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Cardiotoxicity of copper-based antineoplastic drugs casiopeinas is related to inhibition of energy metabolism

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Abstract

Isolated rat hearts were perfused with glucose, octanoate or glucose + octanoate and different concentrations of the copper-based antineoplastic drugs casiopeina II-gly (CSII) or casiopeina III-i-a (CSIII). In isolated perfused hearts with glucose + octanoate, both casiopeinas induced diminution in cardiac work and O_2 consumption with half-maximal inhibitory concentrations (IC_{50}) of 4 (CSII) and 4.6 (CSIII) μM, after 1 h of perfusion. Strong inhibition of the pyruvate and 2-oxoglutarate dehydrogenases as well as total creatine kinase by casiopeinas suggested that ATP generation by oxidative phosphorylation and its transfer towards myofibrils were targets for these drugs. In consequence, the cellular contents of ATP and phosphocreatine were also lowered by casiopeinas. Remarkably, casiopeinas were less toxic than adriamycin (IC_{50} = 2.6 μM), a well-known potent cardiotoxic and antineoplastic drug, which has a wide clinical use. In an open-chest animal, which is a more physiological model than the isolated heart, femoral administration of 1 μM drug revealed that CSII was innocuous very likely due to strong binding to serum albumin, whereas adriamycin induced again a potent cardiotoxic effect (diminution in heart rate and severe depression of systolic blood pressure). Thus, it seems that casiopeinas are a group of new antineoplastic drugs with milder secondary toxic effects than proven drugs such as adriamycin.

Keywords: Cardiotoxicity; Work performance; Oxygen consumption; 2-oxoglutarate dehydrogenase; Pyruvate dehydrogenase; Creatine kinase

Introduction

Significant advance in the treatment of several types of cancer has been achieved by improvements in the radiotherapeutic techniques and in the design of new drugs. However, some of the most effective antineoplastic drugs also promote severe side effects such as acute and chronic nephro- (Goldstein and Mayor, 1983) and cardiotoxicity (Ace, 1992). The most potent cardiotoxic antineoplastic drugs are the anthracyclins such as adriamycin (doxorubicin), daunomycin and epirubicin (Wallace, 2003; Minotti et al., 2004).

The biochemical mechanism of the adriamycin-induced cardiotoxicity seems to be primarily mediated by the generation of reactive free radical molecules (Rajagopalan et al., 1988; Wallace, 2003; Wang et al., 2004), its interaction with mitochondrial cardiolipin and proteins that leads to inhibition of oxidative phosphorylation (Wallace, 2003) and by DNA damage (Minotti et al., 2004).

Cisplatin is one of the most widely used antineoplastic drugs because it has proved to be effective in treating a variety of solid tumors, particularly testicular cancer (Williams and Einhorn, 1982; Ensley et al., 1984). Unfortunately, the clinical success of cisplatin has been limited by its severe side effects on kidney function (Goldstein and Mayor, 1983) and by the development of resistance in many tumor cell lines (Ara et al., 1994). In consequence, new metal-based antineoplastic drugs have

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been designed, seeking to improve their therapeutic properties over those shown by cisplatin.

The copper-based drugs called casiopeinas exhibit higher antineoplastic potency than cisplatin in vitro and in vivo studies of several tumor cell lines (De-Vizcaya-Ruiz et al., 2000; Gracia-Mora et al., 2001). Similarly to adriamycin (Wallace, 2003), casiopeinas II-gly (CSII) and III-i-a (CSIII) showed potent inhibitory effect on mitochondrial functions in experiments with isolated mitochondria and intact cells from AS-30D hepatoma (Marin-Hernández et al., 2003). Therefore, to determine whether casiopeinas have diminished side effects in high energy demanding organs such as the heart, in comparison to well-established antineoplastic drugs such as cisplatin and adriamycin, the effect of casiopeinas II-gly and III-i-a on the heart performance and energy metabolism was analyzed.

Materials and methods

Chemicals. 1,4-Dithio-DL-threitol (DTT), thiamine pyrophosphate, Triton X-100, sodium pyruvate, 2-oxoglutaric acid, glucose, octanoic acid, phosphocreatine, NAD^+, NADH, ADP and ATP were acquired from Sigma Chemicals Co. Hexokinase, glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* and lactate dehydrogenase were from Roche. All other chemicals of analytical grade were from J.T. Baker.

