Notes

ISOLATION OF GLUTATHIONE-S-TRANSFERASE INHIBITORS

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(Received for publication May 27, 1992)

Glutathione S-transferase is one of the most important drug-detoxifying enzymes in the liver. On the other hand, reports have suggested that increased glutathione S-transferase activity may create resistance against a few anticancer drugs, for example, alkylating agents1-4), cisplatin5-7) and doxorubicin8). Inhibitors of this enzyme, ethacrynic acid and piriprost are already known and their potential therapeutic utility in combination with alkylating agents has been suggested by Tew and his colleagues9). Our group has been screening for novel inhibitors of glutathione S-transferase from microbial origin and we recently isolated tryptophanehydrobutyric diketopiperazine10) (TDD) and rishirilide B11) (Fig. 1). Although the structures of these compounds are already known, it seems to be worth while to report their enzymological and biological properties because of their potential usefulness. We report here the inhibitory effect of these compounds on glutathione-S-transferase activity, the kinetic data and the potentiation effect of these compounds on chlorambucil.

Glutathione S-transferase was prepared from doxorubicin-resistant P388 cells. Exponentially growing P388/ADR cells were homogenized using a teflon-glass homogenizer in double distilled water. The homogenized cells were centrifuged at 105,000 x g for 1 hour at 4°C and the supernatant was used for the enzyme assay. The enzyme activity was measured according to the method of HABIG et al.12). 1-Chloro-2,4-dinitrobenzene (CDNB) was used as the second substrate. After screening of microbial culture filtrates, we isolated TDD and rishirilide B. For isolation of TDD, Streptomyces sp. MI513-8F5 was cultured in media containing galactose 2%, dextrin 2%, soy peptone 1%, corn steep liquor 0.5%, (NH4)2SO4 0.2% and CaCO3 0.2% (pH 7.4) for 4 days at 28°C on a reciprocal shaker. The active principle was extracted from broth filtrate (13 liters) with BuOAc and successively purified by Sephadex LH-20 column chromatography (MeOH) and preparative TLC (CHCl3-MeOH = 10 : 1).

UV, FAB-MS, 1H and 13C NMR data revealed that it was identical to TDD. It had already been recognized as a metabolite of Streptomyces spectabilis10). For isolation of rishirilide B, Streptomyces sp. SA-3093 was cultured in media containing glycerol 2%, Bacto soytone 1% and CaCO3 0.4% with a quarter strength artificial seawater (Jamarin S) for 4 days at 28°C on a reciprocal shaker. The active principle was extracted from broth filtrate (8.6 liters) with BuOAc at pH 3.0 and successively purified by silica gel column chromatography (CHCl3-MeOH = 5 : 1), Sephadex LH-20 column chromatography (MeOH) and preparative TLC (BuOH-MeOH-H2O = 4 : 1 : 2).

UV, FAB-MS, 1H and 13C NMR data revealed that it was identical to rishirilide B. It had already been recognized as the inhibitor of α2-macroglobulin11).

Fig. 1. Structures of TDD and rishirilide B.
The inhibitory activity of various inhibitors against glutathione S-transferase are shown in Table 1. Inhibition of TDD against glutathione S-transferase is competitive with glutathione (Fig. 2-a, $K_i = 6.0 \times 10^{-6} \text{ M}$) and competitive with CDNB (Fig. 2-b, $K_i = 1.6 \times 10^{-5} \text{ M}$), too.

Inhibition of rishirilide B against glutathione S-transferase is competitive with glutathione (Fig. 3-a, $K_i = 9.4 \times 10^{-6} \text{ M}$) and competitive with CDNB (Fig. 3-b, $K_i = 1.9 \times 10^{-5} \text{ M}$), too.

Cytotoxic activity was assayed using Walker chlorambucil-resistant and -sensitive cells. Walker cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (Gibco) with or without an inhibitor. After 72 hours, the viable cells were counted by MTT method\(^{13}\). For potentiation tests, practically non-cytotoxic concentration of TDD and rishirilide B were used. TDD and rishirilide B showed little

Table 1. Inhibitory effects of various inhibitors on glutathione S-transferase.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC(_{50}) ((\mu\text{M}))</th>
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<tbody>
<tr>
<td>Ethacrynic acid</td>
<td>16.0</td>
</tr>
<tr>
<td>TDD</td>
<td>26.5</td>
</tr>
<tr>
<td>Rishirilide B</td>
<td>26.9</td>
</tr>
<tr>
<td>TA-3037A(^{13})</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Fig. 2. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by TDD.

(a): ○ I = 0, ● I = 1, ▲ I = 3, ■ I = 6 \(\mu\text{g/ml}\), (b): ○ I = 0, ● I = 1, ▲ I = 5, ■ I = 8 \(\mu\text{g/ml}\).

![Fig. 2. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by TDD.](a) ![Fig. 2. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by TDD.](b)

Fig. 3. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by rishirilide B.

(a): ○ I = 0, ● I = 5, ▲ I = 8, ■ I = 10 \(\mu\text{g/ml}\), (b): ○ I = 0, ● I = 2, ▲ I = 5, ■ I = 8 \(\mu\text{g/ml}\).

![Fig. 3. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by rishirilide B.](a) ![Fig. 3. Lineweaver-Burk plot of inhibition of glutathione-S-transferase by rishirilide B.](b)
potentiation effect on chlorambucil.

Kamei et al. reported piperafizines A and B, potentiators of cytotoxicity of vincristine. It is interesting that both piperafizines and TDD are structurally classified as diketopiperazines.

Potentiation tests with other resistant cells are now under study.

Acknowledgment

The authors wish to thanks Dr. K. D. Tew of Department of Pharmacology, Fox Chase Cancer Center for providing us Walker chlorambucil-resistant and -sensitive cells.

References


