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# Phase I Trial of Echinomycin (NSC 526417), a Bifunctional Intercalating Agent, Administered by 24-hour Continuous Infusion

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**Abstract**—*Echinomycin was administered to 43 patients with advanced cancer in escalating doses ranging from 60 to 2128 mcg/m². The dose-limiting toxicity of echinomycin administered as a 24-h continuous infusion every 28 days was nausea and vomiting beginning at the end of the 24 h infusion and lasting from 3 to 8 days. Other toxicities included sporadic thrombocytopenia and biochemical evidence of liver dysfunction characterized by elevations in SGOT. Peripheral vein phlebitis was noted in 100% of patients, and watery diarrhea of 24–48-h duration was noted in 7% of patients. The maximally tolerated dose of echinomycin was 2128 mcg/m². The recommended dose for phase II trials utilizing the 24-h continuous infusion schedule is 1600 mcg/m² repeated every 28 days with pretreatment antiemetics.*

## INTRODUCTION

ECHINOMYCIN (Quinomycin A, NSC 526417) is a member of the quinoxaline antibiotic family originally isolated from *Streptomyces echinatus* in 1957 [1]. The compound possesses both gram-positive antibacterial [2] and antiviral properties [3, 4]. Its antitumor activity in animal tumor systems and its unique ability to interact with double-stranded DNA has led to the clinical development of echinomycin as an anticancer agent.

Structurally, echinomycin consists of a cyclic octapeptide dilactone with two quinoxaline chromophores connected by a thioacetal bridge [5, 6] (Fig. 1). The biologic activity of echinomycin has been related to its ability to bind intramolecularly with double-stranded DNA by mechanisms which include bifunctional intercalation [7, 8].

Echinomycin's antitumor activity was first observed against rat and hamster tumors in the early sixties [9, 10]. In the National Cancer Institute's biologic screening models, echinomycin demonstrated good antitumor activity against two i.p. implanted murine tumors, the B16 melanoma and P388 leukemia [11]. Activity against the implanted B16 melanocarcinoma was maintained following either i.p. or i.v. administration although the i.p. route was somewhat more effective than i.v. at equal doses. The compound following i.p. administration was active on the single (60% ILS) and daily × 5 (73% ILS) schedules and somewhat more effective following daily administration on days 1–9 (91% ILS). The drug was not active against the three human xenografts (mammary, colon or lung) tested in athymic mice. In the 6-day subrenal capsule assay, ovarian carcinoma showed the greatest sensitivity to echinomycin [12]. When tested in the human tumor cloning system, echinomycin showed minor *in vitro* cytotoxic activity in breast, colon and sarcomas, yet detectable activity in ovarian cancer [13]. When echinomycin and adriamycin were tested simultaneously in the cloning assay, cross-resistance between both agents was expressed in 78% of the specimens [13].

Preclinical toxicity studies were conducted for echinomycin by the i.v. route on a single dose and daily times 5 schedule in both $CDF_1$ mice and beagle

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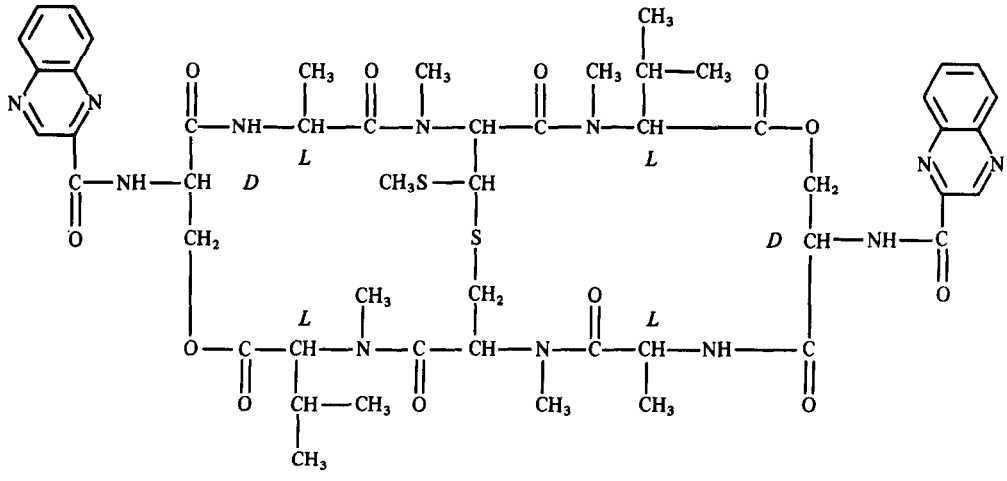

