Biochemical Pharmacology, Vol. 44, No. 4, pp. 839–841, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

PZ-51 (Ebselen) *in vivo* protection against Adriamycin-induced mouse cardiac and hepatic lipid peroxidation and toxicity

(*Received* 9 *March* 1992; *accepted* 20 *April* 1992)

Abstract—Adriamycin® (Adr)-induced cardiotoxicity occurs most likely via an oxidative mechanism of action. Moderation of this activity may result in an improved therapeutic index for this compound. PZ-51, 2-phenyl-1,2-benzoisoselenazol-3(2H)-one, is a selenoorganic compound with thiol-dependent, peroxidase-like activity. We tested this compound alone and in combination with *N*-acetylcysteine (NAC) for its effect on Adr-induced *in vivo* toxicity in Balb/c mice. These studies demonstrated that PZ-51 protects against Adr-induced lipid peroxidation in heart and liver tissue and Adr-induced toxicity in general, as measured by total serum creatine kinase activity and body weight.

Adriamycin® (Adr*) is used as a primary chemotherapeutic agent in the treatment of a broad range of neoplastic diseases. The therapeutic efficacy of Adr in the treatment of human cancer is limited, however, by a variety of side-effects, two of which are bone marrow suppression and cardiotoxicity. Although the pathogenesis of the anthracycline cardiac toxicity remains uncertain, recent studies have suggested that drug-induced oxygen radicals are involved in damaging the heart [1–5]. The finding that cardiac cells have a limited capacity to enzymatically detoxify oxygen radicals [6–8] is further evidence that heart tissue may be particularly susceptible to injury by anthracycline-induced oxygen radicals.

Recently a novel selenoorganic compound, 2-phenyl-1,2-benzoisoselenazol-3(2H)-one (PZ-51: Ebselen), was synthesized. This compound has been found to have antioxidant activity [9], and has been shown to be thiol-dependent and peroxidase-like in activity [10]. It inhibits lipid peroxide accumulation in both microsomal membrane preparations [10] and isolated hepatocytes [11]. Further studies have shown that the combination of *N*-acetylcysteine (NAC) and PZ-51 protects isolated hepatocytes against diquat-induced oxygen radical cytotoxicity [12]. From these previous studies, it appeared that PZ-51, either alone or in combination with NAC, may be a good candidate for moderating the toxicity of Adr. In this paper, the effects of NAC and PZ-51 on Adr-induced lipid peroxidation and toxicity were tested *in vivo* using Balb/c mice.

Materials and Methods

Ebselen was a gift of the Ciba-Geigy Corp. (Summit, NJ). Adriamycin (D 5642) and *N*-acetylcysteine (A 9165) were purchased from the Sigma Chemical Co. (St. Louis, MO).

Lipid peroxidation studies. Female Balb/c mice were treated i.p. as described in Table 1. Forty-eight hours following Adr treatment the animals were killed. Heart and liver tissues were rinsed to remove excess blood, weighed, homogenized and subsequently assayed for lipid peroxidation by the thiobarbiturate acid test [13].

Total serum creatine kinase (CK) activity. Female Balb/c mice were treated identically to those in the lipid peroxidation studies. Blood samples, collected 48 hr post Adr injection, were centrifuged for 15 min in a refrigerated microcentrifuge and the serum was collected. Total CK activity was determined spectrophotometrically using a diagnostic kit purchased from the Sigma Chemical Co. (Diagnostic Kit 47–50).

Body weight change studies. Male Balb/c mice were treated as described in Fig. 2. Food and water were provided *ad lib.* The animals were observed daily and weighed periodically for up to 20 days.

Statistical analysis. Student's *t*-test was used to assess the differences between experimental groups.

Results and Discussion

Lipid peroxidation studies. One of the potentially toxic consequences of oxygen radical generation is lipid peroxidation. Adr has been shown to induce lipid peroxidation in a number of systems [4, 5, 8, 14–16] and therefore studies were conducted to determine whether pretreatment with PZ-51, alone or in combination with NAC, had any effect on Adr-induced lipid peroxidation in our *in vivo* system. The results of these studies are shown in Table 1. Similar results were obtained for both heart and liver tissues. In each case, treatment with Adr resulted in a significant increase in lipid peroxidation over control values. Pretreatment with PZ-51, NAC or the combination of both prior to Adr treatment resulted in a significant decrease in lipid peroxidation in these Adr-treated animals. Lipid peroxidation values for all three of the pretreatment groups were not significantly different from the control values in the cardiac tissue. The combination of PZ-51 and NAC was the most effective in protecting against lipid peroxidation in the mouse hepatic tissue. These studies demonstrate that in this model, pretreatment with PZ-51, NAC or the combination of both is capable of reducing Adr-induced lipid peroxidation.

