Comparative Hematopoietic Toxicity of Doxorubicin and 4′-Epirubicin (43124)

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Abstract. **4′-Epirubicin is an anthracycline analog of doxorubicin which has been shown to be similar to doxorubicin in its anti-tumor activity but significantly lower in its cardiotoxicity. Therefore, it has been proposed as a potential clinical substitute for doxorubicin. Using the hematopoietic colony-forming unit, spleen (CFU-S) assay technique, direct comparison was made of the hematopoietic toxicity of the two drugs *in vivo* in a mouse model, and 4′-epirubicin was found to be significantly ($P < 0.01$) less toxic than doxorubicin. On a milligram per kilogram basis, the dose of 4′-epirubicin required to achieve a given level of hematopoietic progenitor cell kill was approximately 50% larger than that required for doxorubicin. Early CFU-S recovery following 4′-epirubicin exposure was also stronger than that achieved following doxorubicin, as was short-term peripheral white blood cell recovery. These findings confirm previous clinical suggestions that the acute toxicity of 4′-epirubicin toward hematopoietic progenitor cells might be less than that of doxorubicin. At the same time, however, when given in doses near their lethal limit, both drugs were shown to induce a chronic hematopoietic suppression. This was evident in the depressed long-term CFU-S levels following high doses of either drug, as well as in chronically depressed white blood cell levels following high-dose 4′-epirubicin.** [P.S.E.B.M. 1990, Vol 195]

Doxorubicin is one of the most commonly utilized cancer chemotherapeutic agents, with broad application against many diverse tumor types. The extent to which it may be applied in any individual case has been limited, however, by both its cardiac and hematopoietic (myelopoietic) toxicities. In efforts to overcome the limitations imposed by these toxicities, a variety of doxorubicin analogs have been explored (1). Of these, 4′-epirubicin has been reported as being one of the most promising. 4′-Epirubicin differs from doxorubicin only in the epimerization of the 4′-hydroxyl on the daunosamine sugar moiety of the doxorubicin structure, and as such presents a relatively minimal structural departure from doxorubicin (1, 2). Nevertheless, and with particular reference to the problem of cardiotoxicity, pharmacologic studies have suggested that this structural difference may be of paramount importance in reducing the extent of the damage done by the drug to normal tissue (2). At the same time, the anti-tumor activity appears to remain essentially undiminished. In numerous Phase I and Phase II clinical trials, 4′-epirubicin has been reported as being active against a large variety of cancers (1–5), especially breast cancer (1, 3) and non-Hodgkin's lymphoma (1, 4, 5). In all these studies it was reported to be quite comparable to doxorubicin in its antitumor effectiveness, but less cardiotoxic and possibly also less myelotoxic. Direct comparisons of the effects of the two drugs on cardiac function have confirmed the reduced cardiotoxicity of 4′-epirubicin (6–8). In this paper we have focused on its myelotoxicity and present a direct comparison of its effect on hematopoietic progenitor cells, as measured by the *in vivo* 8-day CFU-S technique, against that of doxorubicin.

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Materials and Methods

Animals. Female SJL/J mice (The Jackson Laboratory, Bar Harbor, ME), 12 weeks of age, were used for all aspects of these studies. These were stored at a maximum of five per cage in autoclaved filter-top cages, and provided with autoclaved food, water, and bedding.

Drugs. Doxorubicin (NSC 123127) and 4′-epirubicin (NSC 256942) were provided by Adria Laboratories (Columbus, OH). These were dissolved in normal saline solution and injected via the tail vein on a milligram

per kilogram of body weight basis as is also described under Progenitor Cell Recovery Assay.

Progenitor Cell Survival Assay. Determination of damage to the hematopoietic progenitor cell (CFU-S) compartment was carried out using the method of Till and McCulloch (9) as previously applied by us in earlier studies (10). For each of the various doses of drug indicated in Results, five animals were injected. Two days later, these animals were sacrificed by cervical dislocation, and their spleens were removed and weighed. The spleen cells were gently teased out of the capsule and rendered into single-cell suspensions in Ca^{2+}- and Mg^{2+}-free Hanks' solution. These were pooled, diluted in concentrations of between 5 and 20 $\times 10^5$ cells/ml, and injected into other lethally irradiated (9.5 Gy) SJL/J mice, which served as the recipient assay mice. Eight days later, those recipients were sacrificed and their spleens removed and placed into Bouins' solution for colony fixation and scoring. For each assay, 20–25 recipient mice and three dilutions of drug-treated donor cells were employed. From these, the average numbers of surviving CFU-S per treated donor spleen were determined as a function of drug dose. The resultant data were then analyzed by regression analysis (11).

