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Oxidative damage: The biochemical mechanism of cellular injury and necrosis in choline deficiency

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ABSTRACT

Oxidative stress and damage are characterized by decreased tissue antioxidant levels, consumption of tissue α-tocopherol, and increased lipid peroxidation. These processes occur earlier than necrosis in the liver, heart, kidney, and brain of weanling rats fed a choline deficient (CD) diet. In tissues, water-soluble antioxidants were analyzed as total reactive antioxidant potential (TRAP), α-tocopherol content was estimated from homogenate chemiluminescence (homogenate-CL), and lipid peroxidation was evaluated by thiobarbituric acid reactive substances (TBARS). Histopathology showed hepatic steatosis at days 1–7, tubular and glomerular necrosis in kidney at days 6 and 7, and inflammation and necrosis in heart at days 6 and 7. TRAP levels decreased by 18%, 48%, 56%, and 66% at day 7, with $t_{1/2}$ (times for half maximal change) of 2.0, 1.8, 2.5, and 3.0 days in liver, kidney, heart, and brain, respectively. Homogenate-CL increased by 97%, 113%, 18%, and 297% at day 7, with $t_{1/2}$ of 2.5, 2.6, 2.8, and 3.2 days in the four organs, respectively. TBARS contents increased by 98%, 157%, 104%, and 347% at day 7, with $t_{1/2}$ of 2.6, 2.8, 3.0, and 5.0 days in the four organs, respectively. Plasma showed a 33% decrease in TRAP and a 5-fold increase in TBARS at day 5. Oxidative stress and damage are processes occurring earlier than necrosis in the kidney and heart. In case of steatosis prior to antioxidant consumption and increased lipid peroxidation, no necrosis is observed in the liver.

Introduction

According to the classic definition by Sies (1991) for mammalian cells, systems, and organisms, oxidative stress is an unbalanced situation with increased oxidants or decreased antioxidants. The concept implies both the recognition of a physiological production of oxidants and the presence of an operative antioxidant defense. The production of oxidants is currently understood as a phenomenon continuously fueled by the generation of the free radicals superoxide radical (O_2^{-}) and nitric oxide (NO). The production of both species is mainly mitochondrial and with it accounts for 2–3% of the tissue O_2 uptake. Through a series of first-order and non-enzymatic reactions, these two free radicals, O_2^{-} and NO, produce other oxidizing free radicals and related oxidative species, known as the biochemical free-radical chain reaction (Boveris and Cadenas, 2000; Boveris and Navarro, 2008). The concept of balance applies to the physiological condition with a high effectiveness of tissue antioxidant defenses in maintaining both oxidative stress and cellular damage at a minimum level. Under pathological alterations, the production of the primary free radicals O_2^{-} and NO and of other oxidative species is markedly increased and leads to oxidative stress and damage (Boveris et al., 2008). Regarding antioxidants, a distinction is made between antioxidant enzymes and related co-factors, such as superoxide dismutases, catalase, glutathione peroxidases, and reduced glutathione, whose cellular levels are established by genetic and metabolic regulation, and organic free-radical traps, such as α-tocopherol and ascorbic acid, whose tissue levels depend on the nutritional uptake and were early recognized as vitamins.

At present, oxidative stress is considered an "emergency" cellular situation and a reversible step. The notion is similar to the classic idea of reversible cell injury in cellular pathology, which is prior to the irreversible cell injury leading to cell death by necrosis or apoptosis. There is a current interest in linking the biochemical processes of oxidative stress and damage with the morphological and histological descriptions of cell injury and death. Two main ideas–hypotheses are currently considered. The first hypothesis is related to mitochondrion-dependent apoptosis and considers a sequence with four phases: oxidative stress, mitochondrial dysfunction, apoptosome assembly, and apoptosis, as described by Kroemer et al. (1998) and by Bustamante et al. (2004). The second hypothesis relates to cell necrosis and is thought to be associated with a relatively acute free-radical-mediated process, as the hepatic necrosis following carbon

Abbreviations: CD, choline-deficient or choline deficiency; TRAP, total reactive antioxidant potential; CL, chemiluminescence; TBARS, thiobarbituric acid reactive substances; NO, nitric oxide; NADH, hydrogen nicotinamide dinucleotide; O_2^{-}, superoxide anion; H_2O_2, hydrogen peroxide.

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tetrachloride or paracetamol intoxications. In this case, there is a generalized tissue lipid peroxidation involving all cellular organelles, followed by histological damage of necrosis, and can be partially prevented by antioxidants (Fraga et al., 1984).

Choline deficiency (CD) produces pathological changes in several organs: in the liver, steatosis, cellular death, cirrhosis, and cancer (Zeisel, 1992, 1996; Ghoshal et al., 1993); in kidney, from focal tubular necrosis and widespread cortical necrosis and renal failure (Monserrat et al., 1969; Ossani et al., 2007); in heart, inflammation and necrosis (Carter and Frenkel, 1978; Ariente de García et al., 1981; Sarri et al., 2006), and in brain, morphological alterations (Zeisel, 2004) and functional damage (Zeisel, 2006; Sanders and Zeisel, 2007).