Creatine kinase studies. Measurement of CK released into the blood is used diagnostically to indicate tissue damage. We measured blood serum CK levels in our treated mice as a measure of general tissue damage following Adr treatment to see whether the pretreatment with PZ-51, NAC or the combination of both would decrease this damage. The results of these studies are shown in Fig. 1. The data show that Adr treatment alone significantly increased serum CK levels as compared to control animals ($P < 0.001$). Pretreatment with NAC, PZ-51 or the combination of both significantly decreased serum CK activity in Adr-treated animals ($P < 0.001$). The combination of PZ-51 and NAC was the most effective at reducing serum CK activity. These studies therefore suggest that Adr-induced tissue damage, as measured by serum CK activity, can be inhibited significantly by pretreatment with PZ-51, NAC or the combination of both.

Body weight change studies. The data presented in Fig. 2 show the body weight changes resulting from pretreatment with PZ-51, alone or in combination with NAC, 1 hr prior to Adr treatment as a measure of toxicity. Animals receiving no Adr treatment gained 6–10% of their original body weight. Adr treatment alone resulted in a decrease in body weight throughout the course of the study.

* Abbreviations: Adr, Adriamycin; CAT, catalase; CK, creatine kinase; DMSO, dimethyl sulfoxide; NAC, *N*-acetylcysteine; PZ-51, 2-phenyl-1,2-benzoisoselenazol-3(2H)-one; and SOD, superoxide dismutase.

Table 1. Effects of PZ-51 and *N*-acetylcysteine (NAC) on Adriamycin-induced lipid peroxidation in Balb/c mouse heart and liver tissue

Drug	Pretreatment	Malondialdehyde (nmol/g wet wt tissue) Heart	Liver
None	None	34.7 ± 2.4	70.5 ± 12.9
Adriamycin	None	49.1 ± 6.8*	119.0 ± 17.1*
Adriamycin	NAC	35.0 ± 2.2†	97.1 ± 12.6*†
Adriamycin	PZ-51	37.1 ± 5.0†	85.1 ± 19.4†
Adriamycin	PZ-51 + NAC	34.4 ± 4.9†	73.0 ± 8.7†

Female Balb/c mice were treated i.p. with either control solvent [dimethyl sulfoxide (DMSO)] or PZ-51 (4 mg/kg body wt) and NAC (50 mg/kg body wt) dissolved in DMSO 1 hr prior to i.p. treatment with Adriamycin (20 mg/kg body wt) dissolved in sterile water or sterile water alone. Forty-eight hours following drug treatment the animals were killed. Each value is the mean ± SD obtained from at least ten animals.

* Significantly different from the control group value, no drug, no pretreatment ($P < 0.001$) by Student's *t*-test.

† Significantly different from Adriamycin-treated, no pretreatment value ($P < 0.001$) by Student's *t*-test.

Pretreatment with PZ-51 or PZ-51 and NAC prior to Adr treatment resulted in a recovery of weight not observed in the non-pretreated animals. These pretreated animals, 20 days post Adr treatment, had a significantly ($P < 0.01$) greater percentage of their initial body weight than the Adr-treated animals. These results are consistent with the hypothesis that pretreatment with PZ-51 or PZ-51 and NAC protects against Adr-induced toxicity in Balb/c mice.

The thiol-dependent, glutathione peroxidase-like activity of PZ-51 makes this antioxidant different from those previously tested against Adr [17–21] and potentially the most effective, as it is capable of detoxifying organic hydroperoxides and hydrogen peroxide. Studies using CDF1 mice [7] showed that cardiac tissue had relatively low levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) as compared to liver tissues. Cardiac SOD and CAT activities were 4 and 150 times less, respectively, than their corresponding liver activities. Glutathione peroxidase activities were similar in both tissues. These data lead the authors to conclude that SOD and glutathione peroxidase are the primary antioxidant enzymes involved in cardiac tissue defense against oxidative stress. Further studies [7] showed that a single dose of Adr administered i.p. results in a decrease in glutathione

