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INHIBITION OF CARDIAC NADP-LINKED ISOCITRATE DEHYDROGENASE BY ADRIAMYCIN

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SUMMARY: The inhibitory action of adriamycin against two NADP-linked dehydrogenases of rat heart was investigated. In heart muscle, isocitrate dehydrogenase activity is particularly high, whereas that of glucose-6-phosphate dehydrogenase is very low. Adriamycin inhibited the activity of both mitochondrial and cytoplasmic isocitrate dehydrogenase dose-dependently, but had no effect on glucose-6-phosphate dehydrogenase. The inhibiton by adriamycin was noncompetitive. Pre-incubation of the crude cardiac enzyme preparations with adriamycin enhanced the inhibition of isocitrate dehydrogenase time-dependently for 45 minutes.

INTRODUCTION

Adriamycin and daunomycin, anthracycline antibiotics, possess a high chemotherapeutic effectiveness against acute leukemia and many solid neoplasms(1-4). However, the clinical usefulness of these antibiotics has been limited by their undesirable cardiotoxic side effects(5-9). Several hypotheses have been proposed to explain the mechanism of the cardiotoxicity caused by adriamycin, such as the inhibition of DNA-dependent RNA synthesis(10), the inhibition of Na-K ATPase(11), the increase of ventricular tissue calcium concentration(12), the inhibition of oxidative phosphorylation in mitochondria(13,14) and peroxidation of cardiac lipids(15), but the precise mechanisms remain unknown.

The facts that the NADP-linked ICDH level in muscle is decreased in vitamin E deficient rabbits(16) and adriamycin cardiotoxicity is reduced by prior treatment with vitamin E(15) prompted us to study the effect of adriamycin on cardiac NADP-linked ICDH.

In this paper, we report the effect of adriamycin on two cardiac NADP generating enzymes, ICDH(E.C.1.1.1.42) and G6PDH(E.C.

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Abbreviation used: ICDH, isocitrate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase.

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1.1.1.49) and we suggest that an inhibitory action of adriamycin against NADP-linked ICDH could be one of the most important factors causing cardiotoxicity.

MATERIALS AND METHODS

Materials Male Wistar rats weighing about 200g were used. DL-isocitrate Na_3, glucose-6-phosphate, NAD, ADP, proteinase, and triethanolamine hydrochloride were purchased from Sigma Chemical Co.. Adriamycin was a gift from Kyowa Hakko Co.(Tokyo). A Simazu double beam spectrophotometer(UV-210A) was used for the enzyme assay.

Assay for the enzyme activities NAD- and NADP-linked ICDH were assayed according to the methods of Bernt et al.(17) and Alp et al.(18) respectively. G6PDH was assayed as described by Gloch et al.(19).

Tissue preparations Rats were killed by decapitation. The heart were dissected immediately and minced on ice cold Petri dishes. (1) 20% homogenates were made by loose glass-teflon homogenization using 0.25 M sucrose. Mitochondrial fractions were then prepared according to the methods of Hatefi et al.(20). Final mitochondrial pellets were suspended in 10 mM triethanolamine buffer(pH 7.5) and they were sonicated for 1 min. at an amplitude 6 um. The sonicated fluids were used for the assay of both NAD-linked and mitochondrial NADP-linked ICDH. (2) 20% homogenates were prepared by Polytron at 4°C using 10 mM triethanolamine buffer(pH 7.5). The homogenates were then sonicated under the same condition as described above and were used for the detection of total(mitochondrial plus cytoplasmic) NADP-linked ICDH. (3) 10% homogenates were made by Polytron at 4°C using 0.25 M sucrose. The homogenates were centrifuged at 20,000g and the supernatants were used for G6PDH assay.

Protein determination Proteins were determined by the method of Lowry et al.(21).

RESULTS AND DISCUSSION

Fig.1 shows the comparisons of the activity of NADP-linked ICDH and G6PDH in several organs in the rat. Heart muscle is a particularly rich source of NADP-linked ICDH, whereas G6PDH activity is very low.

NADP-linked ICDH is present both in mitochondria and cytoplasm(17), whereas NAD-linked ICDH and G6PDH are located only in mitochondria(18) and only in cytoplasm(19) respectively. Cytoplasmic NADP-linked ICDH activity was estimated by the subtraction of mitochondrial NADP-linked ICDH activity from the total(mitochondrial plus cytoplasmic) ICDH activity. The rate of mitochondrial and cytoplasmic NADP-linked ICDH activity was 4:1 by protein bases. Adriamycin inhibited approximately equally both

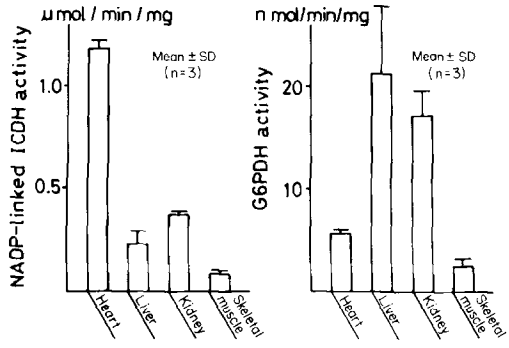


Fig. 1 The comparisons of the activity of total(mitochondrial plus cytoplasmic) NADP-linked isocitrate dehydrogenase and glucoce -6-phosphate dehydrogenase in several organs.