Progenitor Cell Recovery Assay. To achieve maximum hematopoietic suppression while still maintaining optimal long-term survival, a dose level was sought for each drug in which the 5-day mortality was greater than 0%, but less than 10%. For the SJL/J mice used in this study, this dose for doxorubicin was 17.5 mg/kg and for 4′-epirubicin it was 22.5 mg/kg. These two doses were used therefore to compare the recovery of hematopoietic progenitor cells following a single exposure to near-lethal doses of the two drugs. Analysis of recovery utilized the same CFU-S technique as described above, with the exception that in this case the drug dosage was held constant while the time of assay after drug exposure varied.

White Blood Cell Determinations and Survival Comparisons. Using the same two drug dosages as given immediately above, the effects of the two drugs on peripheral white blood cell (WBC) levels were also determined as a function of time after exposure to a single near-lethal dose of drug. Following drug exposure, the mice were bled of 0.02 ml of blood from the tail vein on the days indicated and, with the aid of a Coulter Counter, total WBC counts were determined as a function of time after drug exposure. At the same time, these animals were also monitored for their survival. For these studies, a total of 28 animals were exposed to 4′-epirubicin and 21 were exposed to doxorubicin. Concurrently, 20 untreated animals were used to obtain the control WBC values for normal SJL/J mice. These were bled along the drug-treated mice on the days indicated.

Statistics. Standard biomedical statistical techniques were used for calculation of regression lines and for determinations of P values, means, and standard errors (11). Error values are given as 1 SE, except as otherwise indicated.

Results

Effect on Hematopoietic Progenitor Cell Survival and Recovery. Figure 1 illustrates the hematopoietic progenitor cell survival, reflected by the decrease in number of 8-day splenic CFU-S colonies in response to increasing drug dose. In both cases, the survival patterns followed an exponential decline as a function of increasing dose, similar to that previously described for other cancer chemotherapeutic agents which act on DNA synthesis at the progenitor cell level (12). As indicated by regression analysis of the data, on a milligram per kilogram basis, 4′-epirubicin was significantly less toxic than doxorubicin at a level of $P < 0.01$.

Figure 2 compares the recovery of the 8-day CFU-S colony formers as a function of a single large dose exposure to the drugs: 17.5 mg/kg for doxorubicin and 22.5 mg/kg for 4′-epirubicin. Although the dosage of 4′-epirubicin used exceeded that of doxorubicin, the recovery overshoot for 4′-epirubicin at 10 days was over twice as high as that for doxorubicin. Both drugs, however, induced significant ($P < 0.01$) later residual damage, and for both drugs at these doses the extent of

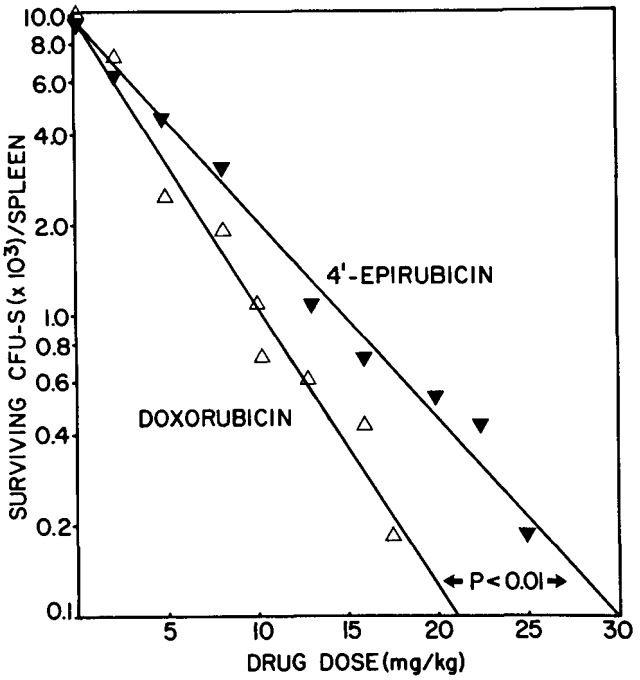

Figure 1. Comparative hematopoietic progenitor cell killing/survival curves for 4′-epirubicin (filled triangles) versus doxorubicin (open triangles), plotted as the log of cell survival versus drug dose. Regression lines were calculated according to the methods described by Colton (11).