*Fig. 1. Structural features of echinomycin* [14].

dogs. In the mouse studies, the range between the $LD_{10}$ (1788 mcg/m$^2$) and the $LD_{90}$ (2171 mcg/m$^2$) was narrow suggesting a steep dose–toxicity curve. The dog was the most sensitive species on the single dose schedule. In the dog, the major clinical signs and symptoms were emesis, diarrhea, gastrointestinal bleeding, hyperthermia and excessive scratching. Hematologic alterations included elevations in red blood cell count, hemoglobin (Hgb) and/or hematocrit (Hct), neutrophilia, lymphocytopenia, reticulocytopenia and increased prothrombin times. Laboratory abnormalities included reversible elevations in liver function tests (SGOT, SGPT, alkaline phosphatase, bilirubin) and decreased blood glucose. Increases in BUN and creatinine were noted but were related to dehydration rather than to a direct nephrotoxic effect. Decreased calcium, sodium, potassium and/or chloride occurred in all dogs in the single dose study. Histologic changes on necropsy included hypocellularity of the bone marrow, moderate centrilobular necrosis of the liver, focal hemorrhagic sites throughout the gastrointestinal system and focal acinar cell degeneration in the pancreas [11].

The purpose of this study was to determine the dose-limiting toxicity and the maximum tolerated dose of echinomycin given as a 24-h i.v. infusion. This schedule was chosen because of the slightly greater antitumor activity of echinomycin when given on a more prolonged schedule and because the toxicity of intercalating agents has been lessened when given on an infusion schedule. Due to the fact that the mouse equivalent lethal dose in 10% ($MELD_{10}$) was significantly toxic in dogs, one-tenth the $LD_{10}$ in mice was not used to determine the phase I starting dose.

## MATERIALS AND METHODS

*Patient selection*

Adults with histologically documented advanced solid tumors refractory to all forms of known effective therapy were candidates for the study. The interval between the subjects prior treatment and institution of echinomycin was at least 4 weeks (6 weeks if prior nitrosourea or mitomycin C) and the patient had recovered from all adverse effects of prior therapy. Other eligibility criteria included a performance status (Southwest Oncology Group) of 3 or less, estimated life expectancy of 12 weeks and adequate organ function as defined by: bilirubin $\leq$2.0 mg%; alkaline phosphate and SGOT $\leq 1.5 \times$ normal; normal prothrombin time; BUN $\leq$20 mg%; creatinine $\leq$2.0 mg%; creatinine clearance $\geq$40 ml/min; normal glucose, electrolytes, calcium, phosphorus, and WBC $\geq$4000/mm$^3$ (granulocytes $\geq$2000/mm$^3$); platelets $\geq$100,000/mm$^3$; Hgb $\geq$10 mg%; Hct $\geq$30%.

Prior to entry, written informed consent was obtained from the patient in accordance with institutional and federal policies.

*Treatment plan*

Echinomycin was supplied by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD, in a sterile 3.5 ml vial containing 0.4 mg of echinomycin as a white vacuum dried film. The drug was dissolved in 0.2 ml of sterile Diluent 12 composed of equal parts of polyoxyethylated castor oil (Cremophor EL) and ethanol. Once dissolution occurred, 1.8 ml of sterile water or 0.9% sodium chloride for injection, USP, was added resulting in a concentration of 0.2 mg/ml of echinomycin with a pH between 4 to 7. At this concentration or after further dilution with 5% dextrose in 0.9% sodium chloride, the solution is stable for at least 8 h at room temperature and ambient light [14].

The treatment schedule consisted of a single dose 24-h continuous infusion repeated every 28 days. To assure maximum potency and sterility, one-third of the total 24-h dose was diluted in 250–500 ml of 5% dextrose and 0.45% sodium chloride and infused over 8-h for three doses. All infusions were

administered through a free flowing i.v. with an IVAC continuous infusion apparatus. During and for 24-h after drug administration, the patients vital signs, ECG and general sense of well being were monitored every 15 min × 4, every hour ×2, then every 6-h unless otherwise warranted.

