RUBIDAZONE VS ADRIAMYCIN: AN EVALUATION OF THEIR DIFFERENTIAL TOXICITY IN THE SPLEEN COLONY ASSAY SYSTEM

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Summary.—Rubidazone, the new semi-synthetic benzol hydrazone hydrochloride derivative of daunorubicin, has proved on a molecular weight basis to be less toxic than adriamycin and similar to daunorubicin in cardiac toxicity studies in the hamster as well as in other in vivo and in vitro test systems. It has proven effectiveness against several animal tumours and human acute leukemias. We have compared the inhibitory effect of rubidazone to that of adriamycin on P388 leukaemia and normal bone marrow colony-forming units (CFU) using the spleen colony assay system in male DBA2 mice. The efficacy ratios (i.e., the ratio of the slopes of the normal bone marrow CFU to leukaemic CFU dose-survival curves) in the spleen colony assay system for rubidazone and adriamycin were 7·8 and 7·5 respectively. This near identity of efficacy ratios for rubidazone and adriamycin correlated with the results of median survival time studies in the leukaemic mice. Their dose–median survival time curves were almost parallel, having nearly identical slopes. Rubidazone’s equal therapeutic index as compared to adriamycin in the spleen colony assay system together with its known decreased toxicity to cardiac muscle cells makes it an extremely promising new anthracycline derivative to study in comparison to adriamycin in human malignancies.

Rubidazone, the new semi-synthetic benzol hydrazone hydrochloride derivative of daunorubicin, isolated by the Rhone-Poulenc Research Laboratory, has proven effectiveness against a variety of experimental tumours (Maral, Ponsinet and Jolles, 1972) and human acute myeloblastic and lymphoblastic leukemias (Jacquillat et al., 1972; Bernard et al., 1972). Although it appears to have similar activity to adriamycin against several mouse tumours (Johnson, 1974), on an equal weight basis rubidazone has been shown to cause less cardio-toxicity than adriamycin in the hamster (Maral et al., 1972) and rabbit (Young, 1974).

We have used the spleen colony assay system for leukaemic and normal bone marrow colony-forming units (CFU) to compare the therapeutic index of rubidazone with that of adriamycin, the presently most active anthracycline compound against a wide variety of human solid malignancies (Blum and Carter, 1974). It was our belief at the initiation of this study that if rubidazone was as active as adriamycin against leukaemic stem cells and showed the same or less evidence of bone marrow damage, it

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might prove to be a worthwhile drug for further clinical studies.

MATERIALS AND METHODS

Mice.—Six- to eight-week-old male DBA₂ mice (Jackson Laboratories, Bar Harbor, Maine) weighing approximately 25 g were used in these experiments.

Mouse tumour line.—P₃₈₈ lymphocytic leukaemia was supplied by Dr John Harris (Department of Radiobiology, University of California, San Francisco) and serially transplanted as an ascites tumour at weekly intervals (10⁶ cells every 7 days in medium 199) (GIBCO, Sunnyvale, California). It was selected for these studies because it predicts the clinical efficacy of anticancer drugs more accurately than other mouse tumour lines (Venditti, 1974).

Chemotherapeutic agents.—Rubidazone in powder form (supplied by the Rhone-Poulenc Industries, Paris, France by R. Maral) was dissolved in sterile water to the desired concentration. The powder was stored in the dark at room temperature. Thin layer chromatography revealed the drug to be in a pure form without significant metabolites. Adriamycin (Adria Laboratories, New Jersey) in powder form was also dissolved in sterile water to the desired concentration. Again, thin layer chromatography revealed a narrow band of the parent compound without significant aglycone or other metabolite moieties.

LCFU assay.—The assay for leukaemic colony forming units (LCFU) was carried out in the following way: On Day 0, 10⁶ P₃₈₈ cells were injected into the tail veins of 5 groups of 5 DBA₂ mice. In 4 of these groups, adriamycin (at 4–9 mg/kg) or rubidazone (at 12–18 mg/kg) was injected i.p. 48 h (Day 2) after tumour cell injection (when the i.p. P₃₈₈ tumour had attained a 93+ % growth fraction) (Harris, Shon and Meneses, 1973). Both adriamycin and rubidazone were injected in a constant volume of 0.01 ml/g body wt. On Day 5 the mice were sacrificed, femurs were isolated, and bone marrow cells were washed out with medium 199. Appropriate dilutions of femoral bone marrow cells were injected in 0.2-ml volumes into the tail veins of groups of 15–20 recipient mice. Nine days later these recipient mice were sacrificed, spleens removed and fixed in Bouin’s solution, the macroscopic LCFU were counted using a dissecting microscope, and the fraction of surviving LCFU per femur (as compared to the controls) determined.

NCFU assay.—Normal bone marrow colony forming units (NCFU) were assayed in a similar way as LCFU except for the following: 3 days after therapy with i.p. adriamycin (at 4–15 mg/kg) or rubidazone (at 15–40 mg/kg) 5 groups of 5 DBA₂ mice (including one control group) were sacrificed and appropriate dilutions of normal femoral bone marrow cells were injected i.v. into groups of 15–20 whole-body-irradiated (720 rad from a 250-kV source) mice. Nine days later these mice were sacrificed, spleens were removed and fixed in Bouin’s solution, the macroscopic NCFU were counted using a dissecting microscope, and the fraction of surviving NCFU per femur (as compared to the controls) determined.

Mean survival time studies.—Groups of 7 DBA₂ mice were given 10⁶ P₃₈₈ ascites cells i.v. in a volume of 0.2 ml and the mean survival times of each of the control and treatment groups was calculated by observing for death at 12-h intervals. The administration schedule of adriamycin and rubidazone was similar to that for the LCFU and NCFU assays.