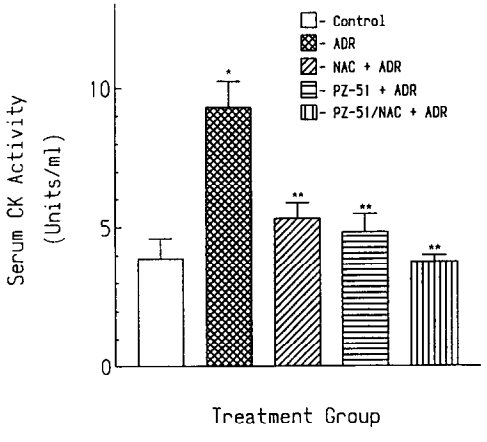

Fig. 1. Serum creatine kinase (CK) activities in Adriamycin-treated Balb/c mice. Female Balb/c mice were treated identically to those described in Table 1. Forty-eight hours post Adriamycin treatment the animals were killed, blood samples were taken, and CK activities were measured in serum. Values are means ± SEM from at least six animals. Key: (*) significantly different ($P < 0.001$) from control group; and (**) significantly different ($P < 0.001$) from Adr-treated group.

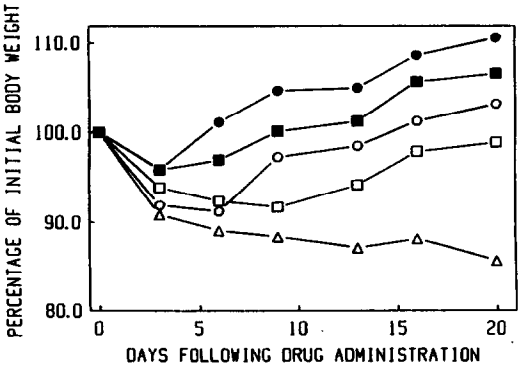

Fig. 2. Percentage of initial body weight of pretreated and drug-treated male Balb/c mice following drug administration. The experimental conditions are similar to those described in Table 1 with the exception that the Adriamycin treatment dosage was 12 mg/kg body wt. Initial body weights (ranging between 20 and 25 g) were taken prior to the antioxidant pretreatment and designated as 100% initial body weight on day 0. Subsequent body weight measurements were compared to the initial weight and expressed as a percentage. Values represent an average obtained from at least twelve animals per group. Key: (■—■) PZ-51, no drug; (●—●) PZ-51 + NAC, no drug; (△—△) Adriamycin, no antioxidant treatment; (□—□) PZ-51 + Adriamycin; and (○—○) PZ-51 + NAC + Adriamycin.

peroxidase activity 24 hr after injection to <44% of control values for cardiac tissue. These decreased enzyme activities were observed for up to 72 hr after drug administration. No effect was observed on SOD activity in these studies. Studies from our laboratory have extended these findings to show that Adr administered therapeutically in Balb/c mice not only causes a decrease in cardiac glutathione peroxidase activity, but also causes a decrease in glutathione reductase activity and reduced glutathione levels.* The peroxidase-like activity of PZ-51 may, therefore, be particularly effective at cardiac protection against Adr-induced toxicity as it may serve as a replacement for the lost glutathione peroxidase activity as well as a supplement for the catalase activity.

PZ-51 was observed to be more effective in protecting against diquat-induced toxicity in hepatocytes when used in combination with NAC [12]. The PZ-51 *in vivo* protective effect is shown in Figs. 1 and 2 where serum CK activities and body weight measurements are recorded. In both studies, the combination of PZ-51 + NAC gave greater protection against Adr toxicity than either agent alone, although the differences were not as dramatic as those observed in the *in vitro* studies [12]. Differences between our *in vivo* results and previous *in vitro* studies may be due to differences in *in situ* sulfhydryl levels and or pharmokinetics. However, all pretreatment regimens did provide significant protection when compared to non-pretreated, Adr-treated animals. It is highly significant that a low dose of PZ-51 alone could moderate Adr toxicity as well as, if not better than NAC alone (in our studies). Cardioprotectant NAC dosages cause patients extreme discomfort through nausea and vomiting. It may be possible to pretreat with PZ-51 alone prior to Adr treatment in order to reduce the Adr toxicity and avoid NAC discomfort.

Acknowledgements—This work was supported by NIH Grant CA-43660 and the Nevada Agricultural Experiment Station.

Department of Nutrition and Cellular and Molecular Pharmacology and Physiology Program
University of Nevada
Reno, NV 89557, U.S.A.

