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Enhancement of Adriamycin-Induced Mortality during Riboflavin Administration and Riboflavin Deficiency in Rats (42767)

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Abstract. Adriamycin-treated rats were monitored for survivorship while consuming a normal diet adequate in riboflavin, a normal diet and receiving daily high-dose injections of riboflavin-5′-phosphate (flavin mononucleotide, FMN), or a riboflavin-deficient diet. Each animal was compared to a corresponding pair-fed, saline-treated control. In Adriamycin-treated rats fed the normal chow diet alone, survivorship declined within 7 days and remained constant after 12 days to about 50%. Adriamycin-treated rats consuming the normal diet and injected with FMN initially showed similar survivorship; however, after 20 days survival fell to 14%. Adriamycin-treated, riboflavin-deficient rats showed within 5 days a precipitous decline in survivorship which leveled to 5%. These results suggest that during Adriamycin treatment, proper riboflavin nutriture may be a crucial determinant of survival.

Adriamycin is an effective drug used for the treatment of a variety of neoplastic diseases. Its potential as a chemotherapeutic agent is limited by the occurrence of both acute and chronic phases of toxicity associated with the gastrointestinal tract, heart, kidney, and liver (1, 2).

Previous work in our laboratory has determined that Adriamycin inhibits the incorporation of riboflavin into flavin adenine dinucleotide (FAD) in both cardiac and skeletal muscle and that this inhibition of FAD biosynthesis by Adriamycin is associated with both the dose and duration of drug therapy (3–5). The inhibitory effect of Adriamycin on FAD formation in both heart and skeletal muscle may have important implications for energy metabolism associated with oxidative phosphorylation in these metabolically active tissues (6). Both the four-membered anthracycline ring of Adriamycin and the three-membered isoalloxazine ring of riboflavin including its physiological derivatives, FMN and FAD, form a 1:1 stoichiometric complex *in vitro* (7). Adriamycin and some of its metabolites can mimic flavins to complete successfully for binding sites in certain flavin-containing enzymes (8, 9). On the other hand, flavoenzymes are responsible for catabolizing anthracycline drugs. Type I flavoenzymes catalyze both the reductive glycosidic cleavage and the formation of free radical intermediates of Adriamycin. Microsomal NADPH cytochrome *P*-450 reductase is the major flavoenzyme responsible for this activity in mammals (10).

Thus, both the action of Adriamycin as well as its rate of degradation may depend upon the availability of riboflavin and the levels of its physiological derivatives. The present investigation was designed to determine whether changes occur in survival of Adriamycin-treated animals which are riboflavin sufficient, supplemented, or deficient.

Materials and Methods. *Animals.* All experiments were conducted with 6-week-old male Sprague–Dawley rats (CAMM Research Lab Animals, Wayne, NJ). Animals were housed individually in stainless-steel cages, maintained on either a normal chow diet or a riboflavin-deficient diet, and allowed free access to water. Body weights and food consumption were recorded daily. The animal care facility is equipped with controlled ambient temperature and a 12-hr day/night lighting cycle.

Chemicals and diets. Adriamycin (doxorubicin HCl) was purchased from Adria Labs (Columbus, OH) as a crystalline powder and reconstituted with saline prior to injection into animals. Riboflavin-5′-phosphate (FMN) was obtained from Sigma Chemical Co. (St. Louis, MO) as a crystalline powder and reconstituted with saline prior to admin-

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istration. The normal chow diet was Purina Rat Chow (Ralston Purina Company, St. Louis, MO), and the riboflavin-deficient diet (<1 ppm riboflavin) was prepared by Dyets, Inc. (Bethlehem, PA). The riboflavin-deficient diet comprised (grams/kilogram) sucrose, 500; vitamin free casein, 200; cornstarch, 150; solkaflox, 50; AIN salt mix (No. 200000), 35; AIN-76A vitamin mix (No. 300050) without riboflavin, 10; DL-methionine, 3; choline bitartrate, 2.

