Mycophenolic Acid and Its Derivatives as Potential Chemotherapeutic Agents

Targeting Inosine Monophosphate Dehydrogenase in *Trypanosoma congolense*

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Running Head: Trypanocidal Activity of MPA and Its Derivatives

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This study aimed to evaluate the trypanocidal activity of mycophenolic acid (MPA) and its derivatives for *Trypanosoma congolense*. The proliferation of *T. congolense* was completely inhibited by adding less than 1 µM of MPA and its derivatives. In addition, the inosine monophosphate dehydrogenase in *T. congolense* was molecularly characterized as the target of these compounds. The results suggested that MPA and its derivatives have the potential to be new candidates as novel trypanocidal drugs.

**Keywords:** African trypanosomosis, Inosine monophosphate dehydrogenase, Mycophenolic acid, Trypanocidal drug, *Trypanosoma congolense*.
Trypanosoma congolense causes animal African trypanosomosis (AAT) in livestock. The lack of effective vaccines makes the use of chemotherapeutic agents the most effective measure for controlling AAT. Limited numbers of commercial drugs have long been used to treat AAT. The emergence of drug-resistant trypanosomes and cases of drug-refractory trypanosomosis have been reported (1-4), underscoring the need for the development of new drugs.

A candidate target for drug development is inosine monophosphate dehydrogenase (IMPDH). This enzyme is very important in the Trypanosoma spp. because it lacks a de novo purine synthesis pathway, which makes the purine nucleotide synthesis in these parasites solely dependent on a salvage pathway in the glycosomes (5-7). IMPDH converts inosine 5'-monophosphate (IMP) into xanthosine 5'-monophosphate (XMP) through this pathway, which is a rate-limiting step in the metabolism of guanine nucleotides (8). Mycophenolic acid (MPA, 1) is a well-known IMPDH inhibitor (Fig. 1). Its enzymatic activity has already been proven in many protozoan parasites (9-14). The anti-protozoan activities of MPA against Babesia spp. have been reported in in vivo and in vitro
Thus, the activity of MPA against IMPDH is expected to lead to a novel strategy for the development of trypanocides. The novel IMPDH orthologue of *T. congolense* (*TcIMPDH*) (accession no. LC094350) was identified from the *T. congolense* re-sequencing data (unpublished data). The recombinant TcIMPDH showed IMPDH activity in vitro (Supplemental Fig. 1-A and B). The nanomolar levels of MPA clearly inhibited NADH production by TcIMPDH in a dose-dependent manner (IC$_{50}$ = 26.2nM) (Supplemental Figure 1-C). The expression profile and cellular localization of TcIMPDH were analyzed by Western blotting and immunofluorescence microscopy. TcIMPDH was expressed in glycosomes as granulated forms throughout the life cycle stages of *T. congolense* (Supplemental Fig. 2). TcIMPDH was expressed at similar levels in bloodstream form (BSF), procyclic form (PCF), and epimastigote form (EMF). In contrast, TcIMPDH expression in the metacyclic form (MCF) was significantly lower than in the other stages ($p<0.05$, Tukey’s multiple comparison test). This result suggests that purine
synthesis is highly important in the proliferative stages of the parasite, but not in the non-proliferative MCF stage.

The aim of this study was to reveal the trypanocidal activities of MPA derivatives for developing an effective trypanocidal drugs. Various inhibitory activities and the cell-differentiation activity of MPA derivatives against mammalian cells have been reported \textit{in vitro}. Some MPA derivatives (2, 4, 9 and 10) have shown particularly significant inhibitory activities against human IMPDH and were observed to induce erythroid differentiation in K562 cells (16, 17). These previous reports suggested that some MPA derivatives might be specific inhibitors for \textit{Trypanosoma}. The chemical structures of the MPA derivatives in this study are shown in Figure 1. We evaluated the trypanocidal activity against \textit{T. congolense}, \textit{T. b. brucei} and \textit{T. evansi} using an ATP-based luciferase viability system (18). To evaluate the trypanocidal activity of 1 and its derivatives \textit{in vitro}, BSFs were cultivated with 1µM of each compound. At 1µM, nine derivatives showed less than 10% anti-\textit{T. congolense} activity (Table 1). In contrast, only three compounds, 1, 2, and 4, inhibited \textit{T. congolense} growth by
99.60±0.38%, 94.46±3.89% and 98.87±0.78% at 1µM, respectively (Table 1).

