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# COMPARATIVE IN VITRO STUDIES OF TIAZOFURIN AND A SELENAZOLE ANALOG

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2-β-D-Ribofuranosylselenazole-4-carboxamide, a selenazole analog of the antitumor agent Tiazofurin, is severalfold more cytotoxic to murine tumor cells in culture than Tiazofurin. Like Tiazofurin, the cytotoxicity of the selenazole analog is reversed by exogenous guanosine, and both nucleosides specifically inhibit IMP dehydrogenase activity in cultured P388 cells. The dose-dependency for this inhibition correlates with the relative cytotoxicities of both drugs, indicating that a more potent inhibition of IMP dehydrogenase by the selenazole analog is primarily responsible for its increased cytotoxicity. The specific inhibition of the isolated enzyme by potential metabolites of the selenazole analog is discussed.

Tiazofurin (2-β-D-ribofuranosylthiazole, NSC 286193) is a C-nucleoside (Figure 1, I) originally synthesized by Srivastava et al. (1). Although RTC[1] possessed only moderate antiviral activity in those earlier studies, recently it demonstrated potent antitumor activity against Lewis lung carcinoma (2). Furthermore, the antiviral and antitumor properties of RTC have been linked with the cellular depletion of guanine nucleotides by both in vitro (1,3) and in vivo studies (4,5). This cellular condition appears to be the result of a specific inhibition of the enzyme IMP dehydrogenase (IMP:NAD oxidoreductase EC 1.2.1.14) (3-6) most likely mediated by a cellular metabolite of RTC (TAD) that is a potent inhibitor of the enzyme (7,8).

RSC, a selenazole analog of Tiazofurin, possesses even greater in vitro cytotoxicity than does RTC and is effective against Lewis lung carcinoma in vivo at slightly lower dose levels than RTC (9). We therefore propose to fur-

Abbreviations: RTC, 2-β-D-Ribofuranosylthiazole-4-carboxamide;
RSC, 2-β-D-Ribofuranosylselenazole-4-carboxamide;
SeAD, selenazole-adenine dinucleotide;
TAD, thiazole-adenine dinucleotide.

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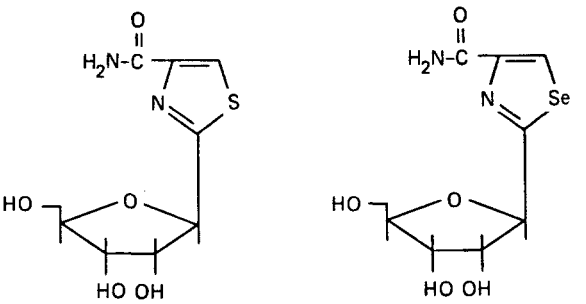


TIAZOFURIN I (RTC) RSC II

Figure 1. Test compounds.

ther investigate the mechanism of action of these compounds by comparing the biochemical properties of RTC and RSC in sensitive tumor cells, and attempt to determine the basis for the increased potency of RSC.

## MATERIALS AND METHODS

RTC, RSC, and various phosphorylated derivatives of these compounds were provided by R. K. Robins (9). [8-$^{14}$C]Hypoxanthine was obtained from ICN Pharmaceuticals, Chemical and Radioisotope Division, Irvine, CA. Other nucleosides and nucleotides were obtained from Sigma Chemical Co., St. Louis, MO.

Cell growth inhibition studies were performed with murine leukemia L1210 and lymphoid neoplasm P388 obtained from American Type Culture Collection and maintained in suspension culture in RPMI-1640 media supplemented with 10% fetal bovine serum (FBS) and 20 mM HEPES buffer. Lewis lung carcinoma cells were maintained in monolayer culture in the same media supplemented with 5% FBS. B-16 melanoma F10 cells were maintained in monolayer culture in Eagle minimum essential medium with Earle's salts supplemented with 10% FBS, 1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, MEM vitamins and 20 mM HEPES buffer. Dialyzed serum was used in the nucleoside "rescue" experiments with P388 cells. The effects of RTC and RSC on the incorporation of [$^{14}$C]-hypoxanthine into purine nucleotides in cultured P388 cells were determined as previously described (6,10).

IMP dehydrogenase was partially purified from P388 cells and assayed by previously described methods (11). The dinucleotide inhibitors, TAD and SeAD, were quantitated by enzyme degradation with nucleotide pyrophosphatase (Sigma Chemical Co., St. Louis, MO) and determination of the amount of AMP formed by high pressure liquid chromatography (HPLC). This analysis was performed on a Partisil-10 SAX anion exchange column, 250 × 4.5 mm (Whatman, Inc., Clifton, NJ) using a Spectra-Physics model 8700 high-pressure liquid chromatograph. The column was eluted isocratically with 0.5 M ammonium formate, pH 3.0, and compounds were detected using a Waters Associates Model 480 variable-wavelength UV detector.