Diet regimen and Adriamycin treatment. In the first experiment, after 4 weeks of feeding on the normal chow diet *ad libitum,* half of the animals received intraperitoneal (ip) injections of FMN (50 mg/kg) and half received isotonic saline daily for the duration of the study. FMN was chosen as the injectable form rather than the parent compound, riboflavin, because of its greater water solubility. Once in the blood stream, FMN is rapidly dephosphorylated to riboflavin prior to cellular absorption. This daily injection represents a pharmacologic dose of about 300 times the recommended daily allowance of riboflavin for the rat. In addition, half of all animals in these groups, received intraperitoneal injections of Adriamycin. All Adriamycin-treated animals received ip injections of Adriamycin twice daily for 3 days, until a cumulative dose of 20 mg/kg body weight was obtained. Pair-fed controls were injected with an identical volume of saline.

In a second experiment, rats were fed a riboflavin-deficient diet for 4 weeks *ad libitum.* After the 4-week period, riboflavin status of the group was determined in erythrocytes by measuring the activity coefficient of glutathione reductase. Half of the animals in the riboflavin-deficient group received injections of either Adriamycin or saline as in the previous experiment.

Statistical analysis. These studies were concluded 30 days after the initial injection of Adriamycin, and the log-rank test (11) was utilized for statistical analysis of the effects of these treatment schedules on survival.

Results. In normal chow-fed animals treated with either Adriamycin alone or Adriamycin and FMN, mean body weights decreased by the 3rd day of the experimental period (Figs. 1A and 1B). By the 6th day, rats

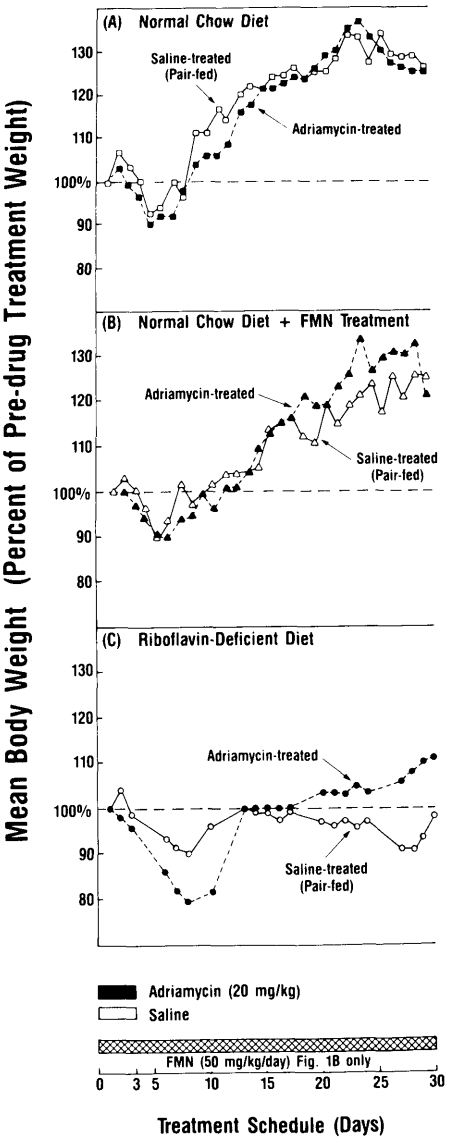

FIG. 1. Effects of Adriamycin upon body weight in rats fed (A) a normal chow diet, (B) a normal chow diet and given daily ip injections of FMN, or (C) a riboflavin-deficient diet. Each group was compared to results in saline-treated, pair-fed controls. All data are expressed as percentage of pre-drug treatment weight. The treatment schedule for Adriamycin, saline, and FMN is shown below the graphs of mean body weight. The normal chow-fed group contained 15 adult male Sprague–Dawley rats each when treatment commenced. The normal chow-fed, FMN-treated groups contained 14 animals each, and the riboflavin-deficient groups contained 21 rats. All rats were weighed daily for the duration of the experimental period.

treated with Adriamycin or Adriamycin and FMN exhibited weight gains similar to their respective saline-treated controls. All of these animals continued to gain weight during the first 3 weeks of the study until body weights appeared to stabilize. By contrast, loss of body weight was recorded after the 1st day of treatment in the Adriamycin, riboflavin-deficient group (Fig. 1C). Weight gain in this group although minimal was detected after the 10th day. Except in the normal chow-fed animals receiving Adriamycin, the other Adriamycin-treated groups had mean body weights which were higher than their respective saline-treated controls. This appeared to be due in large measure to abdominal ascites.

