Effect of Methylmercury on Expression of Immediate Early Gene c-jun mRNA in Rat Brain

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Abstract: In order to probe into the early prediction molecular index and the signal transduction molecular mechanism of methyl mercury chloride (MMC) neurotoxicity, the expression of c-jun mRNA in rat brains induced by different concentration MMC for different times were observed by using reverse transcription polymerase chain reaction (RT-PCR) methods (the control group was physiological saline of 0.9 %, the concentrations of exposure groups were 0.05, 0.5, 5 mg·kg⁻¹ respectively, the sampling times were 20, 60, 240, 1440 min). The result showed the expression of c-jun mRNA in rat brains was prior to the accumulation of mercury, and the expression of c-jun mRNA in rat brains could early predict the neurotoxicity of MMC. IEG (c-jun) participated in the toxicity process of injury by MMC.

Keywords: methyl mercury chloride; immediate early gene; c-jun; gene expression; RT-PCR; early prediction

1  

1.1

WHO   

SD: 200 g ± 5 g.  

1.1.1

RT-PCR  

c-jun mRNA
1.2 RT-PCR

- RNA samples were extracted using the QIGEN RNAextra kit. The RNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer. Quality control (QC) standards were extracted from the same source.
- RT-PCR was performed using the PrimeScript RT Master Mix and the Takara Ex Taq Hot Start polymerase. PCR reactions were performed in a final volume of 25 μL containing 1 μL of cDNA, 0.5 mM of each primer, and 1X Ex Taq PCR buffer. The cycling conditions were as follows: initial denaturation at 95°C for 30 sec, followed by 35 cycles of denaturation at 95°C for 5 sec, annealing at 60°C for 10 sec, and extension at 72°C for 10 sec. A final extension step at 72°C for 10 min was included.
- The primers used for RT-PCR were designed to amplify a fragment of the target gene. The primers were as follows:
  - c-jun: 5′-GGGCTCGCCATCTGAGCCATCC-3′ (forward) and 5′-GCTCTCATCTGGGATGTGGC-3′ (reverse)
  - GAPDH: 5′-CTCCTCCACCTGTCTGCTG-3′ (forward) and 5′-GACGACAGCCGGCTCAAT-3′ (reverse)

Table 1: The mercury concentrations in brains/μg·kg⁻¹

<table>
<thead>
<tr>
<th>Time/min</th>
<th>0 (D1)</th>
<th>0.05 μg·kg⁻¹ (D2)</th>
<th>0.5 μg·kg⁻¹ (D3)</th>
<th>5 μg·kg⁻¹ (D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (T1)</td>
<td>0.0044 ± 0.0002</td>
<td>0.0044 ± 0.0009</td>
<td>0.0173 ± 0.0023</td>
<td>0.0526 ± 0.1164</td>
</tr>
<tr>
<td>60 (T2)</td>
<td>0.0041 ± 0.0005</td>
<td>0.0064 ± 0.0018</td>
<td>0.0302 ± 0.0044</td>
<td>0.0972 ± 0.1153</td>
</tr>
<tr>
<td>240 (T3)</td>
<td>0.0037 ± 0.0010</td>
<td>0.0047 ± 0.0039</td>
<td>0.0196 ± 0.0286</td>
<td>0.0840 ± 0.1400</td>
</tr>
<tr>
<td>1440 (T4)</td>
<td>0.0044 ± 0.0009</td>
<td>0.0100 ± 0.0010</td>
<td>0.0292 ± 0.0119</td>
<td>0.0214 ± 0.0212</td>
</tr>
</tbody>
</table>

1. DNA Marker sizes were 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 bp from bottom to top.

2.2 c-jun RT-PCR

(1) c-jun RT-PCR

DNA Marker sizes were 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 bp from bottom to top. DNA Marker sizes were 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 bp from bottom to top.

2.3 c-jun RT-PCR

(2) c-jun RT-PCR

DNA Marker sizes were 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 bp from bottom to top. DNA Marker sizes were 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 bp from bottom to top.
Table 2 The effects of MMC on the expression of c-jun mRNA in rat brains

<table>
<thead>
<tr>
<th>time/ min</th>
<th>0 (DI)</th>
<th>0.05 mg/kg (D2)</th>
<th>0.5 mg/kg (D3)</th>
<th>5 mg/kg (D4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (T1)</td>
<td>(10 ± 3)</td>
<td>(15 ± 1)²</td>
<td>(34 ± 14)³</td>
<td>(66 ± 3)³</td>
</tr>
<tr>
<td>60 (T2)</td>
<td>(6 ± 4)</td>
<td>(21 ± 10)²</td>
<td>(63 ± 25)³</td>
<td>(43 ± 16)³</td>
</tr>
<tr>
<td>240 (T3)</td>
<td>(5 ± 4)</td>
<td>(46 ± 21)³</td>
<td>(29 ± 10)³</td>
<td>(36 ± 15)³</td>
</tr>
<tr>
<td>1440 (T4)</td>
<td>(1 ± 0.7)</td>
<td>(23 ± 8)³</td>
<td>(37 ± 16)³</td>
<td>(28 ± 9)³</td>
</tr>
</tbody>
</table>

1) $p < 0.05$; 2) $p < 0.01$.

*Fig. 1* Photographs of representative RT-PCR products electrophoresed on 2% agarose gels stained with ethidium bromide.

*Table 2* The effects of MMC on the expression of c-jun mRNA in rat brains
mRNA