Because gastrointestinal toxicity occurred in dogs at 1/10 $MELD_{10}$ (178 mcg/m$^2$), 1/30 the $MELD_{10}$ (1788 mcg/m$^2$) in the dog was used to determine the starting dose of 60 mcg/m$^2$. Dose escalations followed the modified Fibonacchi search scheme [15]. However, because the $LD_{10}$, $LD_{50}$ and $LD_{90}$ in animals spanned a very narrow dose range (1728–2171 mcg/m$^2$ in mice) the escalation scheme was modified further to provide additional safety (Table 1). At least three evaluable patients were entered at each dose level. The first patient at each level was observed for 4 weeks. If toxicity was acceptable, two additional patients were entered. For patients having stable disease and no toxicity at the initial dose level, additional doses were allowed at the highest dose level which had been safely completed in at least three patients not previously treated.

*Study parameters and evaluation*

Prior to the initiation of treatment and subsequent courses, all patients had a complete history and physical examination, hemogram, SMA-12, serum electrolytes, prothrombin time, partial thromboplastin time, amylase, urinalysis, chest roentgenogram, ECG and a 12-h urine collection for creatinine. Measurable lesions were documented on physical examination and with appropriate radiologic studies or scans.

Between treatment courses, patients were followed closely for evidence of toxicity and for biological effects. Physical examinations, hemograms and full chemistry profiles were evaluated at weekly intervals. Standard tumor response criteria were used to measure for antitumor effects. These criteria included: (a) complete remission—disappearance of all clinical evidence of active tumor and tumor-related symptoms for at least 4 weeks; (b) partial response—≥50% decrease in the sum of product of the perpendicular diameters of all measured lesions for at least 4 weeks; (c) stable disease—objective tumor regression not qualifying for partial remission but lasting for at least 4 weeks or a steady state not qualifying as increasing disease; and (d) progressive disease—25% or greater increase in size of any measured lesion or the appearance of any new lesion.

Patients were removed from the study if objective tumor progression occurred following one or more courses of echinomycin or if unacceptable toxicity occurred after drug administration.

*Table* 1. *Dose escalations of echinomycin*

| Dose | mcg/m$^2$ | No. of patients/ No. of courses |
|---|---|---|
| *n* | 60 | 3/5 |
| 2*n* | 120 | 5/8 |
| 3*n* | 180 | 4/11 |
| 5*n* | 300 | 7/9* |
| 7*n* | 420 | 5/6 |
| 9*n* | 540 | 3/6 |
| 11*n* | 660 | 4/4 |
| 13*n* | 780 | 3/7 |
| 15*n* | 900 | 2/2 |
| 20*n* | 1200 | 3/3 |
| 27*n* | 1600 | 8/11 |
| | —1788 mcg/m$^2$ $LD_{10}$ mice | |
| | 1900 | 1/1 |
| 35*n* | 2128 | 3/4 |
| | —2171 mcg/m$^2$ $LD_{90}$ mice | |

*Two patients actually received 240 mg/m$^2$ due to a pharmaceutical admixture error.

## RESULTS

The characteristics of the 43 patients entered on this trial are shown in Table 2. There were 36 males and seven females with a median age of 62 years (range, 32–79 years). Their median performance status was 1 (range 0–3). All but one patient had received prior radiotherapy and/or chemotherapy. Tumor types were predominantly colorectal and lung cancers. With the exception of the 900 and 1900 mcg/m$^2$ dose level, a minimum of three previously untreated patients were treated at each level (Table 1). In parallel studies, other phase I investigators evaluating echinomycin had escalated dosages beyond the 900 mcg/m$^2$ level without inducing toxicity; consequently the number of patients treated at this dosage level was limited to two. The 1900 mcg/m$^2$ level represented a de-escalation

*Table* 2. *Patient characteristics*

| | |
|---|---|
| Number of patients | 43 |
| Men/Women | 36/7 |
| Median age in years (range) | 62 (32–79) |
| Median performance status (range) | 1 (0–3) |
| Number of patients with prior treatment | |
| Chemotherapy alone | 13 |
| Radiation therapy + chemotherapy | 27 |
| Radiation therapy alone | 2 |
| Number of patients without prior therapy | 1 |
| Tumor types | |
| Colorectal | 13 |
| Lung (non small cell) | 10 |
| Unknown primary | 3 |
| Renal | 3 |
| Head and neck | 3 |
| Stomach | 8 |
| Others | |

from the maximal tolerated dose to a potentially more optimum dose for the last patient entered on the study. Seventy-seven courses of the drug were administered. A total of three patients, one each at the dosage levels of 420, 660, 1600 mcg/m$^2$ were not evaluable for toxicity because of early death due to rapid disease progression.

