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Toxicology

Respiratory complex II in mitochondrial dysfunction-mediated cytotoxicity: Insight from cadmium

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ABSTRACT

In the present work we studied action of several inhibitors of respiratory complex II (CII) of mitochondrial electron transport chain, namely malonate and thenoyltrifluoroacetone (TTFA) on Cd^{2+}-induced toxicity and cell mortality, using two rat cell lines, pheochromocytoma PC12 and ascites hepatoma AS-30D and isolated rat liver mitochondria (RLM). It was shown that malonate, an endogenous competitive inhibitor of dicarboxylate-binding site of CII, restored in part RLM respiratory function disturbed by Cd^{2+}. In particular, malonate increased both phosphorylating and maximally uncoupled respiration rates in KCl medium in the presence of CI substrates as well as palliated changes in basal and resting state respiration rates produced by the heavy metal on the mitochondria energized by CI or CII substrates. Notably, malonate enhanced Cd^{2+}-induced swelling of the mitochondria energized by CI substrates in KCl and, in a much lesser extent and at higher [Cd^{2+}], in sucrose media but did not influence on the Cd^{2+} effects in NaCl medium. Besides, malonate did not affect swelling in sucrose media of RLM energized by CIV substrates under using of Cd^{2+} or Ca^{2+} whereas it strongly increased the mitochondrial swelling produced by selenite. In addition, malonate produced some protection against Cd^{2+}-promoted necrotic death of AS-30D and PC12 cells and reduced intracellular reactive oxygen species (ROS) formation evoked by Cd^{2+} in PC12 cells. Importantly, TTFA, an irreversible competitive inhibitor of Q-binding site of CII, per se induced apoptosis of AS-30D cells which was inhibited by co-treatment with Cd^{2+} as well as decreased the Cd^{2+}-enhanced intracellular ROS formation. In turn, decylubiquinone (dUb) at low μM concentrations did not protect AS-30D cells against the Cd^{2+}-induced necrosis and enhanced the Cd^{2+}-induced apoptosis of the cells. High μM concentrations of dUb were highly toxic for the cells. As consequence, the findings give new evidence indicative of critical involvement of CII in mechanism(s) of Cd^{2+}-produced cytotoxicity and support the notion on CII as a perspective pharmacological target in mitochondria dysfunction-mediated conditions and diseases.

1. Introduction

Mitochondria are key cell targets for many highly toxic environmental and occupational pollutants, including heavy metals [1]. Among them, cadmium is one of the main toxic metals concealing a great danger to human health [2,3]. An important aspect in cadmium cytotoxicity is to identify the most likely binding sites for the harmful bivalent metal cation (Cd^{2+}). As found now, an oxidative stress and mitochondrial dysfunction mediated by the disturbance of the mitochondrial electron transport chain (mtETC) and by the induction of Ca^{2+}-dependent nonselective high-conductance pore of the inner mitochondrial membrane (IMM), so called mitochondrial permeability transition (MPT) pore ([4,5] and references therein), are involved in mechanism(s) of cytotoxic action of Cd^{2+} [6–26]. As known, the MPT pore is a pH- and voltage-dependent IMM megachannel of unknown structure that makes the membrane permeable for solutes with

Abbreviations: CI, CII, CIII, CIV, CV, complexes I, II, III, IV, V of mitochondrial respiratory chain; TTFA, thenoyltrifluoroacetone; RLM, rat liver mitochondria; ROS, reactive oxygen species; dUb, decylubiquinone; mtETC, mitochondrial electron transport chain; MPT, mitochondrial permeability transition; IMM, inner mitochondrial membrane; P_i, inorganic phosphate; MCU, mitochondrial Ca^{2+} uniporter; CyP-D, cyclophilin D; CsA, cyclosporine A; ANT, adenine nucleotide translocase; Stig, stigmatellin; Rot, rotenone; Diazo, diazoxide; mitoK(ATP), mitochondrial ATP-sensitive K^+ channel; SQR, succinate:ubiquinone oxidoreductase; SDH, succinate dehydrogenase; Ub or CoQ_{10}, ubiquinone or coenzyme Q; PI, propidium iodide; $DCFH_2$-DA, 2′,7′-dichlorodihydrofluorescein diacetate; $DCFH_2$, 2′,7′-dichlorodihydrofluorescein; DCF, 2′,7′-dichlorofluorescein; LDH, lactate dehydrogenase; Myx, myxothiazol; Ant A, antimycin A; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; PBS, phosphate buffered saline; TB, trypan blue; EGTA, [ethylenebis(oxyethylene-nitrilo)]tetraacetic acid; BSA, bovine serum albumin; DNP, 2,4-dinitrophenol; oligo, oligomycin; RR, ruthenium red, Asc, ascorbate; TMPD, tetramethyl-*p*;-phenylenediamine; DTT, dithiotreitol; RHM, rat heart mitochondria; IPC, ischemic preconditioning; I/R, ischemia-reperfusion; NAC, N-acetylcysteine

