Potential mechanisms of neuroprotection induced by low dose total-body \(\gamma\)-irradiation in C57 mice administered with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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A B S T R A C T

Low dose total-body \(\gamma\)-irradiation (TBI) was reported to confer neuroprotection against MPTP-induced dopaminergic neurotoxicity. After being pretreated with a single low dose (0.5 Gy, 2.0 Gy or 3.5 Gy) TBI, C57BL/6 mice were administered with MPTP (15 mg/kg, four times, 2 h apart) intraperitoneally (i.p.). In the group pretreated with 2.0 Gy TBI, with lower lymphocytes number, neuroprotection was found by High Performance Liquid Chromatography (HPLC) determination of the striatal dopamine. Contrarily, in the group pretreated with 0.5 Gy TBI, with higher lymphocytes number, dopaminergic neuron toxicity was enhanced. So it was probably the decrease of lymphocytes, not the radiation hormesis that rendered the potential neuroprotection. And it was the balance between radiation injury and lymphopenia neuroprotection that decided the effect of low dose \(\gamma\)-irradiation on MPTP-induced dopaminergic neurotoxicity.

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Irradiation is usually harmful to nervous system and can lead to peripheral neuropathy, radiation myelopathy and other nervous injuries [1,14,17]. But recent studies suggested that different low dose total-body \(\gamma\)-irradiation (TBI) rendered neuroprotection [2,9]. And the neuroprotection had been found in different animal models such as inherited glaucoma, optic nerve crush and contusive spinal cord injury. In 2006, Liang et al. [13] reported a potential neuroprotection in Parkinson’s disease models (C57BL/6 mice administered with MPTP) when the mice were pretreated with 3.5 Gy TBI. But the neuroprotection disappeared when the dose rose to 5.5 Gy. And he supposed that two mechanisms might take part in the neuroprotection: increasing of GSH activity induced by low dose irradiation [10], and proliferation of adaptive immunity induced by radiation hormesis [9].

Confused about the neuroprotection in Parkinson’s disease models, we want to know whether the protection is easily repeatable and what the real mechanism of neuroprotection induced by low dose TBI is.

Radiation hormesis is highly controversial. Edward Calabrese found that hormesis usually occurred at doses about five times lower than the toxic threshold. Environmental Protection Agency (EPA) set the acceptable exposure limit which was 20 times lower than the toxic threshold [19]. The dose for radiation hormesis research was usually very low: from 0.025 Gy to 0.2 Gy [4,15,20], which was determined on the toxic threshold of lymphocyte. So, what is the toxic threshold for central nervous system (CNS)? Earlier studies have reported cognitive impairments and deficits in neural functions following brain irradiation in the dose of 5–10 Gy in rats and mice. So in the study on neuroprotection, the TBI dose of radiation hormesis should be lower than 2 Gy. It is a very low dose for nervous system, but a high dose for immune system. So we supposed that the neuroprotection induced by low dose \(\gamma\)-irradiation was probably related to the immune system injury rather than radiation hormesis.

To prove this hypothesis, we investigated the peripheral lymphocytes number, the radiation hormesis and the dopaminergic neuron toxicity induced by MPTP in C57BL/6 mice pretreated with different low doses of total-body \(\gamma\)-irradiation (TBI).

Male C57BL/6 mice, 2 months old and weighting 20–25 g, were kept in a light and temperature-controlled room with food and water ad libitum. Mice were pretreated with a single dose (0 Gy, 0.5 Gy, 2.0 Gy or 3.5 Gy) of TBI which was done with the \(\gamma\)-rays from a \(^{60}\text{Co}\) (Radiation Center, Chongqing) source. Two hours later, MPTP...
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0d</th>
<th>1d</th>
<th>3d</th>
<th>6d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Gy</td>
<td>3.31 ± 0.77</td>
<td>11.92 ± 1.73</td>
<td>8.98 ± 0.49</td>
<td>9.08 ± 0.97</td>
</tr>
<tr>
<td>2.0 Gy</td>
<td>9.28 ± 0.64</td>
<td>5.95 ± 0.56</td>
<td>3.08 ± 0.26</td>
<td>3.23 ± 0.34</td>
</tr>
<tr>
<td>3.5 Gy</td>
<td>9.31 ± 0.58</td>
<td>4.78 ± 0.32</td>
<td>1.72 ± 0.28</td>
<td>2.12 ± 0.38</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. of six animals in each group.

Fig. 1. Effect of TBI on striatal DA and its metabolites levels. There was no significant difference on striatal DA, DOPAC and HVA levels among saline-treated groups pretreated with TBI or not (p > 0.05). After MPTP administration, DA and its metabolites depleted obviously (p < 0.05, versus saline groups). Pretreatment of 0.5 Gy TBI decreased striatal DA and its metabolites level of the MPTP-treated group (p < 0.05). Contrarily, pretreatment of 0.5 Gy TBI depleted striatal DA and its metabolites level of the MPTP-treated group (p < 0.05, versus the non-irradiated group administered with MPTP). There was no significant difference on DA and its metabolites levels between 3.5 Gy irradiated and non-irradiated animals in MPTP-treated groups (p > 0.05). Values represent the mean ± S.E.M. of six animals in each group.