An increased production of O_2^- and H_2O_2 at complex I may be the first step of free-radical-mediated reactions that lead to oxidative stress and damage and afford the pathogenic mechanism involved in the development of these lesions (Hensley et al., 2000; Floyd et al., 2002; Guo et al., 2005). Choline deficiency has been reported to produce oxidative damage in the liver, heart, kidney, and brain, with an increased lipid peroxidation of subcellular organelles and a decrease in tissue antioxidants (Ossani et al., 2007).

The aim of this work was to evaluate the time course of oxidative and histological alterations and damage in the liver, kidney, heart, and brain as a result of CD in weanling rats. This would contribute to the hypothesis of free-radical-mediated lipid peroxidation as the biochemical mechanism of cellular injury and necrosis developed in CD.

Material and methods

Choline deficiency

Weanling male Wistar rats (70.4 ± 1.5 g; 48 animals) from the Center for Experimental Pathology, Department of Pathology, School of Medicine, University of Buenos Aires, were divided into two groups. One experimental group of 24 rats was fed *ad libitum* a CD diet consisting of (g/100 g diet) soybean protein, 20.0; hydrogenated vegetable oil, 14.3; corn oil, 5.7; sucrose, 49.5; cellulose, 4.0; vitamin mixture (choline free), 4.0; salt mixture, 2.0; and L-cystine, 0.5. The other experimental group of 24 control rats was fed the same diet supplemented with 0.35 g/100 g of choline chloride instead of the same amount of sucrose. Both diets were isocaloric. Animals were housed under a 12/12-h light cycle at a temperature of 25 °C and were sacrificed at days 0, 2, 4, 6, and 7. Rats were anesthetized with sodium thiopental (40 mg /kg), abdominal aorta blood samples were withdrawn for plasma determinations, and the organs were rapidly excised. All experimental procedures followed *The Guide for the Use and Care of Laboratory Animals of the American Physiological Society* (Bethesda, MD).

Histology

Liver, kidneys, heart, and brain were removed and weighed. Part of the liver, heart, brain, and kidney were frozen in liquid nitrogen for oxidative stress determinations. The rest of the liver, heart, brain, and kidney were fixed in buffered formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin to analyze histopathological alterations.

Tissue homogenate preparation

Liver, heart, kidney, and brain were excised and weighed, and tissue homogenates were prepared in a medium consisting of 120 mM KCl, 30 mM phosphate buffer, pH 7.4. The tissue–buffer ratio was 1 g/9 ml of buffer. The homogenates were centrifuged at 600 × *g* for 10 min at 4 °C to discard nuclei and cell debris. The supernatant, a suspension of mixed and preserved organelles and plasma membranes, was used as tissue homogenate to determine the indicators of oxidative stress and damage.

Total reactive antioxidant potential

The total reactive antioxidant potential (TRAP) of tissue homogenates and plasma was measured by chemiluminescence. This assay determines the total endogenous water-soluble antioxidants, mainly glutathione, ascorbic acid, bilirubin, and albumin in tissue homogenates and the same antioxidants and uric acid in plasma. The addition of 10 µl of sample to 20 mM 2,2- azobis (2-amidinopropane) (ABAP) in 100 mM phosphate buffer, pH 7.4 and 40 µM luminol decreased chemiluminescence to basal levels and prevented the spike of light emission for a period proportional to the amount of antioxidants present in the sample (induction time, δ). The system was calibrated with Trolox (the hydrosoluble vitamin E analogue). Results are expressed as micromole Trolox per gram of organ, or micromolar Trolox, with 1 g of tissue being equal to 1 ml of water (Lissi et al., 1992).

Homogenate chemiluminescence

The homogenate chemiluminescence derived from lipid peroxidation (homogenate-CL) was measured with a Packard Tricarb model 3355 liquid scintillation counter in the out-of-coincidence mode (Gonzalez Flecha et al., 1991). This assay determines indirectly and with high sensitivity the tissue levels of α-tocopherol by inhibition of the propagation step of lipid peroxidation, as discussed by Gonzalez Flecha et al. (1991). Homogenates were suspended in 4 ml of 120 mM KCl, 30 mM phosphate buffer, pH 7.4 at 0.1–0.2 mg protein/ml. Low-potassium glass vials, 25 mm in diameter and 50 mm in height, filled with homogenate suspensions were used. In the absence of vials, instrument background was 2400 ± 60 counts per minute (cpm) and the emission from the empty vials was 3000 ± 60 cpm. Chemiluminescence measurements with the liver, kidney, and heart homogenates were started by the addition of 3 mM *tert*-butyl hydroperoxide and counting continued until a maximal level of emission was reached, usually after 20 min. In brain, spontaneous homogenate chemiluminescence was followed for about 30 min, when maximal emission was observed. Determinations were carried out at 30 °C. Results are expressed as counts per minute per gram organ.