*Toxicities*

Gastrointestinal toxicity in the form of nausea and vomiting was the dose-limiting toxicity on this schedule. The onset of nausea and vomiting occurred during the latter portion of the 24-h infusion at all dosage levels. Mild-to-moderate nausea of 48-h duration occurred at all dosage levels of 420–1600 mcg/m$^2$ and was attentuated by phenothiazine therapy. Table 3 summarizes the incidence of nausea and vomiting at doses equal to or greater than 1600 mcg/m$^2$. The incidence of nausea and vomiting at 1600 mcg/m$^2$ was 82% (91% mild to moderate, 9% severe). One patient at this level had no nausea or vomiting; however, the patient was receiving dexamethasone 4 mg every 6-h for brain metastases. Two of the 11 courses at 1600 mcg/m$^2$ were accompanied by nausea and vomiting of up to 4 days duration after echinomycin administration. Moderate to severe nausea and vomiting were associated with all courses at the 1900 and 2128 mcg/m$^2$ doses. The duration was prolonged (range 3–8 days) requiring hospitalization for intractable vomiting in two of four patients. A dose reduction from 2128 to 1600 mcg/m$^2$ was necessary in one patient due to severe nausea and vomiting; with the second course, the patient was pretreated with metoclopramide (2 mg/kg) and experienced moderate nausea and vomiting. One additional patient treated at the 1600 mcg/m$^2$ and who was premedicated with prochloperazine and thiethylperazine experienced only mild vomiting.

Hematologic changes consisted of sporadic platelet suppression and thrombocytopenia without an evident dose–response relationship. Four courses (5%) were associated with thrombocytopenia (platelets <100,000/mcl) and were considered to be directly related to echinomycin toxicity (Table 4). A decline of platelets greater than 50,000/mcl occurred in 35 of 77 courses (45%). For those patients who received more than one course of echinomycin, there was no suggestion of cumulative marrow suppressive effect. With one possible exception, echinomycin had no effect on the white blood cell series. One patient treated at the 300 mcg/m$^2$ level became severely pancytopenic. The time to onset for both granulocytopenia [absolute neutrophil count (ANC) <1000] and thrombocytopenia (<100,000/mcl) was day 43 after echinomycin administration. The time to nadir for Hgb/Hct (8.2/24.3), WBC (ANC 144) and platelets (16,000/mcl) was day 43, 47 and day 48, respectively. The aspirate and bone marrow biopsy demonstrated a severely hypocellular marrow. The time to recovery for the patient's granulocytes (ANC >1000) and platelets (>100K) was day 52. Etiology was considered to be either due to echinomycin or secondary to the concomitant administration of cimetidine.

Biochemical evidence of drug induced liver dysfunction, characterized most commonly by elevations in SGOT or alkaline phosphatase occurred in 13 of 73 evaluable courses (Table 5). Elevations of SGOT ranged from 1.5 to 6.1 times the baseline values with a median time to peak elevation on day 8 (range 4–19 day) with return to baseline prior

*Table 3. Echinomycin induced gastrointestinal toxicity*

| Dosage level ≥1600 mcg/ml (No. patients/No. courses) | Severity* of vomiting | Antiemetic† regimen |
|---|---|---|
| 1600 mcg/m$^2$ (8/11) | 0 | Dexamethasone |
| | 1 | — |
| | 1,1 | Thiethylperazine‡/prochloperazine |
| | 1,1 | Metoclopramide (2 mg/kg) |
| | 2 | Thiethylperazine/prochloperazine |
| | 2,2 | Thiethylperazine/prochloperazine |
| | 2 | Metoclopramide (2 mg/kg) |
| | 3 | Metoclopramide (2 mg/kg) |
| 1900 mcg/m$^2$ (1/1) | 3 | Thiethylperazine |
| 2128 mcg/m$^2$ (3/4) | 1,2 | Thiethylperazine/prochloperazine |
| | 2 | thiethylperazine/prochloperazine |
| | 3 | Metoclopramide (10 mg PO) |

*0 = none, 1 = mild (1–2 episodes of vomiting), 2 = moderate (3–4 episodes), 3 = severe (>4 episodes).