As shown in Figs. 2A, 2B, and 2C, all of the Adriamycin-treated groups have accelerated mortality rates compared to their corresponding saline-treated controls. In normal chow-fed, Adriamycin-treated animals, survival declined to 60% within 5 days after Adriamycin injection and continued to decline to 47% by day 23 of the study (Fig. 2A). The survivorship curve of the Adriamycin- and FMN-treated group closely resembled that of the normal chow, Adriamycin-treated group until Day 18 when the percentage surviving began an apparent second decline to 14% (Fig. 2B). Of the three treatment modalities, the Adriamycin-treated, riboflavin-deficient group had the lowest number of survivors (Fig. 2C). Within 10 days after Adriamycin treatment, the decline to 5% survival was precipitious and remained at this level for the duration of the study.

Discussion. The results of this survival study indicate that neither a daily single pharmacologic dose of FMN nor riboflavin deficiency provides a survival advantage for rats treated with Adriamycin. Using the log-rank test (11) to compare survival studies in Figs. 2A and 2C, the accelerated mortality rate which occurs during riboflavin deficiency provides evidence for the importance of riboflavin metabolism perhaps in determining the extent of Adriamycin toxicity.

The overall accelerated mortality rates observed in Adriamycin-treated, riboflavin-deficient animals may provide support for the antioxidant role of riboflavin which is mediated through the glutathione reductase-glu-

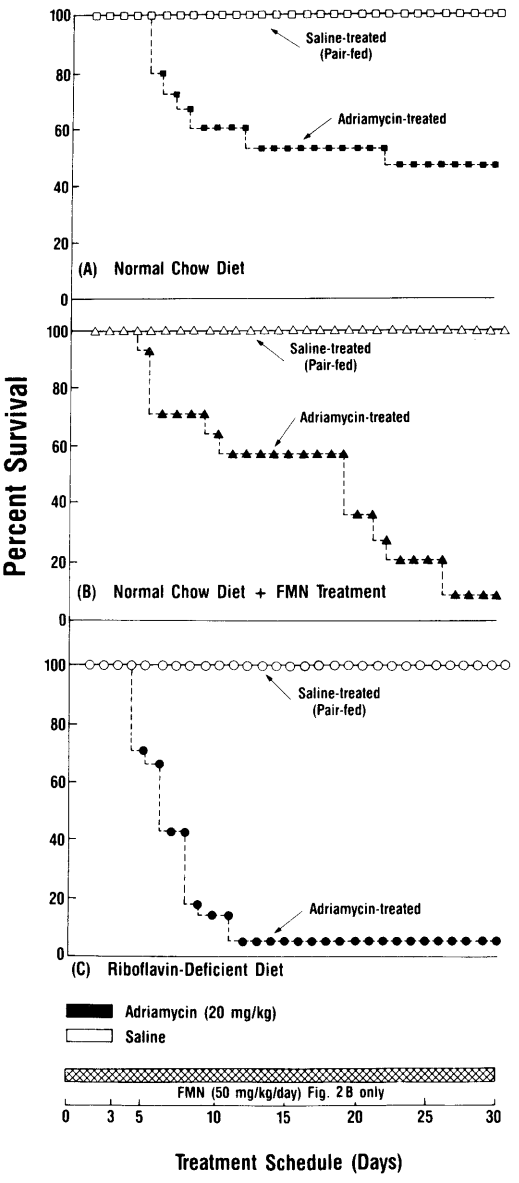

FIG. 2. Effects of Adriamycin upon survival in rats fed (A) a normal chow diet, (B) a normal chow diet and injected with FMN, or (C) a riboflavin-deficient diet compared to results in saline-treated, pair-fed controls. The treatment schedule for Adriamycin, saline, and FMN is shown below the survival graphs. Rats were placed on their respective diets for 4 weeks prior to Adriamycin administration. The differences in percentage survival between the Adriamycin-treated groups and their respective controls are significant: (A) $P < 0.01$; (B) $P < 0.001$; (C) $P < 0.001$. Differences in percentage survival among Adriamycin-treated groups are (A) vs (C) $P < 0.05$; (A) vs (B) not significant.