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6</th>
<th>12</th>
<th>96</th>
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<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>38 ± 8*</td>
<td>61 ± 10*</td>
<td></td>
</tr>
<tr>
<td>MPTP</td>
<td>6</td>
<td>35 ± 7*</td>
<td>64 ± 11</td>
<td></td>
</tr>
<tr>
<td>0.5 Gy + MPTP</td>
<td>6</td>
<td>40 ± 9</td>
<td>60 ± 9</td>
<td></td>
</tr>
<tr>
<td>2.0 Gy + MPTP</td>
<td>6</td>
<td>37 ± 10</td>
<td>63 ± 12</td>
<td></td>
</tr>
<tr>
<td>3.5 Gy + MPTP</td>
<td>6</td>
<td>37 ± 10</td>
<td>63 ± 12</td>
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</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. of six animals in each group.
Fig. 2. Loss of dopaminergic neurons and change of activated astrocytes and microglias in SNpc. The sections were immunostained with antibodies against TH, CD11b or GFAP to show dopaminergic neuron (scale bar, 200 μm), microglia (scale bar, 50 μm) and astrocyte (scale bar, 50 μm). The number of TH-immunoreactive neurons decreased obviously and both of astrocytes and microglias were activated after MPTP administration (P<0.01, versus saline-treated group). But among the MPTP-treated groups with different doses of TBI pretreatment, there was no statistically difference on the total number of TH-positive neurons (P>0.05). Values represent the mean ± S.E.M. of six animals in each group.

by serial section analysis of total number of TH-positive neurons, which was carried out by investigating every sixth coronal section throughout the entire rostral-caudal (RC) axis of the murine, according to the atlas of mouse brain. Microglias and astrocytes were counted in SNpc tissue sections at B-2.92 from each animal. The number of activated microglias was determined by counting CD11b positive microglias with enlarged cell bodies and thick processes. GFAP positive astrocytes with clearly visible cell bodies and
a minimum of two processes were counted as activated astrocytes. Cells were counted at a magnification of 40× (objective).

Results were averaged and differences were analyzed by one-way ANOVA, followed by Newman–Keuls test, and considered significant when p<0.05.

The numbers of blood lymphocytes were counted with haemocytometer manually before TBI and on the first day, third day and sixth day after TBI respectively (see Table 1). Whether being pretreated with TBI or not, there is no difference on lymphocytes number in peripheral blood between mice administered with MPTP and saline. So MPTP could not affect the lymphocytes number in peripheral blood. The number of lymphocytes in peripheral blood was about 9.31±0.77×10^9/L before TBI. After pretreatment with 0.5 Gy TBI, the number of lymphocytes increased in the first 24 h (11.92±1.73×10^9/L) and decreased slowly to the normal level in the following 6 days. But in the group pretreated with 2.0 Gy or 3.5 Gy TBI, the lymphocytes number decreased obviously in the first 24 h and maintained in a very low level in the following 6 days (p<0.05).

Striatal DA and its metabolites levels were determined by HPLC on the seventh day after MPTP or saline administration. There was no significant difference (p>0.05) on content of DA and its metabolites among control groups administered with saline, whether being pretreated with TBI or not. In the groups administered with MPTP whether being pretreated with TBI or not, DA and its metabolites depleted obviously (p<0.01, versus saline-treated groups). There was an obvious decrease of DA, DOPAC and HVA in 0.5 Gy TBI group administered with MPTP compared with its non-irradiated MPTP-treated control by 32.3%, 48.3% and 24.8% respectively. DA of the group administered with MPTP and pretreated with 2.0 Gy TBI was slightly higher (16.7%) than that of the group administered with MPTP without TBI pretreatment (p<0.05), but DOPAC and HVA were similar between the two groups (p>0.05). There was no significant difference on DA and its metabolites levels between 3.5 Gy irradiated and non-irradiated animals in MPTP-treated groups (p>0.05) (Fig. 1).

The loss of TH-immunoreactive neurons in SNpc was obviously after MPTP administration (Fig. 2). However there was no statistical difference on numbers of dopaminergic neurons between irradiation and non-irradiation groups (p>0.05) (Fig. 2). Microglia and astrocytes were counted in SNpc tissue sections at B-2.92 and non-irradiation groups (p>0.05). There was an obvious decrease of DA, DOPAC and HVA in 0.5 Gy TBI group administered with MPTP compared with its non-irradiated MPTP-treated control by 32.3%, 48.3% and 24.8% respectively. DA of the group administered with MPTP and pretreated with 2.0 Gy TBI was slightly higher (16.7%) than that of the group administered with MPTP without TBI pretreatment (p<0.05), but DOPAC and HVA were similar between the two groups (p>0.05). There was no significant difference on DA and its metabolites levels between 3.5 Gy irradiated and non-irradiated animals in MPTP-treated groups (p>0.05) (Fig. 1).