Malondialdehyde determination (TBARS)

Malondialdehyde determination was carried out by the spectrophotometric determination of thiobarbituric acid-reactive substances (TBARS), as described by Fraga et al. (1988). Thiobarbituric acid reacts with malondialdehyde, a product of lipid peroxidation, showing maximal absorbance at 535 nm. The reaction mixture consists of 1 ml of homogenate or plasma, 1 ml of 120 mM KCl, 1 ml of 30 mM phosphate buffer, pH 7.4, 0.05 ml of butylhydroxytoluene 4% w/v in ethanol, 1 ml of trichloroacetic acid 20% w/v, and 1 ml thiobarbituric acid 0.7% w/v. The deproteinized mixture was heated at 100 °C for 20 min. Results ($E = 156\ mM^{-1}\ cm^{-1}$) are expressed as nanomole per gram organ.

Oxidative damage index

Oxidative damage in CD was evaluated by an index using the indicators of oxidative stress and damage as follows: [(TRAP-control/TRAP-CD) + (homogenate-CL-CD/homogenate-CL-control) + (TBARS-CD/TBARS-control)]/3 calculated for each day with data from control and CD rats (Navarro and Boveris, 2007; Boveris and Navarro, 2008).

Protein measurements

Protein levels were assessed using Lowry et al. method (1951) with bovine serum albumin as standard.

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Chemicals

Soybean protein grade II, cellulose (Celufil, non-nutritive bulk), choline-free vitamin mixture, diet 234312, salt mixture (Wesson modification), and L-cystine were purchased from US Biochem. Corp., Cleveland, OH; hydrogenated vegetable oil was from Danica, Buenos Aires; and corn oil (Mazola) was from Refinerías de Maíz, Buenos Aires. Thiobarbituric acid, butylhydroxytoluene, and trichloroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO), *tert*-butyl hydroperoxide was from Aldrich Chem. Co. (Milwaukee, MI). Other reagents were of analytical grade.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). The Student's *t*-test was used for comparison between CD and control groups. Differences were considered statistically significant at $p<0.05$ level.

Results

CD-related histopathological damage to different organs

Weanling rats fed a CD diet during 7 days presented morphological alterations in the liver, kidney, heart, and brain. The histological study showed hepatic steatosis and apoptosis since day 2 (Fig. 1). In the kidney, tubular necrosis was observed at day 6 and glomerular necrosis at day 7, mainly involving convoluted tubules and characterized by pyknosis, karyolysis, and increased eosinophilia (Fig. 1). Renal pathology varied from focal tubular necrosis to massive cortical necrosis, with a macroscopic renal damage characterized by increased size and weight and purplish red discoloration. The whole pathological process leads to acute renal failure and animal death at days 8–9. In the heart, histopathology showed inflammation at day 6 and necrosis at day 7. The latter was associated with inflammatory infiltrates and focal to extensive hemorrhages localized to the posterior–lateral wall of the right ventricle and reaching the interventricular septum (Fig. 1). Although the brain showed small foci of hemorrhage, no morphological alterations due to necrosis or apoptosis were observed in brain cortex or hippocampus at days 1–7 (not shown).

CD-related sequential oxidative damage to different organs

TRAP, a measurement of water-soluble (mainly reduced glutathione) free-radical trapping molecules in the tissues, was performed in the liver, kidney, heart, and brain of CD rats. In the liver and kidney homogenates, TRAP values decreased continuously from day 0 to day 7, when they reached 18% and 48% of the day 0 level (Fig. 2). The decrease in TRAP in the heart and brain started at day 2 and reached the low values of 56% and 66%, respectively, at day 7 (Fig. 3). The $t_{1/2}$, the time required for half maximal changes in TRAP, is indicated in Figs. 2 and 3 and listed in Table 1.

Homogenate chemiluminescence (homogenate-CL), an indicator of tissue lipid-soluble antioxidants, mainly α-tocopherol (Gonzalez Flecha et al., 1991), showed significant increases starting at day 2 and reaching a maximal level at day 4 in the four organs of CD rats. The maximal increases in homogenate-CL at day 4 were 235% in the liver, 121% in the kidney, 98% in the heart, and 300% in the brain; and the increases at day 7 were 97% in the liver, 113% in the kidney, 18% in the heart, and 27% in the brain (Figs. 4 and 5). The $t_{1/2}$ values for changes in homogenate-CL are indicated in Figs. 4 and 5 and listed in Table 1.