tathione peroxidase system. The production of free radicals and peroxides as a result of intracellular catabolism of Adriamycin can overwhelm the antioxidant capacity of cells and may contribute to the toxicities exhibited by anthracyclines. Myers *et al.* (12), working with other antioxidants, reported that the generation of free radicals by anthracycline quinones and hydroquinones may be diminished by pretreatment of animals with α-tocopherol. Selenium–vitamin E administration to Adriamycin-treated rabbits resulted in an increased survival and a decreased incidence and severity of cardiomyopathy (13). In other studies, ascorbate (14) and *N*-acetylcysteine (15) prevented early structural changes of cardiac tissue due to Adriamycin administration and may prevent cellular damage by providing antioxidant and/or free radical scavenging properties.

Other possible explanations for the increased mortality rate of riboflavin-deficient animals treated with Adriamycin may be an associated drug-induced enhancement of dietary riboflavin deficiency (4, 5). Under these conditions, inadequate synthesis of FAD possibly may interfere with mitochondrial electron transport which is needed for maintaining oxidative phosphorylation in metabolically active tissues. Furthermore, Adriamycin competes for binding with flavins on flavoenzymes and may also exacerbate riboflavin deficiency (8, 9).

In comparing survival advantage of Adriamycin-treated animals fed a normal chow diet alone (Fig. 2A) or fed a normal chow diet and administered FMN (Fig. 2B), no statistically significant difference is observed applying the log-rank test. While the log-rank test adequately addresses alterations early in survival, this test is less sensitive to events that occur late when few animals survive (11). Overall observation of these two survival curves (Figs. 2A and 2B) suggests that a biphasic response to treatment may be occurring in Adriamycin-treated, FMN-injected animals. Although these FMN-injected animals exhibit no difference in survival advantage within the initial 18–20 days, FMN injection appears to exacerbate the rate of mortality late in the treatment period. This apparent increase in mortality rate beginning after 18 days during injection with a high dose of FMN may possibly be the result of accelerated catabolism of Adriamycin to toxic free radical intermediates (8, 9). Following this treatment schedule, FMN administration and its subsequent intracellular conversion to FAD would be expected to stabilize several flavoenzymes which catabolize Adriamycin and promote free radical generation. The flavoenzyme NADPH cytochrome *P*-450 reductase transforms Adriamycin to a semiquinone free radical by shuttling electrons from NADPH to molecular oxygen (16). Xanthine oxidase, an FAD-dependent enzyme, can oxidize anthracyclines under anaerobic conditions and generate free radicals which may then react with DNA (17). In mitochondria, after exposure to anthracycline antibiotics, NADH dehydrogenase, an FMN- and FAD-requiring enzyme, generates superoxide anions and hydrogen peroxide (18). In general, these reactive free radicals, superoxides, and peroxides can exhaust the protective capacities of cells which include among other systems the glutathione reductase-glutathione peroxidase and the superoxide dismutase enzyme systems.

Comparable to our studies on Adriamycin-treated animals, other researchers have reported weight gains in animals placed on normal chow diets which contained an adequate amount of riboflavin (19, 20). Similarly, animals treated with Adriamycin in addition to receiving FMN demonstrated weight gain. These weight gains of both groups of animals may be attributable largely to ascites rather than to an increase in body mass. In addition, the results of the present study showed that the combined effects of Adriamycin treatment and riboflavin deficiency retard significant weight gain.

Thus, the significance of riboflavin nutriture is that it may relate to the effects of Adriamycin in a number of ways. These findings, in their entirety, do not exclude the possibility that administration of FMN in doses lower than that used in this study may confer some degree of protection against Adriamycin-induced mortality by providing an optimal balance between deficiency and

excess. Further research is required to establish an optimal level of dietary riboflavin which may maximize chemotherapeutic effectiveness and minimize toxicity and mortality rate. This interaction of a single vitamin with a chemotherapeutic agent also underscores the need to investigate multinutrient management and to consider overall nutritional status of the patient during anticancer therapy.

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