mitochondrial and cytoplasmic NADP-linked ICDH. Fig.2 shows that the total NADP-linked ICDH of the rat heart was inhibited by adriamycin dose-dependently. However, cardiac NAD-linked ICDH and G6PDH were not inhibited by adriamycin. The pyridine-linked dehydrogenase which requires either NAD or NADP as the electron acceptor, is one of the major oxidative-reductive enzymes which participate in the main stream of electron transport from organic substances to molecular oxygen. NADH, the reduced form of NAD, is primarily used for the generation of ATP through the oxidative

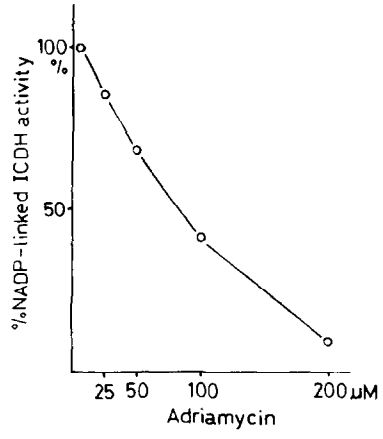
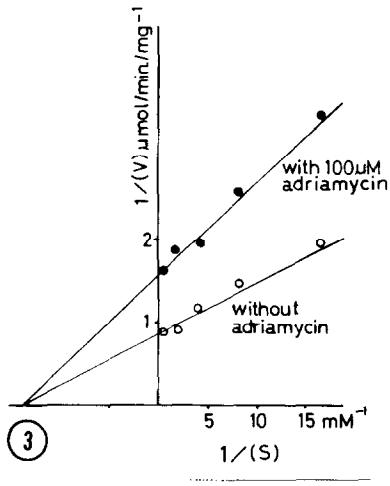


Fig.2 Dose-dependent inhibition of adriamycin against NADP-linked isocitrate dehydrogenase. 20% homogenate of heart(w/v) was prepared as described in Tissue preparations (2) in MATERIALS AND METHODS. App. 0.3% homogenate(diluted by homogenating buffer) was incubated for 30 min. at 37°C with various concentration (shown in abscissa) of adriamycin. The reference cuvette in the double beam spectrophotometer contained the identical solution as the reaction cuvette except for NADP. 100% represents NADP-linked isocitrate dehydrogenase activity after the preincubation for 30 min. at 37°C without adriamycin.



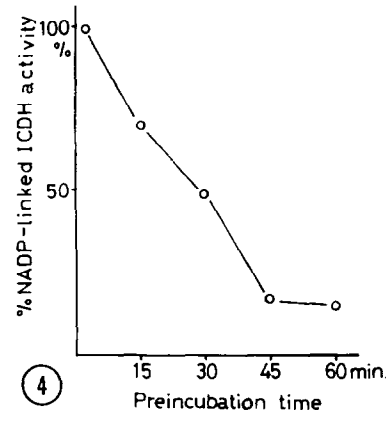


Fig.3 A double-reciprocal plot of enzyme kinetics in the presence and absence of 100 uM of adriamycin.

Fig.4 The enhancement of the inhibition of adriamycin against NADP-linked isocitrate dehydrogenase by increasing preincubation time with heart homogenate prepared as described in Tissue preparation (2) in MATERIALS AND METHODS. App. 0.3% homogenate (diluted by homogenating buffer) with or without 100 uM adriamycin were incubated at 37°C before the enzyme assay for various times. 100% represents NADP-linked isocitrate dehydrogenase activity without adriamycin at each time as indicated in abscissa.

phosphorylation system, whereas NADP, which is reduced by NADP-linked dehydrogenase is used almost exclusively for the reductive biosynthesis. Accordingly, in the case of ICDH, NAD-linked ICDH is important for the citric acid cycle and this is not affected by adriamycin. The role of the high level of NADP-linked ICDH is not yet fully known in heart tissue where the biosynthesis of fatty acid and steroid is not so active. However, the fact that adriamycin inhibits cardiac NADP-linked ICDH may have some relationship to its cardiotoxicity because cardiotoxicity is reduced by prior treatment with vitamin E(15) and vitamin E deficiency induces a decrease in NADPH concentration(22) and a decrease in NADP-linked ICDH levels(17).

Fig.3 shows that the inhibition of NADP-linked ICDH by adriamycin is kinetically distinguished as non-competitive inhibition. The Km value of cardiac NADP-linked ICDH is 66 uM for isocitrate and 30 uM for NADP, respectively.