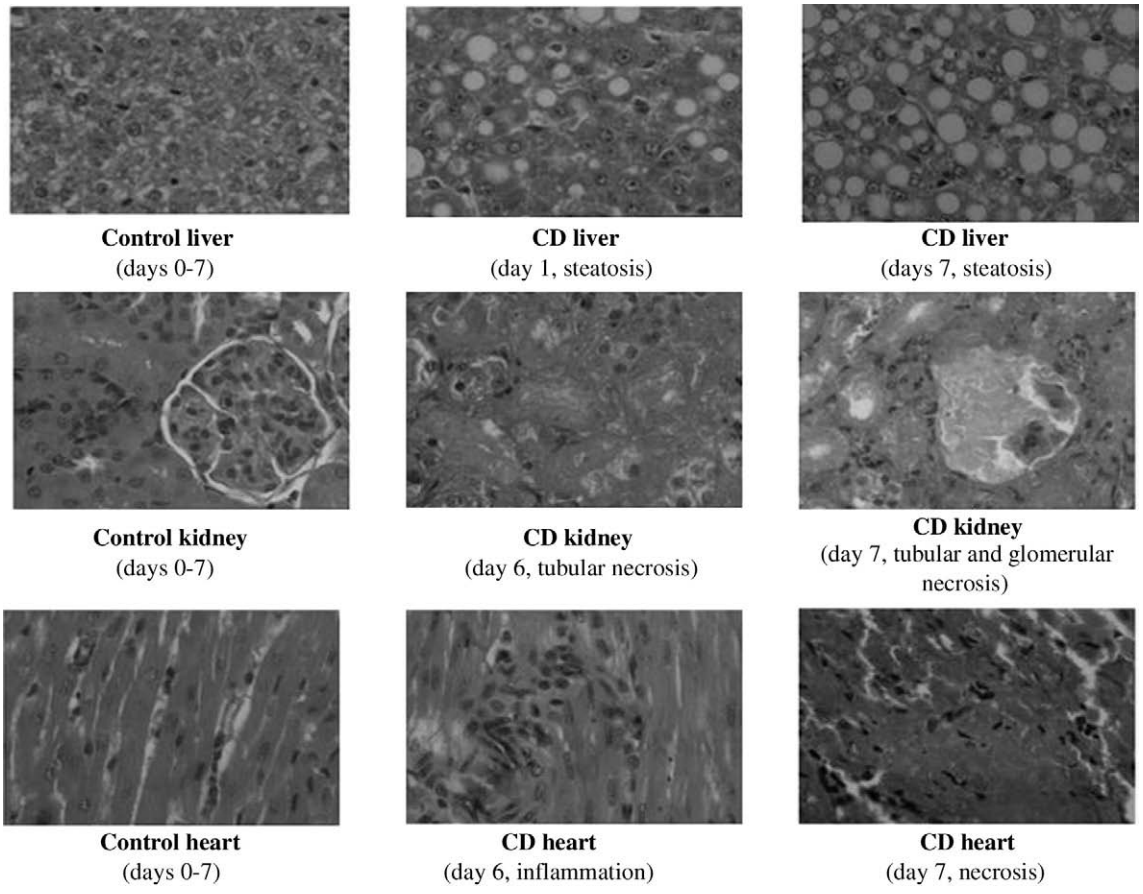

Fig. 1. Histological images of liver, kidney, and heart of rats fed a CD diet and a control normal diet.

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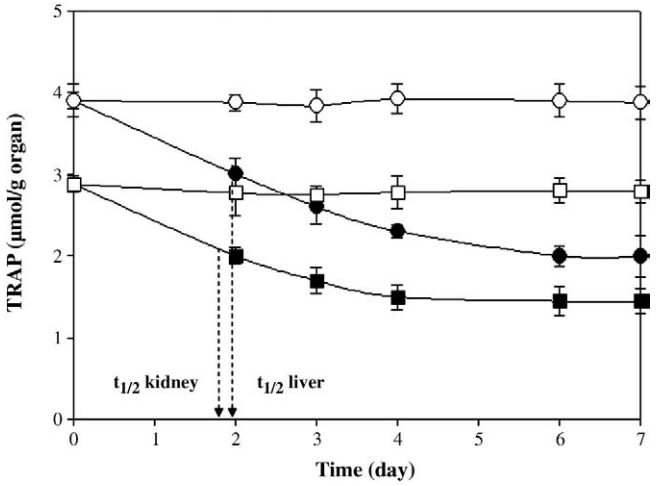

Fig. 2. TRAP levels in liver and kidney homogenates of rats fed CD and control diets. (○) Liver, control; (●) liver, CD; (□) kidney, control; (■) kidney, CD. For changes in CD: $t_{1/2}$ liver, 2 days and $t_{1/2}$ kidney, 1.8 days.

The lipid peroxidation process, assessed by the level of its product malondialdehyde as determined by TBARS, significantly increased in the four organs over the 2- to 7-day period. In the liver and kidney, TBARS levels showed a 3- to 4-fold increase at days 2–3, which was maintained up to day 7 (Fig. 6). In the heart, the increase in TBARS started at day 3 and reached a maximal level at day 4, whereas in the brain, TBARS increases were observed at days 6–7 (Fig. 7). The $t_{1/2}$ values for the observed changes are indicated in Figs. 6 and 7 and listed in Table 1.

Oxidative stress has systemic significance in rats fed a CD diet as indicated by the 33% decrease in TRAP plasma levels (an indicator of the total antioxidant content of plasma) observed from day 2 to 7, with a 5-fold increase in plasma TBARS, and with a $t_{1/2}$ of 3.5 days (Figs. 8 and 9). These facts are considered as the result of the diffusion of lipid peroxidation products from the kidney and liver to blood.

The time course of the changes (expressed as $t_{1/2}$) in the three indicators of tissue oxidative stress and damage (TRAP, homogenate-CL, and TBARS) preceded the time course of the appearance of morphological alterations in the liver, kidney, heart, and brain (Table 1).