Casiopeinas II-gly [(4,7-dimethyl-1,10-phenanthroline) (glycinate) copper (II) nitrate] and III-i-a [(4,4-dimethyl-2,2-bipyridine) (acetylacetonate) copper (II) nitrate] were synthesized as previously described (Ruiz Azuara, 1992). Cisplatin and adriamycin (doxorubicin) were from Sigma and Pharmacia Upjohn, respectively. Solutions of the four antineoplastic drugs in perfusion buffer (see below) were freshly prepared, before starting the experiment.

Animals. Animals were housed in the institutional animal facility, kept under daily cycles of 12 h light/12 h dark and fed ad libitum with standard chow rat and tap water. Experimental manipulations were carried out by following the Instituto Nacional de Cardiología de México guidelines.

Isolated heart preparation. Hearts were excised from male Wistar rats of 300–350 g weight and mounted by the Langendorff technique as previously described (Carvajal et al., 2003). The animals were anesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p.). The chest was opened and the heart quickly removed and placed briefly in ice-cold perfusion buffer to arrest it and prevent ischemic preconditioning (Minhaz et al., 1995). The heart was then suspended from a cannula and perfused retrogradely through the ascending aorta at a constant perfusion pressure of 60 mm Hg and at a constant flow rate of 10 ml/min by means of a peristaltic pump.

The composition of the perfusion buffer was (in mM): 120 NaCl, 23.4 $NaHCO_3$, 4.8 KCl, 1.2 KH_2PO_4, 0.86 $MgSO_4$, 1.25 $CaCl_2$ and oxidizable substrate (10 glucose or 0.1 octanoate or both), of pH 7.4; the temperature was maintained at 37 °C. The perfusion medium was continuously bubbled with a gas mixture of 95% O_2–5% CO_2. After the heart began contracting spontaneously, a latex balloon, connected to a pressure transducer, was inserted into the left ventricle. Once inserted, the balloon was filled with perfusion buffer at a steady diastolic pressure of 10 mm Hg. Two silver electrodes were attached, one at the apex and the other at one atrium, for electrocardiogram monitoring. The pulmonary artery was cannulated and connected to a closed chamber to measure the O_2 concentration in the coronary effluent by means of a Clark-type oxygen electrode. The rate of oxygen uptake was calculated as the difference between the oxygen concentration in the perfusion medium before and after passing through the organ.

Left intraventricular pressure, electrical activity and O_2 uptake were monitored simultaneously (Carvajal et al., 2003) under the conditions detailed in the Results section using a computer acquisition data system SIEVART1 designed by the Instrumentation and Technical Development Department from the Instituto Nacional de Cardiología de México.

Determination of metabolites and enzyme activities. At the end of the in vitro experiments, the isolated beating heart was rapidly removed, frozen in liquid N_2 and powdered in a mortar under liquid N_2. A portion of 0.3 g of the frozen tissue was mixed with 5 volumes of 3% (v/v) perchloric acid/20 mM EDTA. The last suspension was neutralized with KOH/Tris; after centrifugation, the supernatant was stored at −72 °C until use for determination of ATP and phosphocreatine (Bergmeyer, 1983). For determination of enzyme activities, another portion of 0.2 g of the frozen tissue was mixed with a medium that contained 100 mM HEPES pH 7.2, 0.1% Triton X-100, 1 mM EGTA, 0.035 mM phenylmethanesulfonyl fluoride and 0.2 μg leupeptin/ml.

Activity of the pyruvate dehydrogenase complex was spectrophotometrically assayed at 340 nm in 1 ml of a medium that contained 40 mM HEPES pH 7.1, 0.8 mM $MgCl_2$, 0.18 mM thiamine pyrophosphate, 2 mM NAD^+, 10 mM L-lactate, 0.8 mM DTT and 2 μM rotenone (Hansford and Castro, 1985); the reaction was started by addition of 0.08 mM CoA.

Activity of the 2-oxoglutarate dehydrogenase complex was determined by the change in absorbance at 340 nm in 1 ml of a medium that contained 40 mM HEPES pH 7.1, 0.8 mM $MgCl_2$, 0.18 mM thiamine pyrophosphate, 2 mM NAD^+, 0.8 mM DTT, 2 μM rotenone and 10 mM 2-oxoglutarate (Rodríguez-Zavala et al., 2000); the reaction was started by addition of 0.08 mM CoA.

Activity of creatine kinase was determined at 340 nm in 1 ml of a medium that contained 100 mM imidazole pH 7.6,