Pergamon

Biochemical Pharmacology, Vol. 47, No. 11, pp. 2111–2112, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0088-3

SHORT COMMUNICATION

Inhibition of nitric oxide synthase by antineoplastic anthracyclines

(*Received* 16 *September* 1993; *accepted* 24 *January* 1994)

Abstract—Nitric oxide synthase, the enzyme responsible for the synthesis of nitric oxide and citrulline from arginine, was potently inhibited by the anthracycline antibiotics doxorubicin ($K_i = 24\ \mu M$) and aclarubicin ($K_i = 50\ \mu M$). These drugs were non-competitive inhibitors with respect to arginine. This action on nitric oxide synthase may explain some of the cardiovascular and cytotoxic actions of these chemotherapeutic drugs.

Key words: doxorubicin; aclarubicin; nitric oxide synthase; chemotherapeutics; anthracycline antibiotics

Anthracycline antibiotics, such as doxorubicin, are clinically effective against hematological and solid tumors [1]. The cytotoxic action of these drugs may involve a variety of mechanisms including disruption of DNA structure [2] and the generation of reactive oxygen radicals [3]. There is evidence for the oxidation of the quinone moiety of the anthracyclines by xanthine oxidase, NADPH dehydrogenase and purified NADPH–cytochrome P450 reductase [3–6]. Recently, nitric oxide synthase has been shown to be structurally related to NADPH–cytochrome P450 reductase, and to possess a cytochrome reductase activity [7, 8]. We now demonstrate that the anthracyclines are potent inhibitors of this enzyme. This may explain some of the cardiovascular effects of these drugs.

Materials and Methods

Adult male Wistar rats were decapitated, and the cerebella were removed, rinsed and homogenized in 10 vol. of ice-cold 50 mM HEPES buffer, containing 1 mM EDTA, pH 7.4. The homogenates were centrifuged at 10,000 *g* at 4° for 40 min, the supernatant was collected and protein concentration was determined [9]. Nitric oxide synthase activity was measured by monitoring the conversion of tritiated arginine to citrulline [10]. The assay was begun by mixing 25 μL of L-arginine (at 3, 10 or 30 μM), 25 μL of [^{3}H]L-arginine (44 nM, 2.5 μCi/mL, NEN), 25 μL of HEPES buffer containing 4 mM calcium chloride, 0.4 mM NADPH and 1600 units calmodulin/mL, 25 μL of brain supernatant (150–200 μg protein), and 25 μL of doxorubicin or aclarubicin (Sigma). This mixture was incubated at 37° for 3.5 min and the reaction was stopped by adding 2 mL of ice-cold 20 mM HEPES buffer containing 20 mM EDTA, 0.2 mM EGTA, pH 5.5. Citrulline was then separated from arginine with a Dowex-50W cation exchange column and quantified with a liquid scintillation counter.

Results

Under the assay conditions used, nitric oxide synthase activity had a K_m for L-arginine of 5.2 μM, and a V_{max} of 1 nmol/mg protein/min. The inhibition of nitric oxide synthase by doxorubicin and aclarubicin is illustrated in Fig. 1. Both drugs produced a non-competitive inhibition with respect to L-arginine. Doxorubicin ($K_i = 24\ \mu M$) was about twice as potent an inhibitor of nitric oxide synthase as was aclarubicin ($K_i = 50\ \mu M$).

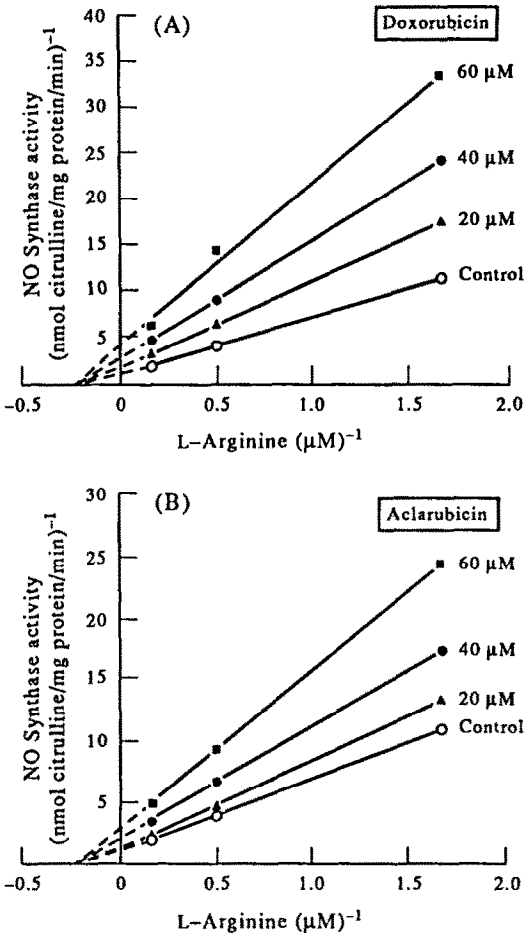

Fig. 1. Double-reciprocal plots of the inhibition of rat brain nitric oxide synthase by anthracyclines. Inhibition with respect to L-arginine is illustrated for doxorubicin (A) and aclarubicin (B) at concentrations of 0, 20, 40 and 60 μM, while L-arginine varied from 0.6 to 6 μM. The figures are representative of four independent determinations, each run in duplicate.

Discussion

A major problem with anthracyclines is their cardiotoxicity [1]. In addition, these drugs have vascular actions as well. High doses of aclarubicin reduce vascular smooth muscle contractility [11, 12]. Of particular interest, however, is the observation that aclarubicin, at concentrations similar to those seen in blood during chemotherapy, inhibits endothelium-dependent relaxation of rat aorta [13]. It also suppresses the increase in cGMP seen in response to acetylcholine, but not that to sodium nitroprusside [13]. These observations suggest an inhibitory effect of aclarubicin on nitric oxide production. Consistent with this hypothesis, we have now demonstrated that aclarubicin and doxorubicin are potent inhibitors of neuronal nitric oxide synthase. Given the similar structure and mechanism of the neuronal, endothelial and inducible nitric oxide synthases [14], it is likely that all of these isoforms would be similarly affected. Inhibition is probably due to the NADPH-dependent reduction of molecular oxygen, with the quinone group of these drugs acting as an electron acceptor. Thus, oxidation of the anthracyclines by nitric oxide synthase, like that seen with NADPH–cytochrome P450 reductase, would be predicted to result in the production of hydrogen peroxide, superoxide anion and hydroxyl radicals [3–6]. Indeed, nitric oxide synthase has been shown to generate both hydrogen peroxide and superoxide under certain circumstances [15, 16]. This might account for the damage to the vascular endothelium induced by these drugs [17], since these cells contain high levels of nitric oxide synthase and would be exposed to high levels of these drugs. A similar action on the nitric oxide synthase in the endocardial cells [18] may be responsible for some of the cardiac toxicity seen with these drugs. Such a mechanism would be consistent with the higher potencies of doxorubicin on nitric oxide synthase and the higher incidence of vascular side-effects seen with this drug compared with aclarubicin.

Oxidation of anthracyclines by nitric oxide synthase may also be important in the chemotherapeutic action of these drugs. The inhibition of endothelial nitric oxide synthase by these drugs in the tumor-associated neovasculature would reduce local blood flow [19]. Furthermore, some tumor cells themselves express nitric oxide synthase [20]. Thus, intracellular generation of active oxygen radicals by the oxidation of anthracyclines by this enzyme may contribute to cytotoxicity.

Acknowledgement—This research was supported by a grant from the Medical Research Council of Canada.

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