

Clinical Pharmacological Trial of Guanazole¹

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SUMMARY

Guanazole was given to 27 patients with advanced neoplastic diseases, mostly acute leukemia, by continuous i.v. infusion in 5-day courses. Toxicity was manifest mainly by myelosuppression, but drug fever, mucositis, rash, and alkalosis were also observed.

Complete remission was achieved in two out of 14 patients with previously treated acute myelocytic leukemia, and an M1 marrow occurred in one out of four patients with advanced acute lymphocytic leukemia. Most of the effects of guanazole appear to be dose related, with a narrow therapeutic index. This is the first evidence of therapeutic activity of guanazole in man.

INTRODUCTION

Guanazole (3,5-diamino-1,2,4-triazole, NSC 1895) (Chart 1) was chosen for Phase I study in man because of its activity against L1210 leukemia in mice (1, 4). It has also shown growth-inhibitory activity against H. Ep. No. 2 cells in culture (1) and against leukemia K1964 and the P815 mast cell tumor (3).

The mechanism of action of guanazole involves inhibition of the enzyme ribonucleotide reductase, which leads to inhibition of DNA synthesis. Like other S-phase-specific drugs, its maximal effectiveness against L1210 leukemia is greatly dependent on the dose schedule (5). The optimal effect in mice was achieved with multiple doses given over a 24-hr period every 4 days (1). Based on these data, we chose a schedule of repeated 5-day courses of continuous i.v. infusions of the drug.

Beagles tolerated doses of 150 g/sq m when given in 24-hr continuous i.v. infusions 1 day a week for 8 weeks, while Rhesus monkeys tolerated doses of 6 g/sq m/day i.v. for 28 days (4). The LD₅₀² by single dose i.v. route in mice was 8.9 to 10.5 g/sq m, and the LD₁₀ was 8.4 to 9.5 g/sq m, while in rats the LD₅₀ was 4.1 to 4.7 g/sq m and the LD₁₀ was 3.8 to 4.4 g/sq m. Thus the dose response curve was shown to be steep. Optimal tumor inhibitory effects in mice by fractionated i.p. doses were seen at 12 g/sq m/day, suggesting that the therapeutic index was low. The main toxic effects of guanazole in animals were on the liver and the marrow.

Studies with guanazole-¹⁴C (3) in mice, rats, and dogs showed that after i.p. and s.c. administration more than 30% appeared in the urine within 3 to 4 hr as the parent compound.

MATERIALS AND METHODS

Guanazole was given to 22 adults from 18 to 69 years of age and 5 children from 9 to 14. There were 14 males. All patients had advanced neoplastic disease, and the disease had relapsed from initial treatment or no generally accepted forms of specific treatment existed. The first 7 patients treated included 5 with advanced lung cancer, 1 with mesothelioma, and 1 with metastatic osteosarcoma. The drug was later given to 14 patients with acute myelocytic leukemia, 4 patients with acute lymphocytic leukemia, and 2 patients with the blastic transformation of chronic myelocytic leukemia.

The drug was administered in 5% glucose in water, 2000 to 3000 ml/24 hr by continuous infusion at a steady rate, in 5-day courses. Between courses there was an interval of at least 9 days. The number of 5-day courses in 24 patients is shown in Table 1. In the early study period, 3 patients received courses shorter than 5 days.

RESULTS

Toxicity. No significant toxicity except fever was seen at any dose of guanazole below 10 g/sq m/day. Twenty-two patients received daily doses in excess of 10 g/sq m; only 2 had no evidence of toxicity.

Fever was the most common side effect (Chart 2). It occurred in 15 patients with equal frequency at all dose levels. In most cases, the temperature rose to approximately 39°, 24 to 28 hr after starting treatment. It usually continued at this level throughout the course and typically fell promptly when the drug was discontinued. Fever did not necessarily occur in subsequent courses.

In some cases the time from starting the drug infusion to the onset of fever became shorter with each subsequent course. This is illustrated in Chart 3. Later we recognized the high frequency of fever without apparent serious effects and continued drug administration despite it.