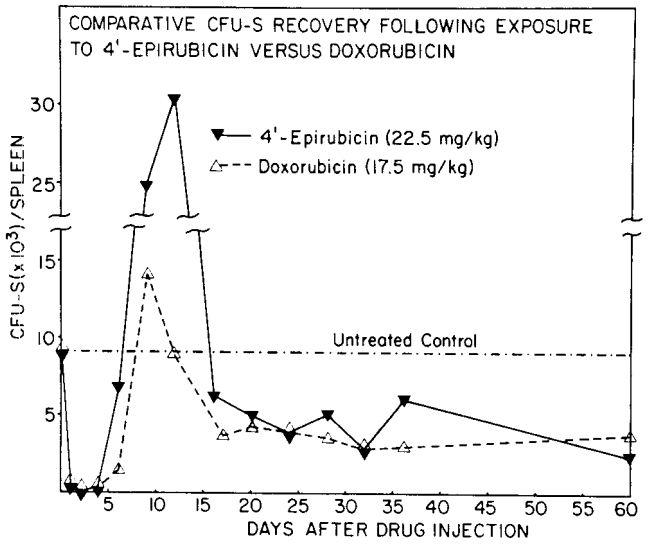

Figure 2. Comparative hematopoietic progenitor cell recovery curves plotted as a function of time following exposure to a large single dose of 4′-epirubicin (22.5 mg/kg, filled triangles, solid line) or doxorubicin (17.5 mg/kg, open triangles, dashed line). The control line represents the pooled data for the splenic CFU-S levels of the normal untreated control mice (extended as a dash-dot line across the period of the study).

this residual damage among the CFU-S was approximately the same.

Effects on Peripheral WBC. The changes seen in peripheral WBC as a function of time after exposure to near-lethal doses of the drugs are shown in Table I. There were no significant differences in the normal control values as a function of the day the control mice were bled. Therefore, the data from these were pooled and are as indicated in the table. In general, the changes in the peripheral WBC of the animals receiving 22.5 mg/kg of 4′-epirubicin reflected the pattern exhibited by the CFU-S, with a sharp early drop and then a rapid and brief recovery to a level significantly above the normal. Thereafter, from 3 weeks to the 3-month termination point of the study, the peripheral WBC fell significantly and remained between two thirds and three quarters of the normal values. By contrast, 17.5 mg/kg of doxorubicin resulted in a lesser initial drop in peripheral WBC, but there was also no early recovery. After this, the WBC remained low through the fourth week, after which normal WBC levels were regained. However, with respect to the 90-day value for the doxorubicin-exposed mice, it should be noted that this value was obtained from only two mice, which were also the only survivors out of the 21 initially exposed, and that all of the mice with low WBC values in this doxorubicin-treated set had expired previously. By contrast, there were 12 survivors out of 28 among the 4′-epirubicin-exposed mice, half of which had severely depressed WBC levels and half of which had WBC that were at or near normal levels.

Effects on Animal Survival. Table II provides the survival data for the same mice as used for the peripheral WBC evaluations. During the first half of the study, through 45 days, survival in both drug-treated groups was comparable and nearly the same as the untreated controls. However, by 60 days, survival in both of these groups had begun to drop significantly below the controls, with the deaths among the doxorubicin-treated mice being far greater, despite the fact that the doxorubicin drug dose was nearly 30% less than that used for the 4′-epirubicin-treated mice. At 90 days, the survival of the 4′-epirubicin-treated mice was 43% and significantly lower than that of the controls at a P value of < 0.0001, but still significantly greater than that of the survival of the doxorubicin-treated mice which, at 10%, differed from the 4′-epirubicin-treated mice at a P value of < 0.001.

Discussion

The results of this study reinforce the previous clinical observations regarding the lesser toxicity of 4′-

Table I. Comparison of the Effect of 4′-Epirubucin (22.5 mg/kg) and Doxorubicin (17.5 mg/kg) on Peripheral White Blood Cells in SJL/J Mice

Days after drug	4′-Epirubicin			Doxorubicin		
	WBC/mm³	% of control	P	WBC/mm³	% of control	P
2	5,039 ± 406	34	<0.001	8,912 ± 725	60	<0.001
7	13,024 ± 903	88	NS	10,049 ± 492	68	<0.001
11	17,016 ± 976	115	<0.05[a]	12,081 ± 822	81	<0.04
14	14,734 ± 766	99	NS	11,900 ± 1,015	80	<0.05
21	10,197 ± 536	69	<0.001	12,893 ± 611	87	NS
28	9,971 ± 562	67	<0.001	9,240 ± 817	62	<0.001
36	11,102 ± 524	75	<0.001	15,471 ± 746	104	NS
90	11,054 ± 1,087	74	<0.006	13,993 ± 275[b]	94	NS

Normal control WBC: 14,851 ± 904 WBC/mm³

[a] Significantly greater than control.
[b] Two survivors.