Statistical analysis.—A simple t test was used to determine statistical significance between the different experimental groups. Dose–response curves for both adriamycin and rubidazone with respect to LCFU and NCFU were constructed on the basis of regression analysis.

RESULTS

We have determined the effect of rubidazone and adriamycin on the survival of both normal bone marrow and leukaemic CFU. Increasing doses of these agents were administered as single i.p. injections to groups of 5 mice and 3 days later the femoral bone marrow cells were assayed for their content of either normal or leukaemic colony forming stem cells. Results obtained with rubidazone and adriamycin are shown in Fig. 1 and 2, respectively, in which the surviving fraction per femur of both normal and leukaemic CFU is plotted as a function of the dose of drug injected. As plotted
in semi-logarithmic form, the dose–response curves for both leukaemic and normal bone marrow CFU for both rubidazone and adriamycin were exponential. The fraction of surviving leukaemic or normal CFU per mouse femur were not significantly different when assayed 1 or 3 days after the administration of either rubidazone or adriamycin.

In order to define either rubidazone’s or adriamycin’s differential effect on leukaemic versus normal bone marrow colony forming units, we have used the term "efficacy ratio" of Valeriote and Tolcn (1972), which is simply the ratio of the slopes of the two dose–survival curves. We have also used Valeriote and Tolcn’s $D_{1/2}$ value as a measure of the slope for both rubidazone and adriamycin, calculated on the exponential portion of the dose response curves. The $D_{1/2}$ value is the dose of drug which reduces the survival of the cell population in question by a factor of one-half. As seen in Fig. 1, the $D_{1/2}$ of rubidazone for LCFU was 1.6 mg/kg; whereas, for NCFU it was 12.4 mg/kg. The resulting efficacy ratio of rubidazone was 7.8 (i.e., 12.4/1.6, or 7.8). In Fig. 2, it can be seen that the $D_{1/2}$ of adriamycin for LCFU was 0.85 mg/kg and for NCFU 6.4 mg/kg, resulting in an efficacy ratio of 7.5. This near-identity of efficacy ratios of rubidazone and adriamycin is reflected in the results of the tumour-bearing mouse survival studies with these agents. In Fig. 3 the median survival times of the DBA4 mice following administration of P388 leukaemia are plotted against the dose in mg/kg of rubidazone or adriamycin used for each of these
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![Graph showing dose-response survival time studies for P388 leukaemia in DBA mice.](image)

**Figure 3.**—Adriamycin vs. rubidazone dose-response survival time studies for P388 leukaemia in DBA mice. Each point represents the mean ± s.e. for 7 mice.

Rubidazone may be a more active drug than adriamycin for the treatment of acute myeloblastic leukaemia (Jacquillat et al., 1972; Bernard et al., 1972; Courts, Ellison and Yates, 1972; Klener, Donner and Kojina, 1973). Bernard et al. (1972) have reported 17 complete remissions in 33 patients with acute myeloblastic leukaemia treated with rubidazone. The median dose to obtain complete remission was 23 mg/kg and there was evidence of congestive heart failure only in patients who had had prior daunorubicin therapy (Jacquillat, 1974). Although EKG changes were seen in 9 of 21 rubidazone-treated patients studied by Chauvergne and Durand (1973), there was no evidence of congestive heart failure in their study.

Young (1974) has presented data on 29 rabbits treated at the National Cancer Institute for cardiac toxicity following anthracycline administration. Dosage schedules included rubidazone at 26-4 mg/m²; adriamycin, 7-7 mg/m²; and daunorubicin, 11 mg/m² given three times per week. The cardiototoxic dose for rubidazone was 1560 mg/m² compared with 250 mg/m² for adriamycin and 400 mg/m² for daunorubicin. For the dosage schedules employed, it took approximately 19.7 weeks to reach the critical cardiotoxic dose with rubidazone compared to 10.8 weeks for adriamycin and 12.1 weeks for daunorubicin.

Using the spleen colony assay system to determine the relative effects of rubidazone or adriamycin on leukaemic or normal bone marrow stem cell growth characteristics, we have shown that these two agents have very similar "efficacy ratios". Though on a mg/kg basis adriamycin appears to be more effective than rubidazone in the inhibition of leukaemic CFU, when balanced with the effects on normal bone marrow CFU, this seeming advantage of adriamycin is cancelled out. Corroborating the results in the spleen colony assay system are the parallel dose–response curves with respect to mean survival time studies for these two anticancer drugs. If the animal data of Young and the human data of Jacquillat and Chauvergne for the incidence of cardiotoxic effects following rubidazone administration prove to be

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**DISCUSSION**

survival studies. Note that the two survival–dose curves are almost parallel.
accurate, then the results of this study indicating equal efficacy for the two drugs with respect to tumour and normal bone marrow stem cell inhibition suggest that rubidazone could be clinically more useful than adriamycin (Bernard et al., 1972; Klener et al., 1973; Jacquillat, 1974). Certainly, our data along with those of previously quoted investigators suggest that rubidazone should be given a thorough clinical trial to determine its true efficacy and cardiotoxicity. Adriamycin has proved extremely useful in the treatment of a variety of solid tumours and acute leukaemia (Blum and Carter, 1974; Courts et al., 1972; Klener et al., 1973); however, its utility is limited by the advent of cardiotoxicity at a relatively low dosage (Lefrak et al., 1973). Because of the cardiotoxic characteristics of adriamycin, much work now and in the future is being carried out to identify anthraacycline-type drugs with equal or greater clinical efficacy but less cardio-toxicity. Rubidazone could prove to be such a drug.

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