Nausea appeared in about half the courses and vomiting in about one-third, usually only on the 1st day of a course. Only rarely was it necessary to add an antiemetic (chlorpromazine) to control vomiting in the 9 patients who were affected. Thrombophlebitis at the site of drug administration, a complication in 8 patients, was more severe at the high doses.

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²The abbreviations used are: LD₅₀, lethal dose for 50%; LD₁₀, lethal dose for 10%.

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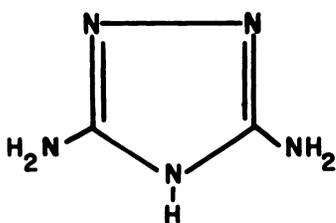


Chart 1. Guanazole (3,5-diamino-1,2,4-triazole, NSC 1895).

Table 1

Number of 5-day courses of guanazole (24 patients)

Dose (g/sq m/day)	No. of courses
5	9
7.5	1
10	6
15	3
20	9
25	16
33	6
40	3
Total	53

Somnolence was seen in 5 patients at high dose levels; it disappeared as soon as the drug was discontinued. A maculopapular skin rash, usually on the extremities, appeared in 8 cases. A biopsy was performed in 3. Perivascular infiltration with mononuclear cells, but no eosinophiles nor fibrinoid necrosis was observed. Reappearance of rash in a subsequent course was seen in some but not all patients. In 1 patient receiving 40 g/sq m/day, severe exfoliative dermatitis limited to the scrotum was observed.

Oropharyngeal ulcerations developed in 6 patients. Hoarseness appeared in 3 patients who received the highest doses of guanazole. Laryngoscopy in 1 revealed laryngeal ulcerations. Moniliasis of the mouth and pharynx was found in 8 cases, accompanied in 3 by retrosternal pain and dysphagia. In 2 of 3 instances, esophageal ulceration due to *Candida albicans* was proved. Ulcerations of the rectal mucosa were seen in 3 cases at doses of 33 to 40 g/sq m/day. Perirectal infections, mainly abscesses, were seen in all 5 children and in 3 adults.

The biochemical toxicity of guanazole was not so striking as the clinical toxicity. Transient enzyme elevations indicative of liver toxicity were seen in 8 patients. In 7 of them, the lactic dehydrogenase was elevated (750 to 2200 units); the alkaline phosphatase in 4 (up to 150 i.u.); the glutamic oxalacetic transaminase in 2 (up to 200 units); and the bilirubin in 2 (less than 2 mg/100 ml). In 3 cases there was an elevation of the blood urea nitrogen to levels less than 40 mg/100 ml, but without concurrent elevation of creatinine. Elevation of the venous pH was seen at doses of 25 g/sq m or more in 13 cases. In 3 patients, the pH was 7.6. There were no clinical manifestations of this alkalosis. The elevated pH can be explained at least in part by the presence of the alkaline guanazole in the plasma, although other contributory mechanisms cannot be excluded. We treated these patients by adding hydrochloric acid, 5 to 15 mEq to each 1000-ml infusion bottle (the pH of the solution then being 5.2 to 5.5),

or by adding ammonium chloride, 75 mg/kg/day by mouth, and the pH returned to a normal range in the succeeding days.

Guanazole is a potent myelosuppressive agent and the effect was dose related (Chart 4). Leukopenia was prominent. The platelet and reticulocyte counts were also depressed, and their effects were accompanied by a lesser degree of marrow hypocellularity on aspiration. Megaloblastosis was seen in most patients at all dose levels, with differential maturation of the nucleus and cytoplasm of nucleated red cells, finer than normal structure of the nuclear chromatin, macrocytosis, bizarre nuclear shapes, and Howell-Jolly bodies. Morphological abnormalities were also common in the developing granulocytic cells. There were giant myelocytes and metamyelocytes, and some of the polymorphonuclear clear leukocytes were large, with irregular abnormal nuclei sometimes appearing hypersegmented.