The oxidative damage index indicate that rats fed a CD diet present maximal oxidative damage to the liver, kidney, and heart homogenates at the fourth day (Fig. 10), while in the brain, oxidative damage increased progressively from day 0 to day 7 (Fig. 10). In plasma, maximal systemic oxidative stress is observed at day 5.

Table 1
Time course of the biochemical and histological changes, expressed as $t_{1/2}$, of TRAP, homogenate-CL and TBARS in liver, kidney, heart, brain in weanling rats fed a CD diet for 7 days.

Organ	TRAP ($t_{1/2}$ days)	Homogenate-CL ($t_{1/2}$ days)	TBARS ($t_{1/2}$ days)	Necrosis (or maximal histological damage) (days)
Liver	2.0	2.5	2.6	1.0 (Steatosis)
Kidney	1.8	2.6	2.8	6.0
Heart	2.5	2.8	3.0	7.0
Brain	3.0	3.2	5.0	–
Plasma	1.2	–	3.5	–

Discussion

Weanling rats fed a CD diet develop acute renal failure and animal death in 7–9 days, (Ossani et al. 2007). It is worth noting that, at variance, adult rats fed CD diets do not undergo either acute renal failure or death unless they have unilateral nephrectomy, or are pregnant and given a CD diet supplemented with sarcosine (Handler, 1946); Woodard, 1970).

The sequence (a) decrease in tissue antioxidants, determined by TRAP and mainly due to decreased intracellular reduced glutathione (GSH); (b) increase of homogenate chemiluminescence, interpreted as a decrease in tissue α-tocopherol (Gonzalez Flecha et al., 1991); and (c) increase in lipid peroxidation, determined by TBARS, was found operative in the liver, kidney, heart, and brain, as a sequential process prior to the irreversible histological damage and necrosis in CD weanling rats. The observation is consistent with the classic view of oxidative stress and oxidative damage where antioxidants decrease and oxidants increase in a progressive and continuous manner. The process sometimes includes an adaptive response with biosynthesis of antioxidant enzymes, which confers reversibility to the oxidative stress situation. The described observation is also consistent with the sequence of the oxidation of hydrosoluble ascorbic acid and liposoluble α-tocopherol and phospholipid peroxidation, as seen in plasma *in vitro* in the Cu^{2+}-catalyzed oxidation of LDL (Esterbauer et al., 1992). It is then clear that the biochemical mechanism for CD-related cell death in the liver, kidney, heart, and brain is a relatively acute free-radical mediated process, quite similar to the hepatic necrosis following carbon tetrachloride or paracetamol intoxications,

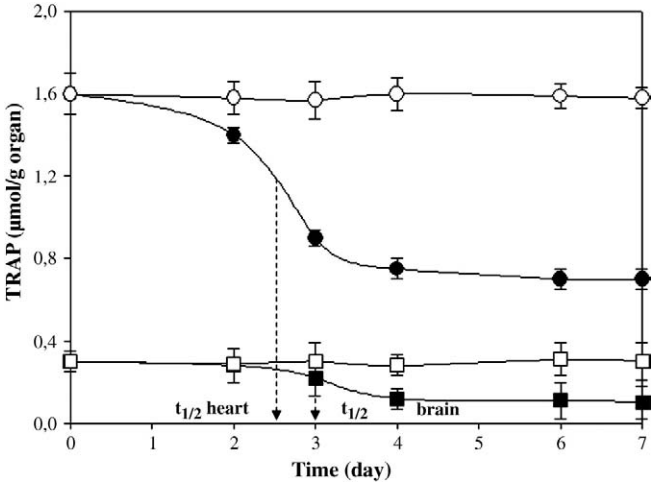

Fig. 3. TRAP levels in heart and brain homogenates of rats fed CD and control diets. (○) Heart, control; (●) heart, CD; (□) brain, control; (■) brain, CD. For changes in CD: $t_{1/2}$ heart, 2.5 days and $t_{1/2}$ brain, 3.0 days.

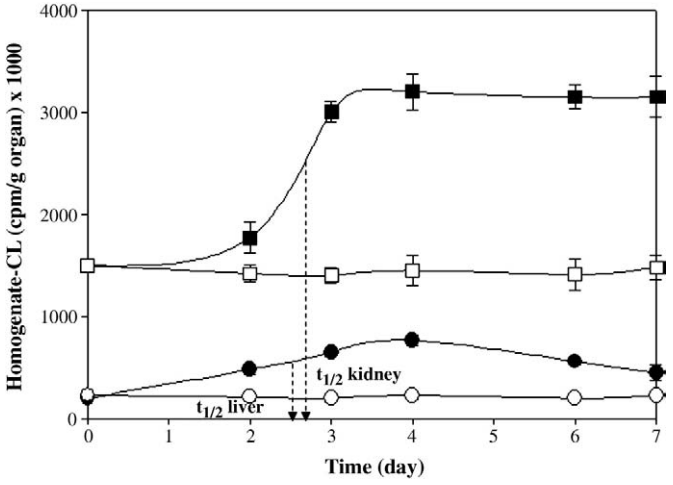

Fig. 4. Homogenate-CL values in liver and kidney of rats fed a CD and control diets. (○) Liver, control; (●) liver, CD; (□) kidney, control; (■) kidney, CD. For changes in CD: $t_{1/2}$ liver, 2.5 days and $t_{1/2}$ kidney, 2.6 days.

