The Carcinostatic Activity of 5-Hydroxy-2-formylpyridine Thiosemicarbazone

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SUMMARY

5-Hydroxy-2-formylpyridine thiosemicarbazone (5-HP) is highly active intraperitoneally against leukemia L-1210, Ehrlich ascites carcinoma, and lymphoma L-5178Y over a broad dose range. 5-HP is also active on leukemia L-1210 and the Ehrlich ascites carcinoma by delayed treatment. Activity by oral routes or subcutaneously is poor. Moderate activity is displayed against the Lewis lung carcinoma and adenocarcinoma 755. 5-HP is the most active antileukemic agent in this series to date.

INTRODUCTION

In previous papers in this series (1–3), it was noted that some thiosemicarbazones of α-(N)-formyl heteroaromatic compounds display carcinostatic activity. These effects are observed only when the appropriate conformational and electronic parameters are present. Manifest activity is also dependent on low toxicity. Toxicity per se may arise from diverse causes and has not proved predictable. It was noted that the introduction of a 3-hydroxy group into 2-formylpyridine thiosemicarbazone decreased toxicity by a factor of 25 and broadened and intensified the antitumor activity (2). To ascertain whether this effect was unique or possibly fairly general, it was necessary to synthesize and test additional related molecules bearing the same substitution features but having different total geometries. If one entertains a chelation model it could be argued, a priori, that 3-hydroxy-2-formylpyridine thiosemicarbazone (3-HP)² (Chart 1) could be acting as an O—N—S or N—N—S ligand in the tridentate mode. 5-HP (Chart 2) was chosen as a test molecule. 5-HP cannot act as an O—N—S ligand except in polynuclear complexes. It can act readily as an N—N—S ligand. Additionally, the hydroxy groups in both 3-HP and 5-HP are essentially phenolic. The notable activity of 3-HP and 5-HP lends encouragement to the hypothesis that additional activities will be found in the general class expressed in Chart 3.

MATERIALS AND METHODS

The biologic methods were the same as those described in the first two papers in this series (1, 2), with the exception that single-dose treatments and treatment to the 50% survival point were also studied on L-1210 leukemia. The strain of Ehrlich ascites carcinoma used in these experiments was different from the one used previously. It is much more susceptible to compounds in this series.

5-HP is a new compound and was synthesized by two methods, illustrated schematically in Charts 4 and 5. The details of these syntheses will be presented elsewhere.

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2The following abbreviations are used: 3-HP, 3-hydroxy-2-formylpyridine thiosemicarbazone; 5-HP, 5-hydroxy-2-formylpyridine thiosemicarbazone; IQ-1, 1-formylisoquinoline thiosemicarbazone; T/C, mean tumor weights of treated/control mice.

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Chart 4. Synthesis of 5-hydroxy-2-formylpyridinethiosemicarbazone (Method 1).
*This compound may be purchased from Aldrich Chemical Co.

Chart 5. Synthesis of 5-hydroxy-2-formylpyridine thiosemicarbazone (Method 2).
**"Activated" manganese dioxide.
RESULTS