Although 1 showed high trypanocidal activity against *T. b. brucei* and *T. evansi*, 2 and 4 showed lower inhibitory activities at 1µM against *T. b. brucei* and *T. evansi* than against *T. congolense* (Table 1). The low plasma membrane permeability of 3, 5, 6, 7, 8, 11 and 12 might account for their low trypanocidal activity; while the low trypanocidal activity of 9 and 10 against all of the tested trypanosome species and of 2 against *T. brucei* and *T. evansi* suggest their low affinity with these trypanosome IMPDHs or the deactivation of these compounds by other species-specific enzymes in cytosol. The IC₅₀ of 1, 2, and 4 to *T. congolense* were 0.10±0.04µM, 0.56±0.21 µM, and 0.16±0.04µM, respectively (Table 2). The IC₅₀ values of these three compounds to MDBK cells were 0.52±0.12, 1.40±0.18, and 0.84±0.21µM, respectively. The selectivity indices of MPA and the two derivatives in *T. congolense* were 5.14, 2.62, and 5.10, respectively (Table 2). However, the higher IC₅₀ values and lower selectivity indices of these 3 compounds were shown in *T. b. brucei* and *T. evansi* (Table 2). The cytotoxicity of these compounds was higher than that of commercial drugs (19).
However, the IC50 values of 1 and 4 for *T. congolense* BSF were comparable to those of two commercially available trypanocides (pentamidine [0.17µM] and diminazene [0.11µM]) against *T. congolense* (18). These results suggested that 1, 2, and 4 might be potential lead compounds in the development of trypanocides, especially against *T. congolense*.

To clarify the mode of action of 1 and 4 in trypanosomes, the effects of guanosine and xanthine supplementation on the trypanocidal effects of these compounds were examined. The IC50 values of 1 and 4 were increased by guanosine in a dose-dependent manner (Table 3), while xanthine supplementation did not alter the IC50 values of either 1 or 4 in *T. congolense* BSF (Table 3). These results suggest that guanosine was transported into the *T. congolense* BSF and converted into GMP as a purine nucleotide source, while no xanthine was transported or converted into XMP by hypoxanthine-guanine phosphoribosyltransferase in *T. congolense*. We therefore concluded that the proliferation inhibitory effects of MPA against *T. congolense* BSF were caused by the inhibition of intracellular TclIMPDH.
Hypoxanthine and inosine were predicted to be the main purine sources in *T. brucei* (20). Hypoxanthine and inosine have also been shown to be present in the blood at higher concentrations than other purines (21), suggesting their roles as the main purine sources in trypanosomes and that they are supplied via the salvage pathway. The concentration of purine bases and nucleosides in the extracellular environment is lower than that in the intracellular environment (21).

*T. brucei* spp. proliferate in blood circulation and then invade the central nervous system through the blood-brain barrier (22, 23), while *T. congolense* only proliferates in blood circulation by adhesion to the vascular endothelium (24).