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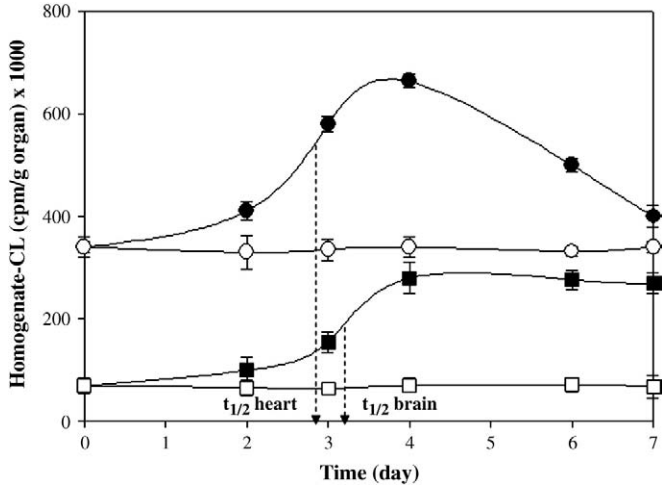

Fig. 5. Homogenate-CL values in heart and brain homogenates of rats fed CD and control diets. (○) Heart, control; (●) heart, CD; (□) brain, control diet; (■) brain, CD. For changes in CD: $t_{1/2}$ heart, 2.8 days and $t_{1/2}$ brain, 3.2 days.

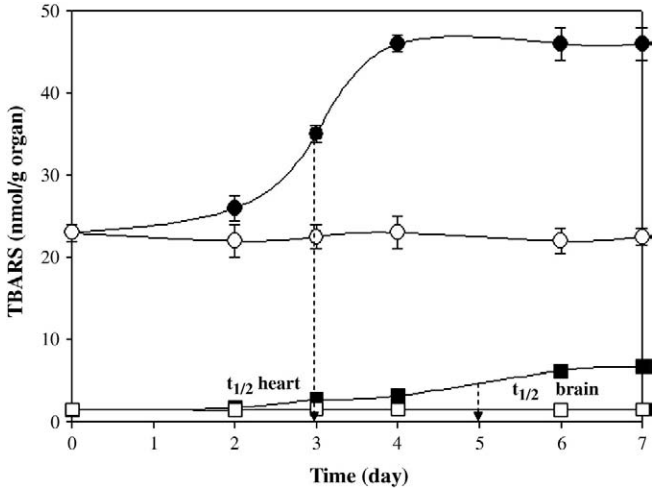

Fig. 7. TBARS in heart and brain homogenates of rats fed CD and control diets. (○) Heart, control; (●) heart, CD; (□) brain, control; (■) brain, CD. For changes in CD: $t_{1/2}$ heart, 3.0 days and $t_{1/2}$ brain, 5.0 days.

with generalized lipid peroxidation of subcellular membranes. In this, situation, mitochondrial and endoplasmic reticulum membranes loose their selective permeability to homeostatically keep cytosolic Ca^{2+} at minimal micromolar levels. Increased cytosolic Ca^{2+} will immediately trigger the "death cascade" with activation of phospholipase A and proteases.

Farber and Sarma (1987) studied the sequence of biochemical and tissue changes associated with the feeding of CD diets to rats during 63 days and found that the effects of oxidative free radicals on the functional integrity of mitochondria and plasma membranes have a major role in the genesis of liver cell injury. Lipid peroxidation was closely related to liver necrosis and oxidative damage in DNA and in mitochondria. Liver triglyceride accumulation started immediately after CD diet uptake and was followed by the onset of lipid peroxidation in nuclear and mitochondrial membranes. Lipid peroxidation in liver nuclei began at day 1 and reached a peak at day 3, whereas in mitochondria lipid peroxidation was first observed at day 3 with a maximum at day 28. Cell death started at day 6 and increased in severity over the following 10 days.

Oxidized lipids and lipid oxidation products have also a signaling function in pathological situations. They have been reported as pro-inflammatory agonists that contribute to cell and neuronal death under conditions in which membrane lipid peroxidation occurs (Esterbauer, 1993). Both necrosis and apoptosis appear involved in the mechanism of cell death associated with CD.

Choline deficiency first affects the liver, which responds with almost instantaneous steatosis in 1 day. Fatty liver, considered a reversible and pre-pathological disturbance, reveals a impairment of phospholipid and lipid metabolism due to the interruption of lipoproteins (LDL and HDL) production and output. Later, cirrhosis or proliferation and cancer could be observed. Oxidative damage in the liver may affect the absorption and transport mechanisms of α-tocopherol in this organ. It has been reported that liver mitochondria from CD rats produce more H_2O_2 per NADH oxidized, which leads to hepatocarcinogenesis (Guo et al., 2005). Impaired electron transfer at complex I and enhanced O_2^- and H_2O_2 generation are likely responsible for the role of oxygen free radicals in cell damage in CD (Floyd et al., 2002). The increased production of O_2^- and H_2O_2 explains the observed decreases in hydrosoluble (TRAP) and liposoluble (homogenate chemiluminescence) antioxidants.