## RESULTS

Table 1 shows the effects of RTC and RSC on cultures of four murine tumor cells. RSC was consistently more cytotoxic than RTC by factors of 5 to 17; the leukemic cell lines P388 and L1210 were the most sensitive to either drug.

Table 1
INHIBITION OF MURINE TUMOR CELL PROLIFERATION
BY RTC AND RSC

| Cells | $ID_{50}$ (μM) RTC | RSC |
|---|---|---|
| P388 | 2.3 | 0.40 |
| L1210 | 4.7 | 0.27 |
| B16 melanoma | 35 | 3.5 |
| Lewis lung carcinoma | 26 | 3.2 |

Because the cytotoxicity of RTC has been linked to the depletion of guanine nucleotides, an attempt was made to "rescue" P388 cells from the cytotoxic effects of RTC and RSC with exogenous nucleosides. The data in Table 2 clearly demonstrate that only guanosine specifically reverses the cytotoxicity of both drugs to these cells, thereby indicating that the impairment of guanine nucleotide biosynthesis is somehow involved in the mechanism of cytotoxicity of both drugs.

The inhibition of specific enzymes in purine nucleotide biosynthesis in intact P388 cells was examined by measuring the effects of both drugs on the incorporation of [$^{14}$C]hypoxanthine into individual nucleotide pools, as previously determined for RTC in Ehrlich ascites cells (6). The pattern of enzyme inhibition by RTC (Table 3) was virtually identical to that previously obtained in Ehrlich ascites, the primary effect being on IMP dehydrogenase, with a secondary inhibition of GDP kinase. The effect on GDP kinase, however,

Table 2
EFFECT OF NUCLEOTIDE PRECURSORS ON CYTOTOXICITY
OF RTC AND RSC TO P388 CELLS

| Precursor[a] | % Reversal of Cytotoxicity RTC[b] | RSC[c] | Toxicity of Precursor (% Inhibition) |
|---|---|---|---|
| Adenosine | 0 | 0 | < 10 |
| Guanosine | 26 | 49 | 15 |
| Uridine | 0 | 0 | < 10 |
| Cytidine | 0 | 0 | < 10 |
| Thymidine | 0 | 0 | 42 |

[a]Precursor concentration, 50 μM.
[b]RTC concentration, 7.5 μM.
[c]RSC concentration, 2.0 μM.

Table 3
INHIBITION OF PURINE NUCLEOTIDE BIOSYNTHESIS IN CULTURED P388 CELLS BY RTC AND RSC[a]

| Reaction | % Inhibition by 10 μM RTC | RSC |
|---|---|---|
| Hypoxanthine-guanine phosphoribosyltransferase | 5 | -12 |
| AMP synthetase + lyase | -7 | -1 |
| AMP kinase | 5 | -1 |
| ADP kinase | 4 | -1 |
| IMP dehydrogenase | 63 | 67 |
| GMP synthetase | 10 | 9 |
| GMP kinase | 7 | 3 |
| GDP kinase | 30 | 1 |

[a]P388 tumor cells were resuspended in serum-free RPMI-1640 medium at 1 × $10^7$ cells/ml; 0.1-ml aliquots were incubated at 37° in 5% $CO_2$ for 30 min, with and without 10 μM of the indicated compounds. Incubations were carried out in triplicate. [$^{14}$C]Hypoxanthine was then added to a final concentration of 0.1 mM, and the incubation was continued for 60 min. The apparent enzyme activities were calculated according to the method described in Ref. 10.

was variable and may not be significant. RSC likewise produced a specific inhibition of IMP dehydrogenase. Moreover, when the dose-dependency of this inhibition was compared (Figure 2), RSC was clearly more effective than RTC in

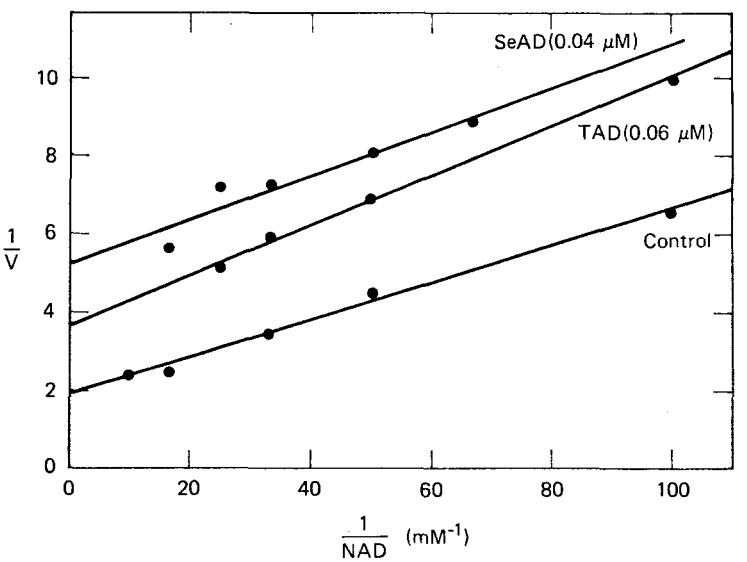


Figure 2. Inhibition of IMP dehydrogenase in P388 cells by RTC and RSC.

