# Clinical Evaluation of Thioguanosine*

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SUMMARY

2-Amino-6-mercapto-9β-D-ribofuranosylpurine (thioguanosine) has been used orally and intravenously in the treatment of 82 patients with leukemias and disseminated tumors.

The oral dose of thioguanosine required to produce toxic or therapeutic responses was less than half the oral dose of thioguanine necessary to produce comparable results calculated on a molar basis. However, the two compounds were equally active when given by the intravenous route, indicating that the difference in oral dosage of the two compounds was due to a difference in absorption.

Remissions have occurred in diseases which might have been expected to respond to the purine antagonists (acute leukemia in children and adults and chronic granulocytic leukemia) and only in cases which had not already become resistant to 6-mercaptopurine. Thioguanosine therefore appeared to have no qualitative or quantitative advantages over the previously available purine analogs.

The purine analogs, 6-mercaptopurine, 6-thioguanine, and 6-chloropurine, have been found to produce temporary remissions in acute leukemia in adults and children and excellent but temporary control of chronic granulocytic leukemia (3–5, 10). The effective oral doses of 6-mercaptopurine (6-MP) and thioguanine are similar—i.e., approximately 2.5 mg/kg daily of 6-MP or 2.0 mg/kg of thioguanine (4). 6-Chloropurine has been found effective in a daily dose of 15–20 mg/kg, but its spectrum of activity is identical with that of the other two compounds, and the relation of the dose producing remission to the dose affecting normal hematopoiesis is also the same. The effectiveness of these purine bases in the treatment of human leukemia has suggested that the nucleosides or nucleotides of these purine analogs would be worthy of clinical trial in order to determine whether they exerted a more selective effect against neoplastic cells than against normal cells or if they might be useful in patients whose disease has become resistant to 6-mercaptopurine or thioguanine.

Fox *et al.* (7) synthesized thioguanosine (2-amino-6-mercapto-9β-D-ribofuranosylpurine) as one of a group of such nucleosides.

In screening against Sarcoma 180 and Adenocarcinoma 755 Clarke *et al.*[1] found thioguanosine (TGR) to have tumor-inhibitory activity comparable on a molar basis to that of thioguanine (TG) when given intraperitoneally. When given by the oral route, however, TGR appeared more active.

Philips *et al.* (12),[2] in preclinical pharmacologic studies, found the $LD_{50}$ of TGR and TG to be similar (on a molecular weight basis) in mice and rats when given intraperitoneally. In both species the dose of either compound was less than that of 6-MP. In dogs, which appeared to be more sensitive than rodents, TGR and TG were equally active on a molecular weight basis when given orally or intravenously, and in that species, too, TG and TGR were more toxic than 6-MP. The manifestations of toxicity of TGR and TG in dogs and rodents were confined to the bone marrow. This is in contrast to the toxicity produced by 6-MP, in

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[1] Dr. D. A. Clarke, personal communication.

[2] Dr. F. S. Philips, personal communication.



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which damage to intestinal epithelium and liver occurred (11, 12).[2]

## MATERIALS AND METHODS

Clinical trials with TGR were conducted in 82 patients with far advanced, nonresectable cancer (Table 1). Of these, 50 were considered to have received adequate therapy as manifested by depression of leukocyte count. In the remainder,

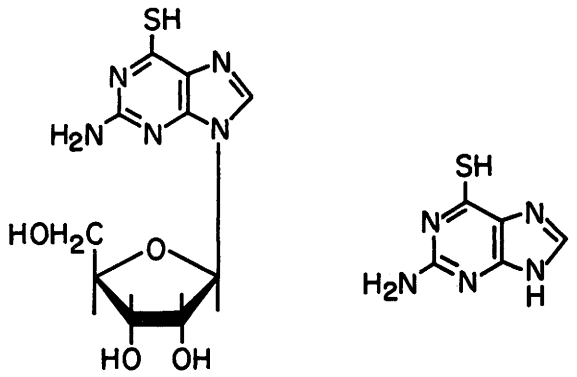


CHART 1.—Structural formulae and molecular weights of thioguanosine and thioguanine.

insufficient TGR was given to produce leukopenia, either because of the death of the patient or because the condition of the patient required a change in therapy.

Either TGR or TG was given orally, once daily, to most of the patients studied, TGR in capsules and TG in the form of tablets.