Chart 5 demonstrates the changes in the peripheral counts of patients with acute leukemia treated by 16 courses of 25 g/sq m/day. The shaded areas represent the range and the dark lines the median, with the count prior to treatment taken as 100%. Leukocytes first decreased on Day 3 and reached a

Guanazole Toxicity By Dose

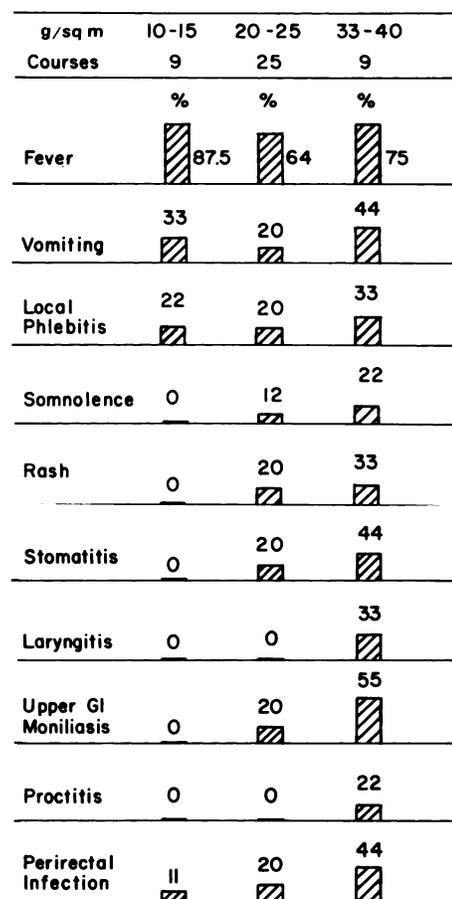


Chart 2. Toxic effects of guanazole administration during 43 courses of drug administration grouped in low, intermediate, and high dose ranges.

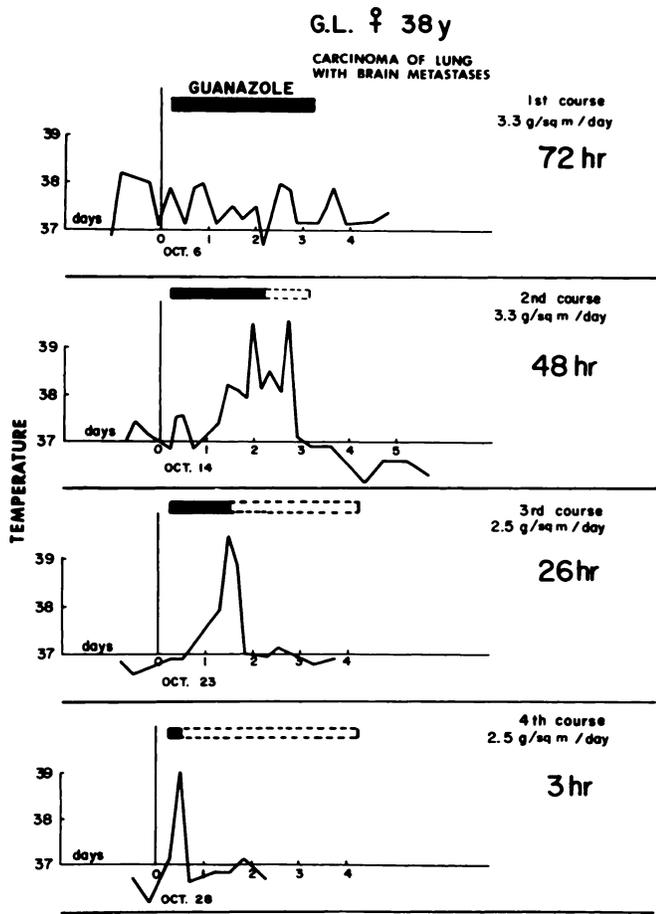


Chart 3. Fever during guanazole administration. Dashed bar, planned course; solid bar, duration of administration.