Fig.4 shows that the inhibition of NADP-linked ICDH by adriamycin is enhanced by the incubation of adriamycin with the cardiac enzyme preparation before the assay of enzyme activity. One possibility is that adriamycin metabolites act as inhibitors of NADP-

linked ICDH. Adriamycin metabolites resulting from the C-7 reductive cleavage of adriamycin which requires NADPH as the sole electron donor for the enzymatic catalysis, are known(23-25) but the estimated endogenous NADPH concentration in cardiac tissue during incubation with adriamycin is negligible(26). These metabolites are unlikely to be formed in our system, although there may be other unknown metabolites. Recently Lucacchini et al. reported evidence of soluble protein binding of adriamycin (27). It is possible that NADP-linked ICDH is inactivated during the incubation by an interaction between adriamycin and the enzyme protein and the binding of adriamycin to this protein may be one of the reasons for the cardiotoxicity. In order to elucidate the enhancement of the inhibition by pre-incubation, further studies required to see if this protein has a high affinity for adriamycin.

NADPH, which is formed in the reaction cuvette during the assay of ICDH activity, may act with adriamycin to form the reduced adriamycin metabolites as described above. To exclude this possibility, exogenous NADPH was added in the reaction cuvettes which contained the same mixture used in the ICDH assay but without NADP and the decreased rate of NADPH in the reaction with adriamycin was investigated. As no decrease of NADPH was observed during the same reaction time as used in the ICDH assay, there is no possibility of this kind of interference.

REFERENCES

1) Di Marco,A.,Gaetani,M.,Dorigotti,L.,Soldati,M.,and Bellini, O. (1964) Cancer Chemother. Rep. 38, 31-38.
2) Di Marco,A. (1967) Pathol. Biol. 15, 897-902.
3) Tan,C.,Etcubans,E.,Wollner,N.,Rosen,G.,Gilladoga,A.,Showel, J.,Murphy,M.L.,and Krakoff,I.H. (1973) Cancer 32, 9-17.
4) Blum,R.H. and Carter,S.K. (1974) Ann. Int. Med., 80, 249-259.
5) Lefrak,E.A.,Pitha,J.,Rosenheim,S.,and Gottlieb,J.A. (1973) Cancer 32, 302-314.
6) Buja,L.M.,Ferrans,V.J.,Mayer,R.J.,Roberts,W.C.,and Henderson ,E.S. (1973) Cancer 32, 771-788.
7) Bristow,M.R.,Thompson,P.D.,Martin,R.P.,Marson,J.W.,Billingham ,M.E.,and Harrison,D.C. (1978) Am. J. Med. 65, 823-832.
8) Cortes,E.P.,Lutman,G.,Wanka,J.,Wang,J.J.,Pickren,J.,Wallance ,J.,and Holland,J.F. (1975) Cancer Chemother. Rep. Part 3, 6, 215-225.
9) Lenaz,L. and Page,J.A. (1976) Cancer Treat Rev. 3, 111-120.
10) Jaenke,R.S. (1974) Lab. Invest. 30, 292-304.
11) Gosalvez,M.,Van Rossum,G.D.V.,and Blanco,M.F. (1979) Cancer Res. 39, 257-261.
12) Olson,H.M. and Capen,C.C. (1977) Lab. Invest. 37, 386-394.

13) Mailer,K. and Peterring,D.H. (1976) Biochem. Pharmacol. 25, 2085-2089.
14) Kishi,T. and Forker,K. (1976) Cancer Treatment Rep. 60, 223-224.
15) Myer,C.E.,McGuire,W.P.,Liss,R.H.,Ifrim,I.,Grotzinger,K., and Youg,R.C. (1977) Science 197, 165-167.
16) Barry,T.A. and Rosenkrantz,H. (1962) J. Nutrition 76, 447-452.
17) Bernt,E. and Bergmeyer,H.U. (1974) Methods of Enzymatic Analysis(edited by Bergmeyer,H.U.) 2, 624-627.
18) Alp,P.R.,Newsholme,E.A.,and Zammit,V.A. (1976) Biochem. J. 154, 689-700.
19) Gloch,G.E. and Mclean,P. (1953) Biochem. J. 55, 400-408.
20) Hatefi,Y.,Jurtshuk,P.,and Haavik,A.G. (1961) Arch. Biochem. Biophys. 94, 148-155.
21) Lowry,O.H.,Rosenbrough,N.J.,Farr,A.L.,and Randall,R.J. (1951) J. Biol. Chem. L93, 265-275.
22) Dhalla,N.S.,Fedelesova,M.,and Toffler,I. (1971) Canadian J. Biochem. 49, 1202-1208.
23) Oki,T.,Komiyama,T.,Tone,H.,Inui,T.,Takeuchi,T.,andUmezawa,H. (1977) J. Antibiotics 30, 613-615.
24) Asbell,M.A.,Schwartzbach,E.,Bulloch,F.J.,and Yesair,D.W. (1972) J. Pharmacol. Ther. 182, 63-69.
25) Handa,K. and Sato,S. (1976) Gann 67, 523-528.
26) Williamson,D.H. and Broanan,J.T. (1974) Methods of Enzymatic Analysis(edited by Bergmeyer,H.G.) 4, 2266-2302.
27) Lucacchini,A.Martini,C.Segini,D.,and Ronca,G. (1979) Experimentia 35, 1148-1149.