Each compound was given intravenously to five patients. Although the solubility of TGR is somewhat greater than TG, both are poorly soluble. The calculated dose was, therefore, measured each day into a 500-ml. bottle of 5 per cent glucose in water. With intermittent shaking, the bottle was warmed slightly in warm running water, and 1 N NaOH was added dropwise until the TGR or TG was completely dissolved. The solution was then brought back to neutrality with acetic acid and promptly administered to the patient by rapid intravenous drip over a period of 45–60 minutes.

Physical examinations and hematologic observations were made daily in hospitalized patients and once or twice weekly in out-patients. Appropriate biochemical determinations were performed twice weekly in the early phases of drug evaluation and subsequently weekly in hospitalized patients. These included blood urea nitrogen, serum uric acid, serum bilirubin, thymol turbidity, serum cholesterol and esters, alkaline phosphatase, prothrombin time, serum proteins, and A/G ratio. In out-patients these biochemical determinations were performed prior to the institution of treatment and subsequently only when specifically indicated.

*Toxicity and side effects.*—No toxicity attributable to TGR was encountered except for evidence of bone marrow depression when the maximum tolerated dose had been given. There were no renal or central nervous system side effects. Nausea and vomiting occurred infrequently with either TGR or TG.

The incidence of liver function abnormalities occurring with TGR administration is similar to that occurring with 6-mercaptopurine, 6-chloropurine, and thioguanine; and, as is the case with those compounds, its role as a possible hepatotoxic agent in man has not been definitely established. Jaundice occurred during or after the administration of TGR in about one-third of the patients with acute leukemia. As has been pointed out in studies of other purine analogs (3–6), the relation of liver damage to drug administration is difficult to establish in this group of patients in whom blood transfusions, parenteral medications, and leukemic involvement of the liver are common. In patients with nonleukemic neoplasms jaundice was much less common, and other abnormalities of liver function correlated well with the presence of liver metastases in the cases in which autopsies were performed.

TABLE 1

PATIENTS TREATED WITH THIOGUANOSINE

| Disease | All patients | Adequately treated | Responses |
|---|---|---|---|
| Carcinoma and sarcoma | 28 | 15 | 0 |
| Chronic granulocytic leukemia | 8 | 6 | 6 |
| Acute leukemia, adults | 21* | 16 | 6† |
| Acute leukemia, children | 25 | 12 | 9 |

* Includes four patients who were in the acute phase of chronic granulocytic leukemia; one partial hematologic remission and one "clinical remission" occurred in these four.

† In addition, three patients had "clinical remissions" which do not satisfy the Cancer Chemotherapy National Service Center criteria for partial or complete hematologic remissions.

Oral ulcerations were occasionally seen in children with acute leukemia treated with TGR. These healed promptly with cessation of therapy.

## RESULTS

The dosage of TGR required to produce leukopenia (WBC, 3,000 or less), when given orally to patients with carcinomas and sarcomas, has been

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compared with the oral dose of TG required to produce similar leukopenia. These are summarized in Table 2. The rate of administration was 1–2.5 mg/kg/day in each group. To compare the doses of these two compounds of different molecular weights, the dosages have been calculated and expressed in mmoles/kg body weight as well as in mg/kg. It is apparent that the average total dose of TGR was less (0.1 mmoles/kg) than the average total dose of TG (0.35 mmoles/kg) required to produce a similar degree of leukopenia. Because of this difference in effective oral dose, a small group of patients was treated intravenously with each compound. As shown in Table 2 the doses of TGR and TG required to produce leukopenia when given intravenously were similar on a molecular weight basis.

TABLE 2

COMPARISON OF ORAL AND INTRAVENOUS THIOGUANOSINE AND THIOGUANINE IN ADULTS WITH CARCINOMAS AND SARCOMAS

| TGR | | TG | |
|---|---|---|---|
| **Oral** | | | |
| Patient no. | Dose (mg/kg) to WBC 3,000 | Patient no. | Dose (mg/kg) to WBC 3,000 |
| 1 | 14 | 7 | 24 |
| 2 | 15 | 5 | 28 |
| 3 | 22 | 9 | 39 |
| 4 | 31 | 10 | 45 |
| 5 | 35 | 11 | 48 |
| 6 | 36 | 12 | 49 |
| 7 | 53 | 7 | 108 |
| 8 | 58 | 13 | 140 |
| Average | 33 (0.1 mmole) | Average | 60 (0.35 mmole)† |
| Median | 33 (0.1 mmole) | Median | 47 (0.28 mmole) |
| **Intravenous** | | | |
| 14 | 10.5 | 17 | 4.5 |
| 15 | 13.0 | 18 | 5.0 |
| 2 | 15.0 | 19 | 5.0 |
| 16 | 22.5 | 20 | 5.5 |
| | | 21 | 7.0 |
| Average* | 15.2 (0.04 mmole) | Average | 5.4 (0.03 mmole) |
| Median | 14.0 (0.04 mmole) | Median | 5.0 (0.03 mmole) |

* One additional patient was treated with TGR intravenously but was excluded from this tabulation because the rate of administration was very slow and the hematologic effects could not be related to the total dose.