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Hormesis occurs when our bodies overcompensate, reaching a new and healthier equilibrium. And possible mechanisms of radiation hormesis occurring include heat-shock proteins, stimulations to the immune system and DNA repair [5,8,18]. To test whether hormesis offered neuroprotection, we investigated the effects of three different doses (0.5 Gy, 2.0 Gy or 3.5 Gy) of TBI in C57BL/6 mice administered with MPTP.

Considering the radiation toxic threshold of lymphocyte and central nerve system, we knew that 0.5 Gy total-body γ-irradiation might increase the number of lymphocytes and it was a suitable dose to test radiation hormesis [19]. Nevertheless, 2.0 Gy total-body γ-irradiation might decrease the number of lymphocytes and it was also a suitable dose to test radiation hormesis [19]. Then if radiation hormesis could confer neuroprotection, what was the role of peripheral lymphocyte?

Our results suggested that 2.0 Gy TBI could render a weak neuroprotection: DA was slightly higher (16.7%) than the MPTP group with no TBI (p<0.05). Yet, in the group pretreated with 0.5 Gy TBI there was no neuroprotection but more injury. If radiation hormesis conferred neuroprotection, both of the two groups pretreated with 0.5 Gy and 2.0 Gy TBI should show the neuroprotection. And 0.5 Gy was a smaller dose for injury, it might cause more neuroprotection. So our results did not support that radiation hormesis had taken part in the neuroprotection.

As the results showed, 0.5 Gy and 2.0 Gy TBI could affect peripheral lymphocytes contrarily. In the group pretreated with 2.0 Gy TBI, with obviously lower lymphocytes number, neuroprotection was found by HPLC determination of the striatal dopamine. Contrarily, in the group pretreated with 0.5 Gy TBI, with higher lymphocytes number, dopaminergic neuron toxicity was enhanced. So it suggested that the number of lymphocytes in peripheral blood was related to MPTP-induced dopaminergic neuron toxicity: more lymphocytes in peripheral blood, and more dopaminergic neuron toxicity induced by MPTP.

However, no neuroprotection was found in the group pretreated with 3.5 Gy TBI, with obviously lower lymphocytes number. How to explain this phenomenon? Compared with 2.0 Gy TBI, 3.5 Gy TBI had more harmful effect on CNS, though it could not injure CNS obviously [4,7,15,16,20]. At the same time, the neuroprotection induced by 2.0 Gy TBI was weak. It could increase the level of striatal DA, but could not increase the number of DA neurons survived. So the neuroprotection was probably just a functional protection on the surviving DA neurons, with lymphocytopenia participating in. So it was probably the balance between the radiation injury and lymphocytopenia neuroprotection that decided the results. This result was different with Liang et al.’s [13]. It might be the different doses of MPTP we used in the two studies that caused the different results. And it was also probably because the 3.5 Gy TBI-induced neuroprotection was too weak that resulted in failure of repetition. In fact, there was seemingly a slight increase of striatal DA metabolites induced by 3.5 Gy pretreatment in our study, though it was not significant.

As we know, the number of lymphocytes could not be affected markedly by MPTP toxicity and pathological changes of Parkinson’s disease. So our study indicated that the change of lymphocytes number in peripheral blood mediated the MPTP-induced dopaminergic neurotoxicity. The immune mechanism of Parkinson’s disease has been focused on for decades. Many investigations suggested that change of T cells subtype, such as CD4+ or CD8+ T cells, could influence the dopaminergic neuron deplletion in Parkinson’s disease. It was probably the change of T cells subtype that resulted in change of cytokines secreting, such as TNF-α and IL-1, which influenced the lesion of dopaminergic neurons [3,6]. Then how did T cells participate in dopaminergic neurons depletion? And where did T cells take the role, in peripheral blood or in SNpc? Researchers found that T cells could infiltrate in SNpc through blood brain barrier in MPTP-induced Parkinson’s disease model. And the neurotoxicity induced by MPTP could be diminished by dexamethasone treatment, which decreased the number of T cell infiltrating in SNpc [11,12]. In accordance with the researches mentioned before, our study suggested that lymphocytes might participate in dopaminergic neurons injury.

In conclusion, low dose TBI does not always play a neuroprotection role against MPTP-induced dopaminergic neurotoxicity. Radiation hormesis was not the main cause of neuroprotection. The number of lymphocytes in peripheral blood was related to dopaminergic neuron injury induced by MPTP and lymphocytopenia might provide neuroprotection. It was the balance between radiation injury and neuroprotection by lymphocytopenia that decided the effect of low dose TBI on the dopaminergic neurotoxicity induced by MPTP.
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