The single dose LD₅₀ (i.p.) is approximately 2200 mg/kg in 22-gm BDF₁ female mice. 5-HP is poorly absorbed when administered by gavage or in the diet. Results by oral routes or subcutaneously are poor, although some activity was noted. The results with L-1210 leukemia are given in Chart 6. 5-HP was administered daily, i.p., starting 24 hours after tumor inoculation and continued until half of the animals had died. When treatment was limited to 10 consecutive doses, essentially similar results were obtained. With both treatment schedules, occasional 60-day survivors were noted. At a dose of 141 mg/kg, good activity is maintained when the initiation of therapy is delayed 72 hours, and a significant activity is obtained even with a 96-hour delay. Table 1 shows the effect of single doses (1000 mg/kg i.p.) of 5-HP on L-1210. 5-HP is as active on ML-1210 (resistant to methylglyoxal bis-guanylylhydrazine) as it is on sensitive L-1210. An attempt to develop a line of L-1210 resistant to 5-HP has been unsuccessful through the 16th transplant generation. The results with Ehrlich ascites carcinoma are given in Chart 7. A high percentage of 60-day survivors is obtained with this tumor over a dose range of 9-100 mg/kg/day i.p. The majority of these survivors were apparently tumor-free at autopsy. Seven groups of 60-day survivors totaling 46 mice were challenged again with Ehrlich ascites carcinoma. Thirteen of these mice (28%) survived an additional 60 days. Delayed therapy was effective and yielded many 60-day survivors. However, the nonsurvivors died early. In no case were there any 60-day survivors in the control groups. The activity on the L-5178Y lymphoma is shown in Chart 8. 5-HP, at 100 mg/kg/day i.p., is not significantly active on Sarcoma 180. Significant but modest activity is shown against adenoscarcinoma 755 at 141 mg/kg/day i.p. (T/C = 0.17, 0.44, 0.38) but not at higher or lower doses. With the Lewis lung carcinoma a moderate activity is also shown over a narrow dose range. Typical T/C values at 100 mg/kg/day i.p. are 0.16, 0.41, and 0.26.

BDF₁ female mice given 5 daily i.p. injections of 141 mg/kg and autopsied on the 7th day showed moderate to marked variations in size of ventricular red cells. There was considerable hyperplasia of the erythroid system in the bone marrow. Visceral tissues were normal. When treatment was extended to 10 days with autopsy on the 12th day, essentially the same features were noted; however, the variation in red cell size was more moderate. When leukemic mice were treated for 5 days with the same drug dose starting 24 hours after tumor inoculation, the visceral tissues were normal. The ventricular blood and bone marrow had an increased number of poorly differentiated cells and numerous red cell fragments. When treatment of leukemic mice was extended to 10 days, at the same dose of 5-HP, and sacrifice was on Day 12, the picture was essentially similar except that leukemic infiltration was more marked. When untreated leukemic mice were autopsied on the 7th day, leukemic infiltration of tissues was general and massive.

DISCUSSION AND CONCLUSIONS

The theoretical basis for the development of this series of compounds has been discussed in earlier papers (1–4). The most active compounds to date are 5-HP, 3-HP, and IQ-1. There are distinct and significant differences in the quantitative aspects of the antitumor profile. The order of potency against L-1210 leukemia is 5-HP > 3-HP > IQ-1, and this order is reversed with the Lewis lung carcinoma. With adenoscarcinoma 755 the order of activity is 3-HP > IQ-1 > 5-HP, and in the L-5178Y lymphoma the order is 3-HP > 5-HP > IQ-1. None are active on Sarcoma 180. The potency against Ehrlich ascites carcinoma is high in all cases, but an exact comparison against a single subline has not yet been completed.

A preliminary report has been presented on the hematologic effects of 3-HP (5). 5-HP behaves similarly, but other members of this series are devoid of this peculiarity. These hematologic effects are reversible when drug treatment is stopped. A preliminary report of the antileukemic activity of 5-HP was presented earlier (4).
Chart 7. Results of treatment with 5-hydroxy-2-formylpyridine thiosemicarbazone on groups of 20 Swiss mice inoculated on Day 0 with Ehrlich ascites carcinoma (2 × 10^6 cells i.p.). Control groups totaled 60 mice. In the experiments indicated by solid lines, the mice received 11 daily treatments starting 24 hours after tumor inoculation. The dotted line indicates treatment started 72 hours after tumor inoculation and continued for 9 days. mkd, mg/kg/day.

Chart 8. Results of treatment with 5-hydroxy-2-formylpyridine thiosemicarbazone on groups of 20 mice inoculated on Day 0 with L5178Y lymphoma (1 × 10^6 cells i.p.). Eleven daily treatments were given starting 24 hours after tumor inoculation. Control groups totaled 40 mice.

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