Hematologic Toxicity by Dose of Guanazole

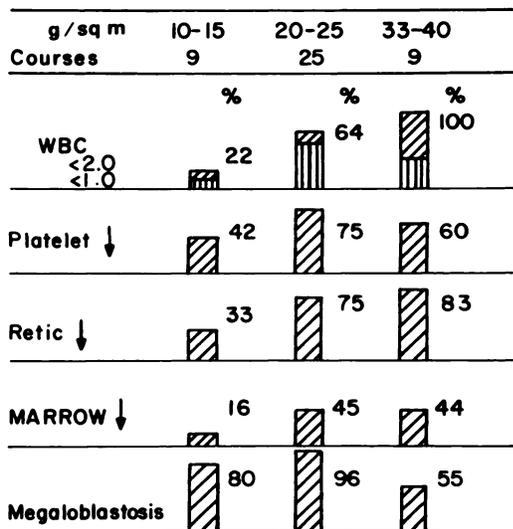


Chart 4. Hematological toxic effects of guanazole administration during 43 courses of drug administration grouped in low, intermediate, and high dose ranges. Leukopenia, thrombocytopenia, reticulocytopenia, and marrow hypocellularity were encountered with striking megaloblastosis. Retic, reticulocyte.

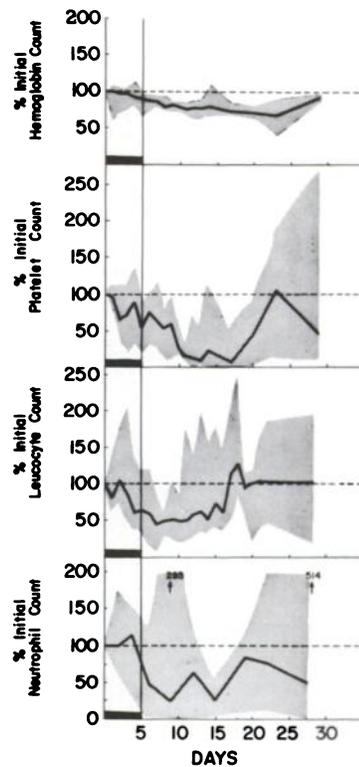


Chart 5. Median hematological values and range as affected by guanazole during 16 courses of 25 g/sq m in patients with acute myelocytic leukemia. Pretreatment value taken as 100%.

nadir on Day 6. Platelet and hemoglobin changes lagged by 2 to 5 days. The recovery period lasted about 10 days.

Therapeutic Response. A complete remission was achieved in 3 patients with acute leukemia, 2 with acute myelocytic leukemia, and 1 with acute lymphocytic leukemia. One patient with acute myelocytic leukemia (Case 1) attained remission after a single course of 25 g/sq m/day X 5. The 2nd patient with acute myelocytic leukemia entered remission on Day 64 following treatment with 40 g/sq m/day X 5 after prior failure to respond to previous courses of 25 and 33 g/sq m/day X 5. The remission was preceded by an "overshoot" of marrow myeloblasts representing a cohort of young normal myeloid cells which spontaneously decreased to normal levels after withholding the drug (2). The remission lasted 11 weeks. The remission in a 9-year-old boy with acute lymphocytic leukemia, a very short one, was achieved with a dose of 25 g/sq m/day X 5 after no beneficial response to 20 g/sq m/day X 5.

Case Report. In Case 1 (Chart 6), acute myelocytic leukemia was diagnosed in this 52-year-old woman 5 months prior to treatment with guanazole. A complete remission had been achieved with cytosine arabinoside and thioguanine, with relapse 6 weeks later while on maintenance treatment with the same drugs. At that time, the marrow was normocellular and contained 37% myeloblasts and 6% promyelocytes. She was started on guanazole, 25 g/sq m/day X 5. On Day 3 of treatment, the bone marrow became hypocellular with a decrease in the percentage of blasts, followed by a progressive fall of the peripheral count. By Day 30, the bone marrow had

