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Acetaldehyde targets superoxide dismutase 2 in liver cancer cells inducing transient enzyme impairment and a rapid transcriptional recovery

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ABSTRACT

Alcohol is undoubtedly, the main toxic agent that people consume by recreation and the abuse is associated with liver damage, mainly by the overproduction of reactive oxygen species and the toxic effects of its first metabolite acetaldehyde. It is known that acetaldehyde targets mitochondria inducing redox imbalance and oxidative stress. Mitochondrial superoxide dismutase transforms superoxide radical into hydrogen peroxide, which in addition, is transformed in water by other enzymes. In the present study we demonstrate that acetaldehyde transiently impairs SOD2 activity in HepG2 cells, the decrease in the enzyme activity was associated to a reduction in the protein content, which was rapidly recovered, to basal values, by synthesis *de novo* in a mechanism mediated by NF-κB and PKC. The SOD2 impairment was not associated with adduct formation. The recovery on SOD2 activity in HepG2 cells can represent survival advantage for cancer cells, the results shown that SOD2 could be considered a therapeutic target in liver cancer.

1. Introduction

The liver is constantly subjected to dramatic redox changes due to its function in metabolism and detoxification. Several insults from air, food, or drinks are constantly transformed in the liver producing detoxification intermediaries that usually are even more toxic than the first ones, among these, reactive oxygen species (ROS), such as superoxide anion ($O_2^{-\cdot}$) or hydrogen peroxide; and in the case of ethanol, its first metabolite acetaldehyde (Ac), are responsible of cell damage. Alcoholic liver disease (ALD) is still one of the main causes for liver-related deaths (Druesne-Pecollo et al., 2009) worldwide, important advantages have been taken last years to understand how ethanol induces liver damage, however, it is not completed understood the mechanism of cellular impairment mediated by Ac.

Adduct formation has been proposed as one of the main mechanism of damage (Setshedi et al., 2010), however Ac can affects cells by multiple ways, such as the upregulation of genes involved in the initiation or progression of liver disorders, for example the collagen gen that we have previously reported (Gutierrez-Ruiz et al., 2001a,b), in addition to liver fibrosis (Arellanes-Robledo et al., 2013), Ac has been linked to mutations (Brooks, 1997), hepatocellular carcinoma (Setshedi et al., 2010) and oxidative stress (Farfan Labonne et al., 2009), but is not clear what is the mechanism of ROS generation induced by Ac.

Superoxide dismutases (SOD) are enzymes that catalyze the dismutation of $O_2^{-\cdot}$ into hydrogen peroxide and oxygen; in mammals there are three forms located predominantly in cytosol (SOD1), in mitochondria (SOD2) (Karnati et al., 2013) and in plasma membrane (SOD3) (Zelko et al., 2002). SOD2 is critical to control the oxidative stress in ALD due to mitochondria are the main cellular target of alcohol and Ac (Lluis et al., 2003). It has been reported that overexpression of SOD2 prevents alcohol-induced liver injury in experimental animals (Wheeler et al., 2001), even more, polymorphisms in the SOD2 gene have been associated to the severity of fibrosis in non-alcoholic fatty liver injury, and in the risk to

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develop cholestasis in drug-induced liver injury (Al-Serri et al., 2012; Lucena et al., 2010).

The relevance of SOD2 is clear, and the advantages than SOD2 confers to normal cells also welfare cancer cells. Previously we reported that Ac targets SOD2 in primary rat hepatocytes, but the enzyme activity is partially recovered and did not reach normal values (Farfan Labonne et al., 2009). In the present work we were focused to figure out the effect of Ac on SOD2 in the hepatoblastoma cell line HepG2, with the purpose of approaching the consequence of Ac presence in transformed cells and its impact on liver cancer.

2. Material and methods

2.1. Materials

The cell permeable NF-κB peptide inhibitor SN50 was purchased from Merck-Millipore (Darmstadt, Germany), and was used as previously reported (Gomez-Quiroz et al., 2008); cycloheximide (Clx) was purchased from Cell Signaling (Danvers, MA, USA), anti-SOD2 was from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and anti-actin from NeoMarker (Fremont, CA, USA). All others chemicals were from Sigma–Aldrich (San Louis, MO, USA).

2.2. Cell culture

The HepG2 cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were routinely growth in monolayer culture in Williams E medium supplemented with 10% fetal bovine serum (Hyclone Lab Inc., Logan UT), 1% antibiotics in a humidified atmosphere of 5% CO_2/95% air. Medium was replaced twice a week and cells were trypsinized and diluted every 7 days at a ratio of 1:3.

All experiments were carried out using 225,000 cells/cm^2 seeded in 10-cm dishes. In order to lessen Ac evaporation all dishes were wrapped with parafilm during time of treatment.

2.3. Mitochondria isolation

After treatments cells (20×10^6) were incubated and homogenized in SHE-1 buffer (0.25 M sucrose, 5 mM HEPES, 1 mM EGTA, pH 7.4) and mitochondria and cytosolic fractions were isolated following protocol previously reported (Farfan Labonne et al., 2009)

2.4. SOD activity

SOD activity was determined by the method of Winterbourn et al., 1975). It is bases on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium by superoxide anion. Mitochondrial SOD activity was assayed by the same method using protein extracts from isolated mitochondria.

