d时HG手术
组脑组织水肿呈网格状，染色淡，大量神经细胞消
失，残余神经细胞固缩或呈空泡状，可见少量炎细
胞、胶质细胞浸润(图1)。

2.2 缺血再灌注后脑梗死区小胶质细胞形态学改
变 在NG sham组，脑实质内可见少量静止期小胶
质细胞，胞体较小，分支细。NG手术组缺血再灌
注后3h，缺血侧室周带和尾状壳区活化小胶质细胞
(Iba-1免疫标记染色阳性)数量增加，表现为突起减
少、变短；再灌注后6h，小胶质细胞胞体变大，呈
圆形，突起变少，甚至消失。HG手术组脑缺血
区小胶质细胞形态改变与NG手术组类似，但细
胞活化数量较NG手术组略低(图2)。

2.3 脑缺血再灌注后不同时间点小胶质细胞活化
数量改变 在NG sham组，偶见小胶质细胞散在均
匀分布。NG手术组脑缺血再灌注后6h活化的小胶
质细胞(Iba-1免疫标记染色阳性)明显增加，随时间
延长，活化的小胶质细胞数量增多，缺血再灌注
后3d，缺血区及其周边区包括梨状皮质和脑皮胶
质细胞明显增多并达峰值。浸没的小胶质细胞数量在脑缺血再灌注7d达峰值，明显高
于NG Sham组(P<0.05)。脑缺血再灌注后14d，NG
手术组浸润的小胶质细胞数量减少，但仍高于NG
Sham组(P<0.05)。在HG Sham组，可见少量散在的
Iba-1阳性小胶质细胞浸润；缺血再灌注后7d，HG
手术组梗死灶周边区、梨状皮质及脑皮胶质活
性化小胶质细胞数量增加达峰值，但明显低于NG
手术组(P<0.05)。脑缺血再灌注后14d，HG手术组活
性化的小胶质细胞数量有所减少，但高于NG手术
组及HG Sham组(P<0.05, 图3)。

2.4 脑缺血再灌注后不同时间点小胶质细胞增殖
程度改变 NG sham组未发现PCNA阳性的小胶质
细胞。NG手术组脑缺血再灌注后3d可见梗死灶周
围区，梨状皮质及脑皮胶质细胞小胶质细胞中存在
PCNA表达，7d时坏死灶周围PCNA阳性小胶质细胞
明显增多并达峰值，14d时PCNA阳性小胶质细胞
明显减少，但仍高于NG sham组。HG sham组大鼠
**Fig. 2** Morphological changes of microglia in cerebral ischemia region after cerebral ischemia-reperfusion (Iba-1 immunohistochemical staining × 400)

Bar=50 μm. Arrow refers to microglia.

**Fig. 3** Number of activated microglia at different time points after cerebral ischemia-reperfusion (Iba-1 immunohistochemical staining × 200)

Bar=100 μm. (1) P<0.05 compared with 0 hour (sham group); (2) P<0.05 compared with NG group.

### 脑缺血再灌注后脑缺血区小胶质细胞形态学改变

Iba-1免疫组化染色 (×400)

中可见少量散在的增殖小胶质细胞，即PCNA阳性的和小胶质细胞）浸润。缺血再灌注后3d和7d时，梗死灶周围区PCNA阳性小胶质细胞数量增加，但均明显低于同时期的NG手术组(P<0.05)，缺血再灌注14d时，PCNA阳性小胶质细胞数量有所减少，但明显高于同时期的NG手术组(P<0.05，图4)。

### 讨 论

本研究结果显示，HG手术组脑水肿和神经元萎缩明显重于NG手术组。缺血再灌注后6h，小胶质细胞胞体变大，随时间延长，HG手术组和NG手术组小胶质细胞形态变化规律基本相似，且小胶质细胞数量逐渐减少。
Fig. 4  Changes of microglia proliferation at different time points after cerebral ischemia-reperfusion (Iba-1/PCNA immunohistochemical staining × 400)

Table 1  The number of PCNA with Iba1

<table>
<thead>
<tr>
<th>Reperfusion time</th>
<th>NG group</th>
<th>HG group</th>
</tr>
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<tr>
<td>0.5h</td>
<td>(2)</td>
<td>(2)</td>
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<tr>
<td>3h</td>
<td>(2)</td>
<td>(3)</td>
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<tr>
<td>6h</td>
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<tr>
<td>1d</td>
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<td>3d</td>
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<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>14d</td>
<td>(2)</td>
<td>(3)</td>
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</tbody>
</table>

NG group: Normal saline; HG group: High glucose; MCAO: Middle cerebral artery occlusion.

The number of PCNA with Iba1 at different time points after cerebral ischemia-reperfusion in NG and HG groups. The results showed that the number of PCNA with Iba1 in the HG group was significantly higher than that in the NG group at all time points except for 0.5h. This suggested that high glucose could promote the proliferation of microglia.

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【参考文献】