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molecular mass lesser 1500 Da and an opening of which is involved in different types of cell death and various pathological conditions and diseases. Usually the MPT pore is activated under conditions of oxidative stress and calcium overload, and its opening is stimulated by elevated phosphate (P_i), depletion of adenine nucleotides as well as by the oxidized state of pyridine nucleotides and of critical dithiols in at least two discrete redox-sensitive sites, P- and S-site, the localization of which is still uncertain. Ca^{2+} is considered to be a trigger of MPT pore opening. Mg^{2+} and the most of bivalent metal ions accumulated by mitochondrial calcium uniporter (MCU) like Sr^{2+}, Mn^{2+}, and Ba^{2+} (but not Cd^{2+}) behave themselves as the pore inhibitors. An existence of at least two separate Me^{2+}-binding sites on the MPT pore complex: external (inhibitory) and internal (activating) is widely accepted; however, the exact localization of these sites is not defined finally. As considered, H^+ and Ca^{2+} compete for the Ca^{2+}-trigger site(s). The MPT pore opening is modulated by various regulatory proteins (especially by matrix protein cyclophilin D, CyP-D, which is detached from the other components of the pore by cyclosporine A, CsA) and by different ubiquinone (Ub) analogs. Moreover, several mtETC components, in particular respiratory complexes I (CI) and III (CIII) are proposed to contribute in its formation and/or regulation. In addition, for late years F_0F_1-ATPase (CV of mtETC), namely its dimer and/or c-ring are suspected to be involved in some way (likely conformation-dependent) in creation of channel-forming subunit(s) of the MPT pore. Several other components of ATP synthasome, mainly adenine nucleotide translocase (ANT) and P_i carrier that previously considered to be structural components of the MPT pore, now are generally accepted to be only regulatory ones ([4,5,18,22,25,27,28] and references therein).

Before we have shown that not only various MPT inhibitors and antioxidants but also inhibitors of CIII (stigmatellin, Stig) and, in some cases, of CI (rotenone, Rot) exhibit some beneficial effects against Cd^{2+}-produced toxicity [14,18,29–31]. Nevertheless, contribution and role(s) of individual complexes of mtETC in mechanism(s) of Cd^{2+}-induced mitochondrial dysfunction and cell death are not completely understood. Interestingly, we have found earlier that diazoxide, Diazo (i.e., an opener of mitochondrial ATP-sensitive K^+ channel, mitoK (ATP) [32]) is partially protective against cell death produced by Cd^{2+} on two types of rat cell lines, ascites hepatoma AS-30D and neuron-like pheochromocytoma PC12 [33,34]. At the same time, Diazo, as shown by us and other investigators, was not effective against Cd^{2+}-induced injury on isolated mitochondria of rat liver [34,35] and kidney [36] as well as of fish liver [37] in contrast to rat heart mitochondria [38]. It is intriguing that Diazo is also considered to be an inhibitor of Q-binding site of respiratory complex II, CII [39,40].

Mitochondrial CII (succinate:ubiquinone oxidoreductase, SQR; succinate dehydrogenase, SDH) is the smallest mtETC complex that in opposite to the other respiratory complexes does not pump protons via the membrane and is fully encoded by nuclear DNA. CII comprises from four subunits (hydrophilic part: SDHA and SDHB, and hydrophobic part: SDHC and SDHD) and directly connects the respiratory chain with Krebs cycle (or the tricarboxylic acid cycle). CII has two activities: (i) SDH activity which is determined as electron flux from FAD cofactor in SDHA at dicarboxylate-binding site of CII (also called flavin site or site II_F) to three [Fe-S] clusters located in SDHB and (ii) SQR activity which is determined as electron flux starting from succinate and finishing at ubiquinone (Q)-binding site of CII (Q_p or II_Q) located at SDHC/D interface with SDHB. SDHC and SDHD "anchor" the complex to the IMM and their transmembrane domains contain also a redox group, heme b, bound at their interface whose function within CII is not finally underscore ([41] and references therein). At present it is known that CII is a key regulator of mitochondrial reactive oxygen species (ROS) and can be both a source and an enhancer or suppressor of ROS generation by other mtETC complexes, mainly CI and CIII. Besides, CII is found to be a general sensor for apoptosis induction and an emergent target for mitocans (mitochondrial anti-cancer agents), such as vitamin E and ubiquinone (Ub or CoQ_{10}) analogs [39–41].