CHRIS A. PRITSOS†
MICHAEL SOKOLOFF
DANIEL L. GUSTAFSON

REFERENCES

1. Bachur NR, Gordon SL and Gee MV, Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. *Mol Pharmacol* **13**: 901–910, 1977.
2. Davies KJA and Doroshow JH, Redox cycling of anthracyclines by cardiac mitochondria. I. Anthracycline radical formation by NADH dehydrogenase. *J Biol Chem* **261**: 3060–3067, 1986.
3. Doroshow JH and Davies JKA, Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical. *J Biol Chem* **261**: 3068–3074, 1986.
4. Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K and Young RC, Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. *Science* **197**: 165–167, 1977.
5. Minnaugh EG, Trush MA and Gram TE, Stimulation by Adriamycin of rat heart and liver microsomal NADPH-dependent lipid peroxidation. *Biochem Pharmacol* **30**: 2797–3804, 1981.
6. Moore HW, Bioactivation as a model for drug design: Bioreductive alkylation. *Science* **197**: 527–532, 1977.
7. Doroshow JH, Locker GY and Myers CE, Enzymatic defenses of the mouse heart against reactive oxygen metabolites: Alterations produced by doxorubicin. *J Clin Invest* **65**: 128–135, 1980.
8. Goodman J and Hochstein P, Generation of free radicals and lipid peroxidation by redox cycling of Adriamycin and daunomycin. *Biochem Biophys Res Commun* 77: 797–803, 1977.
9. Müller A, Cadenas E, Graf P and Sies HA, A novel biologically active seleno-organic compound–I. Glutathione peroxidase-like activity *in vitro* and antioxidant capacity of PZ 51 (Ebselen). *Biochem Pharmacol* **33**: 3235–3239, 1984.
10. Wendel A, Fausel M, Safayhi H, Tiegs G and Otter R, A novel biologically active seleno-organic compound—II. Activity of PZ 51 in relation to glutathione peroxidase. *Biochem Pharmacol* **33**: 3241–3245, 1984.
11. Müller A, Gabriel H and Sies H, A novel biologically active selenoorganic compound—IV. Protective glutathione-dependent effect of PZ 51 (Ebselen) against ADP-Fe induced lipid peroxidation in isolated hepatocytes. *Biochem Pharmacol* **34**: 1185–1189, 1985.
12. Cotgreave IA, Sandy MS, Berggren M, Moldéus PW and Smith MT, *N*-Acetylcysteine and glutathione-dependent protective effect of PZ51 (Ebselen) against diquat-induced cytotoxicity in isolated hepatocytes. *Biochem Pharmacol* **36**: 2899–2904, 1987.
13. Wills ED, Evaluation of lipid peroxidation in lipids and biological membranes. In: *Biochemical Toxicology* (Eds. Snell K and Mullock B), pp. 127–152. IRL Press, Washington, DC, 1987.
14. Mimnaugh EG, Gram TE and Trush MA, Stimulation of mouse heart and liver microsomal lipid peroxidation by anthracycline anticancer drugs: Characterization and effects of reactive oxygen scavengers. *J Pharmacol Exp Ther* **226**: 806–816, 1983.
15. Mimnaugh EG, Trush MA, Bhatnagar M and Gram TE, Enhancement of reactive oxygen-dependent mitochondrial membrane lipid peroxidation by the anticancer drug Adriamycin. *Biochem Pharmacol* **34**: 847–856, 1985.
16. Thayer WS, Evaluation of tissue indicators of oxidative stress in rats treated chronically with Adriamycin. *Biochem Pharmacol* **37**: 2189–2194, 1988.
17. Lenzhofer R, Magometschnigg D, Dudczak R, Cernie C, Bolebruch C and Moser K, Indication of reduced doxorubicin-induced cardiac toxicity by additional treatment with antioxidant substances. *Experientia* **39**: 62–64, 1983.
18. Olson RD, MacDonald JS, vanBoxtel CJ, Boerth RC, Harbison RD, Slonim AE, Freeman RW and Oates JA, Regulatory role of glutathione and soluble sulfhydryl groups in the toxicity of Adriamycin. *J Pharmacol Exp Ther* **215**: 450–454, 1980.
19. Freeman RW, MacDonald JS, Olson RD, Boerth RC, Oates JA and Harbison RD, Effect of sulfhydryl-containing compounds on the antitumor effects of adriamycin. *Toxicol Appl Pharmacol* **54**: 168–175, 1980.
20. Van Fleet JF and Ferrans VJ, Evaluation of vitamin E and selenium protection against chronic Adriamycin toxicity in rabbits. *Cancer Treat Rep* **64**: 315–317, 1980.
21. Doroshow JH, Locker GY, Ifrim I and Myers CE, Prevention of doxorubicin cardiac toxicity in the mouse by *N*-acetylcysteine. *J Clin Invest* **68**: 1053–1063, 1981.

* Gustafson DL, Swanson JD and Pritsos CA, Modulation of glutathione and glutathione dependent antioxidant enzymes in mouse heart following doxorubicin therapy. Manuscript submitted for publication.

† Corresponding author. Tel. (702) 784-6443; FAX (702) 784-6449.