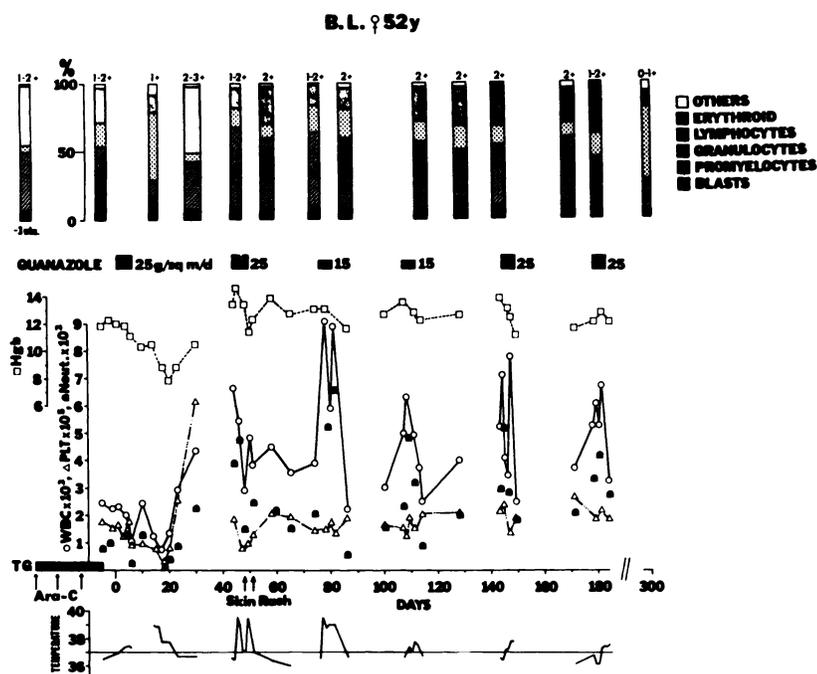


Chart 6. Effect of guanazole in acute myelocytic leukemia in Case 1. See text. *PLT*, platelets; *Neut.*, neutrophils.

recovered with repopulation by normal elements, and with less than 5% myeloblasts plus promyelocytes. The patient had no toxic side effects and was asymptomatic. Peripheral blood values increased to satisfy complete remission status. Maintenance courses of guanazole were begun once monthly. The first course was given at 25 g/sq m/day \times 5. Fever to 39.5° and skin rash inclined us to reduce the dose to 15 g/sq m/day \times 5, but subsequently she tolerated the 25 g/sq m/day dose without side effects. The patient remained in complete remission for 16 months and 14 courses.

DISCUSSION

As an inhibitor of ribonucleotide reductase, guanazole induces biochemical lesions in cells in the S phase of the cell cycle (1). Other S phase-specific drugs which are effective in mice with L1210 leukemia are highly dose schedule-dependent. This has been shown to be true for guanazole also by Brockman *et al.* (1), who found that the best schedule in mice with L1210 leukemia was i.p. administration of the drug every 3 hr for 24 hr every 4 days.

Based on these observations, but with adaptation made for a longer cycle time of human cells, repeated administration in courses of 5 days duration was the schedule chosen in this study.

The most prominent toxic effect was myelosuppression, which was dose related. The other clinical side effects were evanescent, and disappeared shortly after the drug was discontinued. In contrast to the animal experience, hepatotoxicity was not frequent and, when present, was mild and transient. As in the animal, elevation of blood urea nitrogen was not accompanied by concurrent elevation of creatinine. It is possible that guanazole interferes with determination, causing a false elevation of the blood urea nitrogen. Elevation of the blood pH was common, particularly in the high dosage levels, due at least in part to the basicity of guanazole. This

complication was handled easily either by adding hydrochloric acid to the solution or by administering ammonium chloride by mouth.

The beneficial responses to guanazole also appear to be dose related. The 2 remissions of acute myelocytic leukemia were achieved at 25 and at 40 g/sq m/day \times 5 (after failure of the latter patient to respond to lower doses). In acute lymphocytic leukemia the single M1 marrow was obtained at a dose of 25 g/sq m/day \times 5 after no response to 20 g/sq m/day \times 5.

Guanazole constitutes a new drug with demonstrated activity in advanced acute myelocytic leukemia discovered during the Phase I clinical pharmacology exploration. Further studies of its use in human neoplastic disease alone or in combination may disclose a wider spectrum of antitumor effectiveness.

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