† The average and median doses for TG are high, partially because of the very large amounts tolerated by patients No. 7 and 13. If the data are recalculated, excluding those two patients, the average dose of TG is 39 mg/kg (0.23 mmoles/kg) and the median dose is 42 mg/kg (0.25 mmoles/kg), somewhat less than before but still significantly higher, on a molecular weight basis, than the oral dose of TGR.

No clinical improvement or regression of tumor was seen in patients with carcinomas and sarcomas.

Beneficial therapeutic results were seen in chronic granulocytic leukemia and in acute leukemia. In chronic granulocytic leukemia, adequate trials were carried out in six patients. Each of these demonstrated improvement as manifested by a fall to normal in the leukocyte count, a reduction in spleen size, and a rise in hemoglobin in those cases in which it has been depressed. Here, too, the oral dose of TGR required to produce a given fall in leukocyte count was less than the oral dose of TG in a similar group of patients. Table 3 illustrates the dosage data in a small number of patients with chronic granulocytic leukemia treated with each compound.

TABLE 3

COMPARISON OF ORAL THIOGUANOSINE AND THIOGUANINE IN CHRONIC GRANULOCYTIC LEUKEMIA

| THIOGUANOSINE | | THIOGUANINE | |
|---|---|---|---|
| Patient no.* | Dose (mg/kg) to WBC <20,000 | Patient no. | Dose (mg/kg) to WBC <20,000 |
| 22 | 18 | 28 | 27 |
| 23 | 28 | 29 | 34 |
| 24 | 30 | 30 | 35† |
| 25 | 34 | 23 | 38 |
| 26 | 37 | 28 | 58 |
| 27 | 77 | 28 | 166 |
| | | 31 | 110 |
| | | 31 | 118 |
| Average | 37 (0.12 mmole) | Average | 73 (0.43 mmole) |
| Median | 32 (0.10 mmole) | Median | 48 (0.28 mmole) |

* Two additional patients were given small doses of TGR which was discontinued before any hematologic effects were seen.

† Fall in WBC without clinical improvement. This patient was previously resistant to 6-mercaptopurine.

Nineteen adults with acute leukemia were treated with TGR. Of these, three were also given prednisone before sufficient TGR had been given to evaluate. A fourth showed no evidence of remission after several months of treatment with TGR and then developed a hematologic remission a few days after prednisone was added to her treatment. This case is considered adequately treated but a failure. There were, therefore, sixteen adult acute leukemic patients who were considered to have received adequate therapy. Six complete or partial hematologic remissions occurred

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in the sixteen adequately treated cases. These are summarized in Table 4. The gradings of the remissions are those established by the Cancer Chemotherapy National Service Center for the Acute Leukemia Cooperative Study Groups (2), although these cases were not included in those studied. Comparison with three cases in whom remissions were obtained with TG indicates that

TABLE 4

COMPARISON OF ORAL THIOGUANOSINE AND THIOGUANINE IN ACUTE LEUKEMIA IN ADULTS*

| THIOGUANOSINE | | THIOGUANINE | |
|---|---|---|---|
| Patient | Dose (mg/kg) (to remission) | Patient | Dose (mg/kg) (to remission) |
| 32 | 11 | 38 | 17 |
| 33 | 20 | 39 | 20 |
| 34 | 30 | 40 | 38 |
| 35 | 35 | | |
| 36 | 56 | | |
| 37† | 12 | | |
| Average | 22 (0.07 mmole) | Average | 25 (0.15 mmole) |
| Median | 25 (0.08 mmole) | Median | 20 (0.12 mmole) |

* Includes only patients in whom partial or complete hematologic remissions occurred.