the inhibition of IMP dehydrogenase. The maximum inhibition by either drug was 65 to 70% at concentrations above 10 μM. The $ED_{50}$ (50% of the maximum effect) was 0.7 μM for RSC, 4 μM for RTC. These values are in the same range as the respective $ID_{50}$ values for the cytotoxicities of the drugs against P388 cells (Table 1), thus indicating that impairment of IMP dehydrogenase may primarily account for the relative antiproliferative activity of both drugs.

Previous studies have reported the phosphorylation of RTC and the further metabolism of RTC-5'-monophosphate to a dinucleotide analog of NAD, TAD, which is a potent inhibitor of IMP dehydrogenase (7,8). To determine whether the increased cytotoxic effects of RSC could be linked to a similar mode of action, we undertook a comparative evaluation of the nucleosides, 5'-mononucleotides, and the dinucleotide "NAD" analogs of both RTC and RSC for their relative abilities to inhibit IMP dehydrogenase from P388 cells. The nucleosides and 5'-mononucleotides of both drugs were poor inhibitors of the isolated enzyme ($K_i$s > 100 μM), although RSC-5'-phosphate ($K_i$ = 140 μM) was slightly more effective than RTC-5'-phosphate ($K_i$ = 300 μM). In contrast, TAD and SeAD are potent inhibitors of the enzyme (Figure 3). With NAD as the variable substrate, the inhibition by TAD is noncompetitive in nature ($K_i$ = 0.07 μM), whereas for SeAD ($K_i$ = .02 μM) the inhibition is more uncompetitive

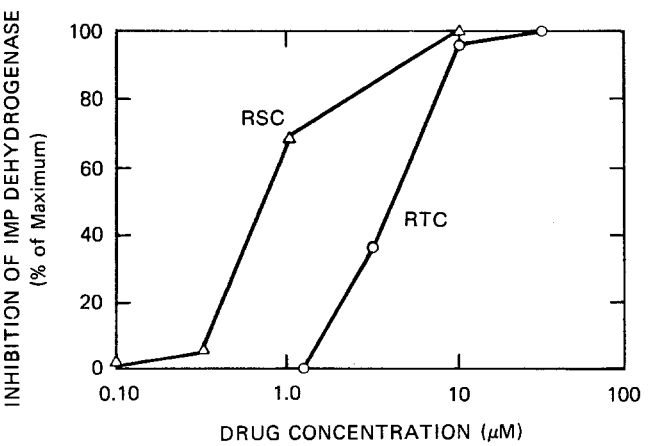


Figure 3. Inhibition of partially purified IMP dehydrogenase from P388 cells by TAD and SeAD. Velocity is expressed as nmoles of NADH formed per minute.

in nature. the corresponding $K_i$ values with IMP as the variable substrate are 0.05 and 0.02 μM, respectively.

## DISCUSSION

The results obtained in this study demonstrate that the biochemical effects of RSC, the selenazole analog of RTC, on guanine nucleotide biosynthesis in P388 tumor cells are qualitatively similar to those of RTC. The increased cytotoxicity of RSC relative to RTC in P388 and in other murine tumor cell lines is paralleled by an increased ability of RSC to inhibit IMP dehydrogenase in these cells. The two activities are further linked by the ability of guanosine, to rescue cells from the cytotoxic effects of both compounds.

Studies with isolated IMP dehydrogenase reveal that whereas the nucleosides and 5'-nucleotides of both RTC and RSC are relatively poor inhibitors of the enzyme ($K_i$s > 100 μM), TAD and SeAD, the "NAD" analogs of RTC and RSC, are potent inhibitors of the enzyme ($K_i$s < 0.1 μM), which is in agreement with previous findings on RTC (7,8). SeAD is slightly more active than TAD in this regard, which could partially account for the increased cytotoxicity of RSC toward tumor cells.

Studies are in progress to determine the relative ability of tumor cells to metabolize RTC and RSC to the NAD analogs as well as other metabolites, and whether the nature and extent of this metabolism affects the relative cytotoxicities of these compounds.

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