Different techniques, such as HE, propidium iodide, *in situ* tailing, and nick translation have been used to describe apoptosis *in vivo* in CD (Matoso et al., 1998). In the kidney, lipid peroxidation is a process prior to necrosis; it was observed at prenecrotic and necrotic stages in

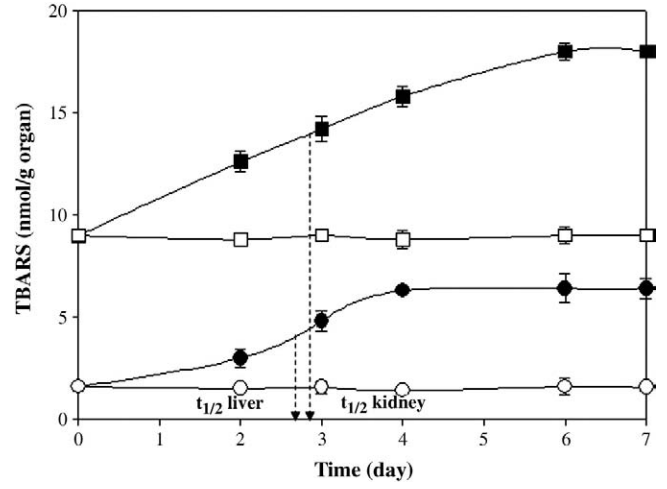

Fig. 6. TBARS in liver and kidney homogenates of rats fed CD and control diets. (○) Liver, control; (●) liver, CD; (□) kidney, control; (■) kidney, CD. For changes in CD: $t_{1/2}$ liver, 2.6 days and $t_{1/2}$ kidney, 2.8 days.

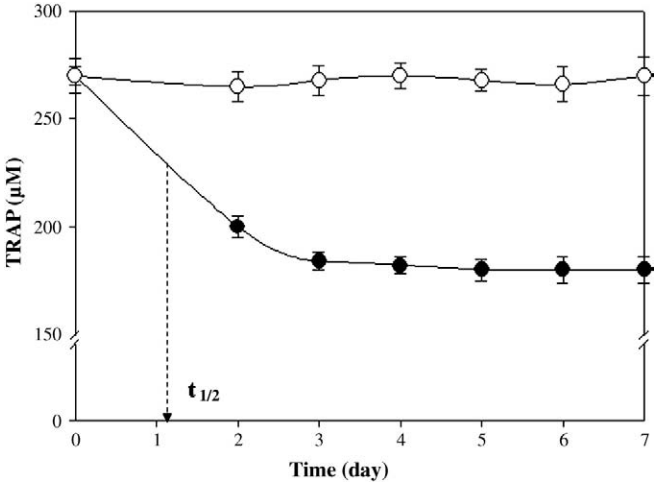

Fig. 8. Plasma TRAP in rats fed CD and control diets. (○) Control; (●) CD, $t_{1/2}$ of the change in plasma TRAP, 1.2 days.

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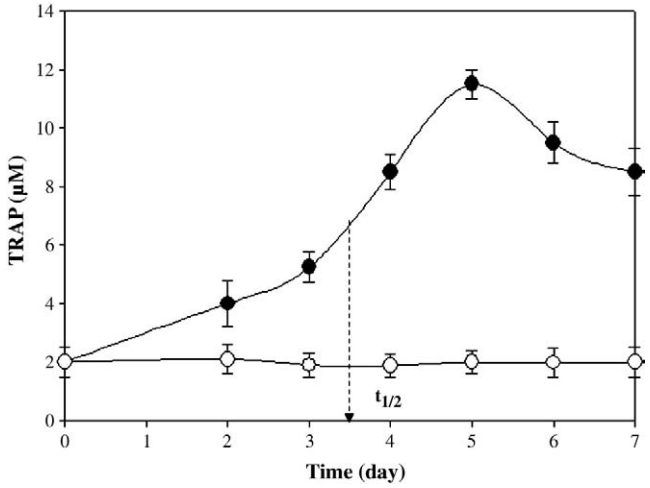

Fig. 9. Plasma TBARS in rats fed CD and control diets. (○) Control; (●) CD, $t_{1/2}$ = of the change in plasma TBARS, 3.5 days.

CD weanling rats with oxidative damage and lipid peroxidation in subcellular membranes (Ossani, 2007; Monserrat et al., 1969).

In the heart, oxidative stress (TRAP and homogenate chemiluminescence) and damage (TBARS) clearly preceded the morphological alterations, inflammation and necrosis that were observed upon the development of CD pathological signs.