†Antiemetics administered on a PRN basis except for (‡)—administered pre-echinomycin.

*Table 4. Platelet suppressive effects of echinomycin*

| Dosage level (mcg/m²) | No. of courses | Magnitude of platelet change* Mean decrease | (range) |
|---|---|---|---|
| 60 | 5 | 20K† | (0–39) |
| 120 | 8 | 23 | (0–96) |
| 180 | 11 | 36 | (0–141) |
| 300 | 9 | 112 | (0–310) |
| 420 | 5 | 55 | (0–98) |
| 540 | 6 | 45 | (0–98) |
| 660 | 3 | 81 | (52–138) |
| 780 | 7 | 60 | (0–128) |
| 900 | 2 | 108 | (29–187) |
| 1200 | 3 | 30‡ | (0–63) |
| 1600 | 10 | 103‡‡ | (0–263) |
| 1900 | 1 | 206 | — |
| 2128 | 4 | 240‡§ | (94–350) |

*Baseline platelet count minus nadir.
†Thousand (K).
‡No. of courses associated with thrombocytopenia (platelets <100K); (§patient with DIC).

to the next course. Patients remained asymptomatic without evidence of a cumulative effect with repetitive doses.

Other toxicities included fever as high as 102°F which occurred post-infusion in four patients treated at the 1600 mcg/m² (2 patients) and 2128 mcg/m² (2 patients) level. Phlebitis at the infusion site or extending above the infusion site occurred universally at all dosage levels. The extent of induration was more prevalent at the higher dosage levels and lasted approximately 1–2 weeks. Extravasation of echinomycin occurred at the 540 and 1600 mcg/m² levels on three separate occasions without evidence of tissue necrosis. Watery diarrhea lasting 24–48-h was noted in three of the patients. There were no anaphylactoid reactions to echinomycin administration. There was one drug related death at the 2128 mcg/m² dosage level. A 67-year-old male with Duke's D colon cancer metastatic to the liver received 3873 mcg of echinomycin over 24-h. During and after infusion the patient experienced nausea and vomiting which persisted for 3 days. Eighteen hours post infusion, his temperature increased to 102.5°F; cultures were negative. The third day post echinomycin, he developed watery diarrhea and subsequently became hyponatremic, hypochloremic and hyperglycemic. His platelet count dropped from a baseline of 364,000/mcl to 160,000/mcl. By day 5, the platelets had dropped to 39,000/mcl with a positive disseminated intravascular coagulation (DIC) screen. His liver function tests (SGOT, SGPT and LDH) began to rise with evidence of acute renal failure (creatinine >3 mg/dl, BUN >90 mg/dl). Over the period of the next 7 days, the patient received numerous transfusions with platelets, fresh frozen plasma and packed red blood cells until his DIC stabilized. During the following week, the patient's renal function continued to deteriorate necessitating renal dialysis. The LDH, SGOT and bilirubin were elevated as high as 3, 18 and 30 times the patient's baseline values, respectively. His liver function eventually returned to baseline with the exception of the bilirubin which remained elevated. The patient expired 23 days post echinomycin infusion of renal and hepatic failure. Significant findings at autopsy included severe centrilobular and mid-zonal necrosis of the liver and normal cellularity of the bone marrow. There were no ischemic changes found in the kidney, however, there were focal aggregates of inflammatory infiltrates composed of mononuclear cells and/or eosinophils.