Table II. Survival of SJL/J Mice Following Exposure to a Single Dose of 4′-Epirubicin or Doxorubicin

Drug and dose	Survival after drug (%)					
	15 days	30 days	45 days	60 days	75 days	90 days
Control, no drug (20)[a]	100	100	100	100	100	100
4′-Epirubicin, 22.5 mg/kg (28)[a]	96	93	93	75	46	43
Doxorubicin, 17.5 mg/kg (21)[a]	95	95	95	29	14	10

[a] Number of mice in group.

epirubicin versus that of doxorubicin. In particular, and with specific respect to those hematopoietic progenitors reflected by the CFU-S assay, the milligram/kilogram dose of 4′-epirubicin required to achieve the same killing effect as doxorubicin was nearly 50% higher. Since the molecular weight of 4′-epirubicin (579.88) is only about 10% greater than that of doxorubicin (543.54), this difference in the dosage required to achieve an equivalent CFU-S cell killing effect cannot be explained on the basis of molecular weight disparities alone. Therefore, it must represent a true lessening in the hematopoietic toxicity of the drug as a result of its epimerization.

In contrast to its reduced hematopoietic toxicity, other evidence has suggested that 4′-epirubicin nevertheless retains significant antitumor activity. Clinical studies done elsewhere have indicated that on an equimolar basis its effectiveness closely approaches that of doxorubicin when used as a single agent in treating soft tissue sarcomas (13) and for the palliation of advanced breast cancer (3). When used in combination chemotherapy along with cyclophosphamide, vincristine, and prednisone against non-Hodgkin's lymphoma, 4′-epirubicin has been reported to be equivalent in effectiveness to doxorubicin and to be so with lesser toxic side effects to the normal tissues (4, 5). Likewise, similar findings have been reported for advanced ovarian cancer when used in combination with cisplatin and cyclophosphamide (14). Therefore, 4′-epirubicin recommends itself as an effective potential substitute for doxorubicin.

Its lessened toxicity toward normal tissues also suggests that it may be possible to use 4′-epirubicin in higher doses than doxorubicin. However, on the basis of the hematopoietic progenitor cell survival data of this paper, the potential for increasing its dosage would certainly not be unlimited. With respect to short-term hematopoietic effects, the maximum allowable increase in this limit would be on the order of 50% (Fig. 1). However, considering that similar levels of long-term hematopoietic suppression were achieved with 22.5 mg/kg of 4′-epirubicin and 17.5 mg/kg of doxorubicin (Fig. 2 and Table I), the real limit may be only half of that, on the order of 25%. In setting a lower maximum dose for 4′-epirubicin, the survival data suggest that the drug could be expected to be better tolerated, especially as compared with doxorubicin, and this is borne out in that the 43% survival of the 4′-epirubicin exposed animals in this study exceeded the 10% survival of the doxorubicin exposed mice with a P value of < 0.001 (Table II). Relative to this, in earlier comparative animal survival studies, Casazza (15) had estimated the single-dose exposure LD_{50} for mice to be between 10 and 13 mg/kg for doxorubicin and about 50% higher than that for 4′-epirubicin (16–19 mg/kg), which agrees well with the results presented here for hematopoietic progenitor cell survival (Fig. 1). However, for humans he found the maximum tolerable dose to be only on the order of 22% higher (75–90 mg/m^2 of 4′-epirubicin given every 3 weeks). Therefore, from the aspect of its potential clinical application, the maximum dosage of 4′-epirubicin may have to be adjusted within this lower range rather than at the higher limit that is suggested by the comparative hematopoietic progenitor cell survival curves of Figure 1.

In summary, the data indicate that 4′-epirubicin is significantly less toxic than doxorubicin to hematopoietic progenitor cells, as well as to the whole animal, as reflected by direct CFU-S assay and the 90-day animal survival. They thus suggest that there is a potential for applying comparatively higher doses of 4′-epirubicin in cancer therapy. However, based on other considerations, the maximum increase that might be tolerated may be somewhat less than the 50% predicted by the hematopoietic progenitor cell survival assay. Finally, it is apparent that at extremely high doses both drugs may induce a long-term depression among the hematopoietic progenitor cells.

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