2.5. Nuclear protein extraction and Electrophoretic Mobility Shift Assay (EMSA)

Nuclear protein extraction was performed in HepG2 cells treated or not with Ac. Protein was quantified and subjected to NF-κB EMSA analysis according the protocol previously reported (Gomez-Quiroz et al., 2008)

2.6. ROS determination

HepG2 cells were seeded in borosilicate chamber slides, treated as indicated and immediately incubated for 15 min, in the dark, and room temperature with dihydroethidium (DHE, 50 μM) for determination of superoxide anion ($O_2^{\cdot-}$), detecting ethidium fluorescence (Zhai et al., 2011). Samples were covered and observed by multiphoton confocal microscope (Carl Zeiss, LSM 780 NLO, Jena, Germany) at excitation wavelength of 485 nm and emission wavelength of 570 nm for DHE-derived ethidium fluorescence.

2.7. Western blotting

Total protein was isolated from HepG2 cells, with M-Per tissue protein extraction reagent (Pierce, Rockford, IL. USA), containing 1% halt protease inhibitor mixture (Pierce), 100 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 mM sodium ortho-vanadate. One hundred μg of total protein were separated on NuPAGE novex 4–20% gels (Invitrogen), transferred to polyvinylidenedifluoride membranes (Invitrogen), and probed with anti-SOD2 (Santa Cruz Biotechnology, SC-133134) according manufacturer's instruction. Membranes were incubated with anti-mouse horseradish peroxidase-conjugated secondary antibody.

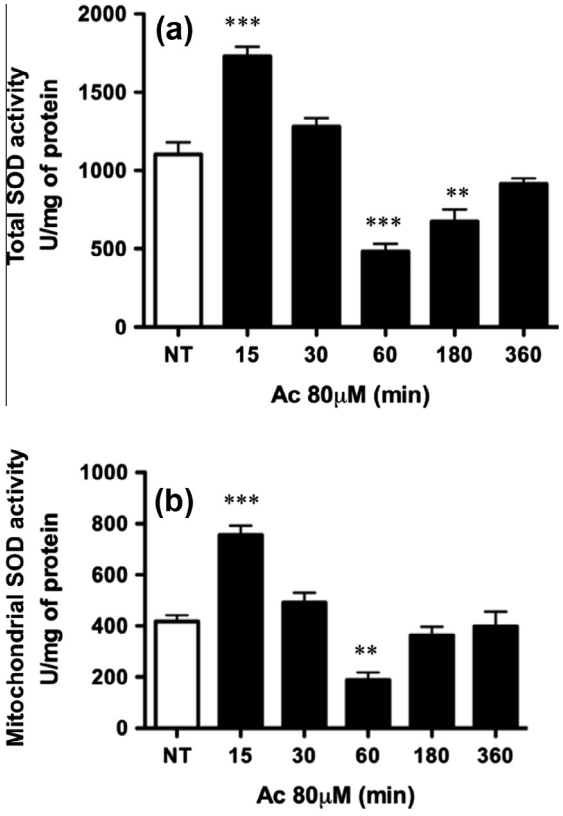

Fig. 1. Acetaldehyde brings a biphasic response in SOD activity. HepG2 cells were treated with acetaldehyde (80 μM) for 15,30,60 180 and 360 min after that SOD activity was assayed in (a) whole cell (total SOD activity) and, (b) mitochondria extracts (mitochondrial SOD, or SOD2). Enzyme activity was assayed as depicted in Material and Methods section. Each column represents mean ± SEM of three independent experiments carried out in triplicate. Differences were considered significant at $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$ vs NT cells.

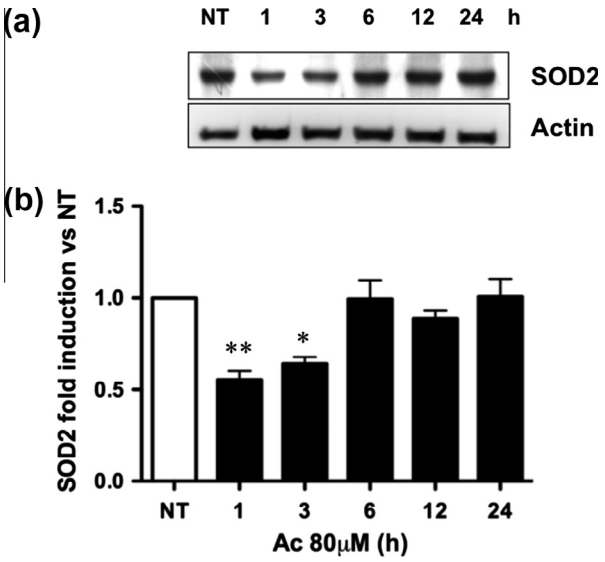

Fig. 2. Acetaldehyde induces a downregulation of SOD2 content. HepG2 cells were treated with acetaldehyde (80 μM) for 1,3,6,12 and 24 h. Whole cell lysate was obtained and subjected to Western blotting. (a) Representative image of the immunoblot, (b) densitometric analysis of protein content relative to actin used as loading control. Each column represents mean ± SEM of three independent experiments. Differences were considered significant at $^{*}p \leqslant 0.05$ vs NT cells.