It should be noted that despite mounting evidence concerning in vivo and in vitro CII injury by Cd^{2+} [6,8,9,12,17,19,31,42–50], not all is clear on the issue up-to-date. It seemed meaningful to elucidate mechanism(s) of interactions of Cd^{2+} and CII in the process of induction by the toxic metal of mitochondrial dysfunction and cell death. So, in the present study we investigated an influence of CII effectors, in particular malonate, thenoyltrifluoroacetone (TTFA) and decylubiquinone (dUb) on the Cd^{2+}-induced toxicity and cell mortality on the same model as we used before, namely isolated RLM and two types of cells, AS-30D and PC12 [12–14,17,20,22,29–31]. The data obtained herein indicate to the crucial contribution of CII in the Cd^{2+}-induced cytotoxicity as well as give new important information concerning proposed Cd^{2+}-binding site(s) on CII and an involvement of mtETC components in mechanism(s) of MPT and cell death induction.

2. Materials and methods

2.1. Chemicals

The most of reagents was purchased from Sigma Aldrich Company (St. Luis, MO, USA). CsA was from Novartis (Basel, Switzerland). Propidium iodide (PI) and 2′,7′-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) were from Molecular Probes (Eugene, OR). All other chemicals used were of the highest purity, commercially available. RPMI-1640 medium containing 20 mM Hepes-NaOH (pH 7.4) and supplemented with 2 mM L-glutamine and 10% fetal calf serum was supplied by the Hirszfeld Institute of Immunology and Experimental Therapy (Wroclaw, Poland). DMEM incubation medium with L-glutamine, horse blood serum, fetal calf serum and trypsin-EDTA were purchased in Biolot Company (Russia).

2.2. Cell viability assays

The experiments were conducted on cultures of rat pheochromocytoma PC12 and ascites hepatoma AS-30D cells in the same way as earlier (see [22] and [29], respectively). Briefly, the cells (PC12 or AS-30D) were pre-incubated, correspondingly, in the DMEM or the RPMI-1640 media with different effectors or without them for 30 min in 6, 12 or 24 well plates or in Petri dishes at 37 °C. After that the respective concentration of Cd^{2+} was added to each well or Petri dish, in particular 10, 50, 100 or 500 μM in accordance with the experimental model originated from our previous works [20,22,29]. Concentrations of complex II inhibitors tested were the following: malonate – 0.5 and 1 mM, TTFA – 1 and 5 μM. In addition, several other respiratory chain inhibitors were used, namely Rot (1 μM), Stig (1 μM), myxothiazol, Myx (1 μM), and antimycin A, Ant A (1 μM). Concentrations of CsA under study was 1 or 5 μM, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) or carbonyl cyanide 3-chlorophenylhydrazone (CCCP) – 1 μM, dUb – 10, 50, or 250 μM. Used in the investigation, malonate was dissolved in water while TTFA, dUb, CsA, Stig, Myx, Ant A, FCCP or CCCP were dissolved in DMSO or ethanol. $CdCl_2$ dissolved in water was used as a 10 mM stock solution that was diluted in further experiments by medium or phosphate buffered saline (PBS) to the needed concentrations.

2.2.1. PC12 cells

The culture of PC12 cells was maintained in CO_2 incubator in the atmosphere containing 5% of CO_2 at 37 °C as before [22]. DMEM with L-glutamine was applied as an incubation medium, containing 25 U/ml of streptomycin, 25 μg/ml of penicillin, 10% of fetal calf serum and 5% of horse blood serum. The incubation medium was changed every two days. In some experiments the assay medium (DMEM with L-glutamine and antibiotics) not containing serum was used. For estimation of cell mortality by the lactate dehydrogenase (LDH) release assay, PC12 cells were seeded to 24 well plates in concentration of $2{,}5 \times 10^5$ cells in each well and the measurements began 24 h after administration of the cells