*Table 5. Biochemical induced liver dysfunction*

| Dosage level (mcg/m²) | No. of evaluable patients | No. of patients with increased SGOT/Alk Phos | SGOT* Baseline | SGOT* Peak value | Toxicity† grade | Alk Phos* Baseline | Alk Phos* Peak value | Toxicity† grade |
|---|---|---|---|---|---|---|---|---|
| 180 | 4 | 2 | 41 | 75 | 1 | 172 | 245 | 1 |
| | | | 21 | 52 | 1 | • | | |
| 300 | 7 | 1 | 60 | 90 | 1 | • | | |
| 540 | 3 | 1 | 43 | 66 | 1 | • | | |
| 780 | 3 | 1 | 80 | 130 | 1 | 91 | 604 | 3 |
| 1200 | 3 | 2 | • | • | | 129 | 359 | 2 |
| | | | 30 | 110 | 2 | • | | |
| 1600 | 7 | 3 | • | • | | 144 | 336 | 2 |
| | | | • | • | | 118 | 353 | 2 |
| | | | 66 | 402 | 3 | • | | |
| 2128 | 3 | 2 | 22 | 98 | 2 | • | | |
| | | | 18‡ | 77 | 2 | 64 | 201 | 2 |
| | | | 10‡ | 59 | 3 | 69 | 198 | 2 |

*Normal ranges SGOT 7–40 MU/ml; alkaline phosphatase (Alk Phos) 30–115 MU/ml.
†SGOT, Alk Phos: Grade 1 (≤2.5 × baseline), grade 2 (2.6–5 × baseline), grade 3 (5.1–20 × baseline).
‡Same individual treatment on separate occasions.
•No change.

*Response*

One patient with a fibrosarcoma of the hand metastatic to the lung treated with 1200 mcg/m$^2$ of echinomycin had a brief mixed response.

## DISCUSSION

Echinomycin is the first bifunctional intercalating agent to reach phase I trials. One-thirtieth of the $MELD_{10}$ in the dog was chosen as the initial starting dose. A total of 43 patients received 77 courses of echinomycin in doses ranging from 66 to 2128 mcg/m$^2$. The dose-limiting toxicity of echinomycin as a 24-h continuous infusion every 28 days was nausea and vomiting. The onset of nausea and vomiting usually occurred at the end of the 24-h infusion and lasted for 3–8 days. Patients failed to respond to phenothiazines or metoclopramide after the nausea and vomiting had begun. In spite of pretreatment with metoclopramide and a dose reduction (2128 → 1600 mcg/m$^2$), one patient continued to experience nausea and vomiting with subsequent courses. The onset at the end of infusion suggests the possibility of accumulation of echinomycin over time. The pharmacokinetic characteristics of echinomycin, particularly clearance, are unknown. Attempts to develop a sensitive HPLC and biological assay were unsuccessful. Other toxicities included sporadic thrombocytopenia, an isolated episode of DIC and biochemical evidence of reversible liver dysfunction. There was one drug-related death due to renal and hepatic failure. Severe centrilobular and mid-zonal necrosis of the liver was found on autopsy which is consistent with the preclinical toxicology necropsy findings in the dog.

Three additional phase I trials with echinomycin have been performed administering the drug on a weekly × 4 schedule [16], by a single bolus dose every 4 weeks schedule [17] and on a daily × 5 bolus schedule [18]. The dose-limiting toxicity for all four studies was nausea and vomiting. Other toxicities noted in this study are similar to those observed on the other schedules with the notable exception of phlebitis and lack of allergic reactions. Allergic reactions have been associated with other cremophor containing antineoplastic agents [19]. The volume and length of infusion may have accounted for the high incidence of phlebitis but the lack of allergic reactions on our schedule.

Three patients with metastatic sarcomas were reported to have achieved disease stabilization on the weekly × 4 schedule [17]. With the brief response in fibrosarcoma in our study coupled with the human tumor cloning data, echinomycin should be evaluated in patients with sarcoma in phase II trials. The recommended doses of echinomycin for phase II studies are 450–500 mcg/m$^2$/day for the daily × 5 schedule, 1500 mcg/m$^2$ for the single bolus dose schedule and 1200 mcg/m$^2$/week for the weekly × 4 schedule. Since the clinical toxicities of echinomycin are relatively equal on four schedules and the greater preclinical antitumor activity observed with the days 1–9 schedule could possibly be attributed to administration of a larger total dose of drug, the weekly × 4 schedule would appear to be the appropriate schedule for phase II trials. The recommended phase II dose of echinomycin utilizing a 24-h continuous infusion schedule is 1600 mcg/m$^2$. Because of the incidence and severity of nausea and vomiting associated with echinomycin, patients should be pretreated and continued for at least 24-h with an antiemetic regimen such as metoclopramide plus dexamethasone, indicated for highly emetogenic agents.

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