In conclusion, MPA and its derivatives might therefore also inhibit trypanosome proliferation *in vivo*, particularly in *T. congolense*. 
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References


Fig. 1. The structures of mycophenolic acid and its derivatives

MPA (1): $R^1 = \text{OH}$
2: $R^1 = \text{NHOH}$
3: $R^1 = \text{N}
4: $R^1 = \text{N}
5: $R^1 = \text{N}
6: $R^1 = \text{N}
7: $R^1 = \text{N}
8: $R^1 = \text{N}
9: $R^1 = \text{N}
10: $R^1 = \text{N}
11: $R^2 = \text{CH}_3\text{CO}$
12: $R^2 = \text{H}$
The trypanocidal activity of MPA (1) and 11 MPA derivatives (see Fig. 1) at a concentration of 1µM was evaluated for *T. congolense*, *T. b. brucei* GUTat 3.1 strain and *T. evansi* Tansui strain. Five hundred ng/mL of pentamidine was used as a 100% inhibition control (Pentamidine). HMI-9 media with 0.25% DMSO was used as a 0% inhibition control (Control). The inhibition rate (%) was calculated from 3 independent experiments, and expressed as the mean inhibition rate (%) ± standard deviation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition rate (%)</th>
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<tbody>
<tr>
<td></td>
<td><em>T. congolense</em></td>
</tr>
<tr>
<td>1</td>
<td>99.60±0.38</td>
</tr>
<tr>
<td>2</td>
<td>94.46±3.89</td>
</tr>
<tr>
<td>3</td>
<td>2.36±8.64</td>
</tr>
<tr>
<td>4</td>
<td>98.87±0.78</td>
</tr>
<tr>
<td>5</td>
<td>4.65±15.29</td>
</tr>
<tr>
<td>6</td>
<td>1.45±10.94</td>
</tr>
<tr>
<td>7</td>
<td>4.59±15.12</td>
</tr>
<tr>
<td>8</td>
<td>3.59±14.06</td>
</tr>
<tr>
<td>9</td>
<td>0.06±8.66</td>
</tr>
<tr>
<td>10</td>
<td>3.15±8.43</td>
</tr>
<tr>
<td>12</td>
<td>3.03±12.91</td>
</tr>
</tbody>
</table>

Pentamidine 99.93±0.07 99.96±0.06 99.94±0.07
Control 0.00±1.74 0.48±1.58 -0.24±2.25
Table 2. The IC50 value and selectivity index of MPA (1) and MPA derivatives 2 and 4 against *T. b. brucei* and *T. evansi*

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>Selectivity indexa</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. congolense</em></td>
<td><em>T. b. brucei</em></td>
<td><em>T. evansi</em></td>
<td>MDBK cell</td>
<td><em>T. congolense</em></td>
<td><em>T. b. brucei</em></td>
<td><em>T. evansi</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.10±0.04</td>
<td>0.62±0.05</td>
<td>0.61±0.002</td>
<td>0.52±0.12</td>
<td>5.14</td>
<td>0.84</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.56±0.21</td>
<td>&gt;2.5</td>
<td>&gt;2.5</td>
<td>1.4±0.18</td>
<td>2.62</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.16±0.04</td>
<td>1.26±0.009</td>
<td>1.38±0.10</td>
<td>0.84±0.21</td>
<td>5.10</td>
<td>0.67</td>
<td>0.61</td>
<td></td>
<td></td>
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</table>

All of the values were calculated from 3 independent experiments and expressed as the mean ± standard deviation. IC50: 50% inhibitory concentration; a, the mean IC50 of MDBK cells/the mean IC50 of trypanosomes; ND, not determined.
Table 3. The effects of guanosine and xanthine on parasite proliferation under the IMPDH inhibition by MPA (1) and N-(2,3,5-triazolyl) mycophenolic amide (4)

<table>
<thead>
<tr>
<th>xanthine (µM)</th>
<th>IC₅₀ (µM) with guanosine</th>
<th>IC₅₀ (µM) with xanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>50</td>
<td>0.29±0.19</td>
<td>0.50±0.31</td>
</tr>
<tr>
<td>0</td>
<td>0.07±0.006</td>
<td>0.13±0.02</td>
</tr>
</tbody>
</table>

The IC₅₀ values of MPA (1) and 4 supplemented with 250, 50 and 0µM of guanosine or xanthine. All of the values were calculated from 3 independent experiments and are shown as the mean ± standard deviation. IC₅₀, 50% inhibitory concentration.