Effect of 
N,N'-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine 
on Vaccinia Virus Replication in vitro and in vivo

Brief Report

By

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With 1 Figure

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Summary

The inhibitory effect of N,N'-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (compound TSKI—VI) and methisazone (Marboran) on the growth of vaccinia virus (IHD strain) was studied in vitro and in vivo. The therapeutic indices of both compounds determined in vivo were similar, but TSKI—VI was found more efficient in vitro.

N,N'-bis(methylisatin-β-thiosemicarbazone)-2-methylpiperazine (compound TSKI—VI) belongs to the so called Mannich bases (Fig. 1). Its chemistry and some antiviral properties have been discussed earlier (3, 4, 8, 12). The aim of the present work has been to obtain further information on the inhibitory effect of TSKI—VI on the vaccinia virus multiplication in experiments on mice and to compare antiviral activity of the compound with that of N-methylisatin-β-thiosemicarbazone (methisazone) (Fig. 1) both in vitro and in vivo. The latter compound has been introduced in the prophylactic treatment against smallpox and is still used in the therapy of post-vaccination complications (2, 5, 9).

The first intracerebral passage of vaccinia virus (IHD strain) in newborn mice, following a passage in the chorioallantoic membrane of chick embryos was used for tests in vivo. Stock suspensions of TSKI—VI and methisazone were prepared by grinding the compound in a glass mortar and suspending it in saline. Median plaque reduction doses (PRD₅₀) and maximum well tolerated doses (WTD) of the investigated compounds in 48 hours cultures of chick embryo fibroblasts (CEF), expressed in μM concentrations, and therapeutic indices (Th.i.) were determined by the methods described elsewhere (4).
Table 1. Effect of TSKI—VI and methisazone on vaccinia virus (IHD strain) replication studied in vitro and in vivo

<table>
<thead>
<tr>
<th>Compound</th>
<th>PRD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>WTD</th>
<th>Th.i. (a)</th>
<th>Toxic effect in mice LD&lt;sub&gt;0&lt;/sub&gt;</th>
<th>Protective effect against lethal virus infection in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>s.c.</td>
<td>i.p.</td>
<td>PD&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>TSKI—VI</td>
<td>2.1</td>
<td>60.8</td>
<td>28</td>
<td>2000</td>
<td>6.1</td>
</tr>
<tr>
<td>Methisazone</td>
<td>1.8</td>
<td>7.9</td>
<td>4.4</td>
<td>2000</td>
<td>3.5</td>
</tr>
</tbody>
</table>

PRD<sub>50</sub> — Median Plaque Reduction Dose, concentration of tested compound, in μM, in Parker-Methylcellulose-overlay at which the number of vaccinia virus plaques in CEF cultures is reduced to 50 per cent of their number in controls.

WTD — Well Tolerated Dose, maximum non toxic concentration of tested compound, in μM, in Parker-Methylcellulose-overlay at which there were no morphological changes in CEF cultures stained supravitally with 0.1 per cent Neutral Red after 48 hours incubation at 37°C.

PD<sub>50</sub> — Median Protective Dose, in mg/kg of body weight, i.e. a dose of tested compound given subcutaneously (s.c.) or intraperitoneally (i.p.) twice a day for 5 days to 10g Swiss mice infected intracerebrally with 10<sup>6</sup>LD<sub>50</sub> of vaccinia virus and protecting 50 per cent of animals.

EMPD — Estimated Minimum Protective Dose, in mg/kg of body weight, i.e. a dose of tested compound protecting all mice infected and treated as above.

LD<sub>50</sub> — The highest nonlethal dose, in mg/kg of body weight, i.e. a dose of tested compound tolerated by all mice uninfected and treated as above.

Th.i. — Therapeutic index; Th.i. (a): WTD/PRD<sub>50</sub>; Th.i. (b): LD<sub>50</sub>/PD<sub>50</sub>; Th.i. (c): LD<sub>50</sub>/EMPD

Note — Statistical analysis carried out using RTBA, RTB2 and RTB4 computer programmes (3, 11)

<sup>a</sup> From ref. 4
<sup>b</sup> From ref. 3