In the brain, choline is required for the synthesis of neuronal phospholipids and is a precursor for the biosynthesis of acetylcholine (Zeisel, 1992). Antioxidant consumption and oxidative stress were observed in brain in CD prior to brain lipid peroxidation damage (Table 1). No major histological alterations were observed in brain cortex or hippocampus, probably due to a brain slower response in terms of cell damage as compared to the liver, kidney, and heart. Apoptosis is a normal process during brain development and is modulated in some tissues by the availability of choline. Brain and hippocampus damage caused by CD has been attributed to apoptosis, and it was observed that it induces apoptotic cell death in neuronal-type cells and in brain by p53- and by NF-kB-dependent signaling pathways (Holmes-McNary et al., 1997; Holmes-McNary et al., 2001).

Pregnancy and lactation are periods when maternal choline reserves are depleted and choline availability in embryogenesis and the perinatal period is critical for the development of spinal cord and brain, in general, and hippocampus, in particular. Choline is needed for neural tube closure in early pregnancy and for the development of brain and hippocampus memory centers (Sanders and Zeisel, 2007). Multiple mechanisms participate to ensure choline availability to the fetus and the neonate. Large amounts of choline are delivered to the fetus across the placenta, where choline transport systems pump it against a concentration gradient, since choline concentration in amniotic fluid is higher than in maternal blood, with fetus and neonate choline plasma levels being greater than in adults. There are two sensitive periods in embryonic days 12 to 17 and in postnatal days 16 to 30, when choline supplementation (1 mmol/day) produces lifelong enhancement of spatial memory (Zeisel and Niculescu, 2006). Choline deficiency in late pregnancy is associated with irreversible changes in hippocampal function in adults, including altered long-term potentiation and memory (Zeisel and Niculescu, 2006).

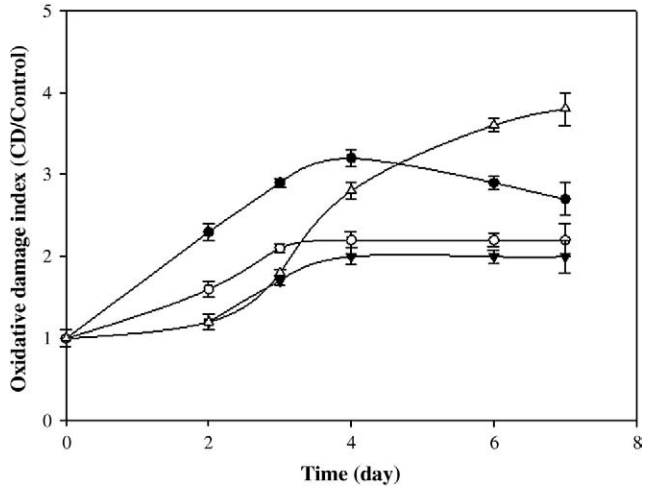

Fig. 10. Time course of oxidative stress and damage (CD/control) in CD. (●) Liver, (○) kidney, (■) heart, and (□) brain.

High choline intakes (about four times the normal levels) during days 11 to 17 of gestation in rats increase proliferation and decrease apoptosis in hippocampal progenitor cells, and enhance the long-term potentiation of visual, spatial, and auditory memory (by about 30%) in adult animals. Indeed, adult rodents have a decrement in memory with age that is not observed in the offspring exposed to high choline when *in utero* (Zeisel, 2000; Zeisel and Niculescu, 2006).

Decreased levels of TRAP and homogenate-CL and increased TBARS in the liver, kidney, heart, and brain in CD reveal a condition of oxidative stress and damage in these organs. The phenomenon reaches the characteristics of systemic oxidative stress, as evidenced by plasma decreased TRAP and increased TBARS (Figs. 8 and 9), which can be understood as the diffusion to plasma of the products of lipid peroxidation from the well-perfused liver and kidney. The condition of oxidative stress in plasma or systemic oxidative stress is simultaneous to the oxidative stress and damage in the liver and kidney and prior to the morphological irreversible damage to the organs in CD (Table 1).

Peripheral or systemic oxidative stress is a condition involving one or more target organs, or the whole body. Two hypotheses exist regarding the origin of plasma oxidants, pro-oxidants and the oxidation products: the first hypothesis refers to a tissue origin and the second one supports the notion of an intravascular origin. The potential consideration of a double origin, according to the number of chemical species involved, may be taken as a third possibility. Regarding tissue origin, soluble and diffusible metabolites, such as H_2O_2, NO, ROOH, and MDA, diffuse to the extracellular space and blood. This hypothesis is supported by the increases in plasma TBARS seen in the experimental situation of oxidative stress focused on one or more organs, as it is the case of liver and kidney in CD.

The results described in this study clearly indicate that lipid peroxidation plays a biochemical mechanistic role in the pathogenesis of CD.

Conclusion

Decreased antioxidant content and increased lipid peroxidation are earlier biochemical alterations that precede and lead to histological cell death by necrosis in CD. It is also clear that the process of lipid peroxidation is preceded by a decrease in hydrophilic and hydrophobic antioxidants. The reported observations indicate that CD, which can be modulated by the severity of choline withdrawal, is a relatively slow experimental model suitable for mechanistic studies of the role of oxidative free radicals in chronic cell injury and neoplasia.

The authors declare that there are no conflicts of interest.

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