† Acute phase of chronic granulocytic leukemia.

the total dose producing remission was similar. The percentage of hematologic remissions seen in these patients is similar to that which might be expected with 6-mercaptopurine. In Table 5 are shown the data on the nine children with acute leukemia in whom remissions were obtained with TGR. It is of interest to note that 6-mercaptopurine had not previously been used in seven of the patients who developed remissions and had been used inadequately in the other two. No remissions were seen in the few mercaptopurine-resistant cases which were treated with TGR.

## DISCUSSION

The effectiveness of 6-mercaptopurine in producing temporary remissions in acute leukemia and chronic granulocytic leukemia has led to the experimental and clinical trial of other purine analogs, among which are 6-thioguanine, 6-chloropurine, and an imidazolyl derivative of thioguanine. Except for differences in effective dosage, none of these compounds has shown any greater degree or spectrum of activity than the prototype compound, 6-mercaptopurine.

Because of the evidence in animal systems that purine polynucleotide biosynthesis is accomplished principally or totally with nucleotides of purines and purine precursors rather than with the free bases (8), it has been suggested that nucleosides or -tides of purine analogs might be more active as chemotherapeutic agents than their free bases. Thioguanosine, the first of these nucleosides to become available for clinical trial, appears to have a spectrum of antitumor activity similar to that of the previously investigated purine analogs—i.e., its useful activity is confined to chronic granulocytic and acute leukemias.

The "greater activity" of TGR as compared with TG based on the effective oral dosage appears to be related to better absorption from the gastrointestinal tract. This is probably due to the fact that, although both TGR and TG are sparingly soluble, TGR is approximately twice as soluble in aqueous solution as is TG, and therefore its better absorption might have been predicted. The similarity of the intravenous dosage of the two compounds appears to confirm that the difference in oral dosage was due to a difference in absorption. These studies indicate that in man TG and TGR are equally active or are equally well converted to an active form.

The clinical trials do not suggest that TGR has any practical superiority over other purine analogs currently available, and this, too, might have been predicted from the preclinical data. Smallness of the effective dose of a drug cannot be considered an advantage unless accompanied by evidence of dissociation of effects on normal and neoplastic cells. In clinical trials of the purine analogs (as well as other types of chemotherapeutic agents) which have become available, no such dissociation has been seen, and a reduction in the size of the

TABLE 5

RESPONSES TO ORAL THIOGUANOSINE IN ACUTE LEUKEMIA IN CHILDREN

| Patient no. | Oral dose (mg/kg) | Remission | Previous 6-MP |
|---|---|---|---|
| 41 | 22 | Complete | 0 |
| 42 | 38 | Partial | 0 |
| 43 | 61 | Partial | Inadequate |
| 44 | 110 | Complete | 0 |
| 45 | 152 | Complete | 0 |
| 46 | 25 | Partial | 0 |
| 47 | 17 | Partial | Inadequate |
| 48 | 32 | Partial | 0 |
| 49 | 16 | Partial | 0 |
| | Average | 52 (0.17 mmole) | |
| | Median | 32 (0.10 mmole) | |

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dose necessary to affect leukemic cells has in each instance been accompanied by a parallel reduction in the size of the dose necessary to affect normal hematopoietic cells. The initiation of clinical trials of future members of this series of compounds might better be based on evidence of a selective activity on neoplastic vs. normal cells than on enhanced drug toxicity at a smaller dose.

An additional factor that would lend true superiority to one member of a class of compounds over other members would be the demonstration that a compound can produce remissions in patients (or animals) with leukemia which has developed resistance to one or more related compounds. This has not been shown in animals in the case of TGR, and the present data suggest that there is cross-resistance in man between TGR and 6-MP. Leukemic patients in this series who developed a remission were those whose disease has not become resistant to 6-MP. Each patient who had previously developed resistance to 6-MP failed to respond to TGR. Since an important mechanism of development of resistance to 6-MP or TG by the leukemic cell appears to be the failure of the resistant cell to convert the purine base to its ribonucleotide (the active form) (1) and, since preformed ribonucleotides appear incapable of penetrating cell membranes, it is difficult to foresee the circumvention of purine analog resistance by the development of new purine analogs.

A final theoretical advantage of a new member of an old class of compounds is the elimination of undesirable side effects (as distinguished from toxicity directly related to the desired biologic activity). In the case of the alkylating agents, new compounds have provided real clinical advantages over older ones in terms of ease and route of administration, although the presence of true differences in biologic activity is questionable. In the case of the purine analogs which have been clinically useful, side effects have been minimal, and new additions to this group have added little to their clinical usefulness or ease of administration.

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