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Chapter 7

Redox Reactions in the Physiopathology of the Liver

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Abstract

Electron fluxes are constant within cellular metabolism. Donating or accepting electrons, either naked or as hydrogen atoms, is one of the most important properties of bioenergetic networks. These redox reactions fulfill key physiological phenomena such as cellular growing, phenotypic differentiation, nutritional adaptations and redox-dependent cellular signaling, but when they became unregulated, serious pathologies such as degenerative diseases and metabolic disorders arise. The liver being an important metabolic organ, redox reactions play a strategic role in its main functions: processing of nutrients, fasting response, xenobiotic managing and circadian activity. However, liver is also very sensitive to compounds that disturb redox state such as ethanol, CCl_4, aflatoxins, among others, as well as to stressors such as hypercaloric diets, endocrine disruptors and stressful life situations. This chapter reviews concepts related to redox reactions in the liver, including metabolic aspects of reactive oxygen species (ROS), prooxidant and antioxidant subcellular systems, alterations produced by hepatotoxins, adaptations to experimental surgical protocols such as portacaval anastomosis, and participation in cancer. It is out of question that for a better comprehension of the physiopathological events in the liver and other metabolic organs, the more complete understanding of the roles played by redox reactions will be a necessity.

Keywords: metabolism, ROS, antioxidant, prooxidant, hepatocyte

1. Introduction

Living organisms are dynamic and complex systems with the notable capacity to continuously preserve their structural identity, but at the same time, to display functional and

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morphological adaptations in daily terms (circadian rhythms), during ontogeny, as well as in the context of evolutionary progression. Since living beings are thermodynamic open systems, they are empowered to exchange matter and energy. This competence sustains the intricate intertwine that make up the metabolic networks present in every cell [1].

For the last 2 billion years (after the transition of oxygenic photosynthesis), in our planet the bioenergetic cycle has been defined by two principal and complementary processes: photosynthesis and respiration [2]. Indeed, every other chemical and energetic transformation that takes place in the cellular milieu is included within this biochemical cycle (**Figure 1**). Inspection of the formula shown in **Figure 1** rapidly indicates that carbon-containing molecules oscillate from a reduced (as glucose) to an oxidized state (as carbon dioxide). The corollary is that in a general sense, metabolism can be visualized as a collection of reduction-oxidation (redox) reactions [3].

Electrons flux is in the pith of the formal definition of redox reactions: it establishes that molecules accepting electrons are reduced whereas those that give up electrons are oxidized. Therefore, redox reactions always occur between redox pairs. As Albert Szent-Gyorgyi, the prestigious Nobel Prize winner biochemist in 1937, quoted: (1) understanding metabolism is to figure out the direction taken by the electrons in transit among molecules and (2) the secret of life is to take advantage of the correct flow of the electrons [4]. Electrons in transit can be mobilized alone (as in the iron-sulfur complexes and cytochromes within mitochondrial and microsomal electron transport chains) or joined with protons as hydrogen atoms (as in biochemical transformation among acids, aldehydes and alcohols).

Redox potential (ΔE, quantified in volts) is a physicochemical parameter that measures the capacity to either release or accept electrons within a chemical reaction. It characterizes the extent of free Gibbs energy (ΔG) and the direction of the electron flow in each redox reaction ($\Delta G = nF\Delta E$, n is the electrons transferred and F is Faraday's constant). Molecular entities with higher (more positive) redox potential have the facility to oxidize molecules with a lower (more negative) redox potential [5]. Spontaneous biochemical transformations involve the release of metabolic energy as the electrons move from reactions with negative redox potential (oxidation of glucose into 2 pyruvates + $4e^-$, −720 mV) to reactions with positive redox potential (reduction of O_2 with $2e^-$ into water, +820 mV).

1.1. Liver as a paradigmatic metabolic organ

Certainly, every cell and tissue in the organism shows metabolic activity. However, the liver has a special consideration since it is the principal organ in the biochemical processing of nutrients and xenobiotics. Distinctive metabolic pathways, such as gluconeogenesis, ureagenesis, assembly of lipoproteins, synthesis of ketone bodies, metabolism of foreign chemical substances, lipogenesis, cholesterol formation, glutamine synthesis, and others, take place in the different population of hepatocytes: periportal (with high [O_2] and oxidative metabolism) and pericentral (with low [O_2] and less oxidative metabolism) [6].

Hepatic metabolism comprises synthetic (anabolic) and degradative (catabolic) pathways, each one being regulated by particular factors, including: (1) cellular compartments (oxidative

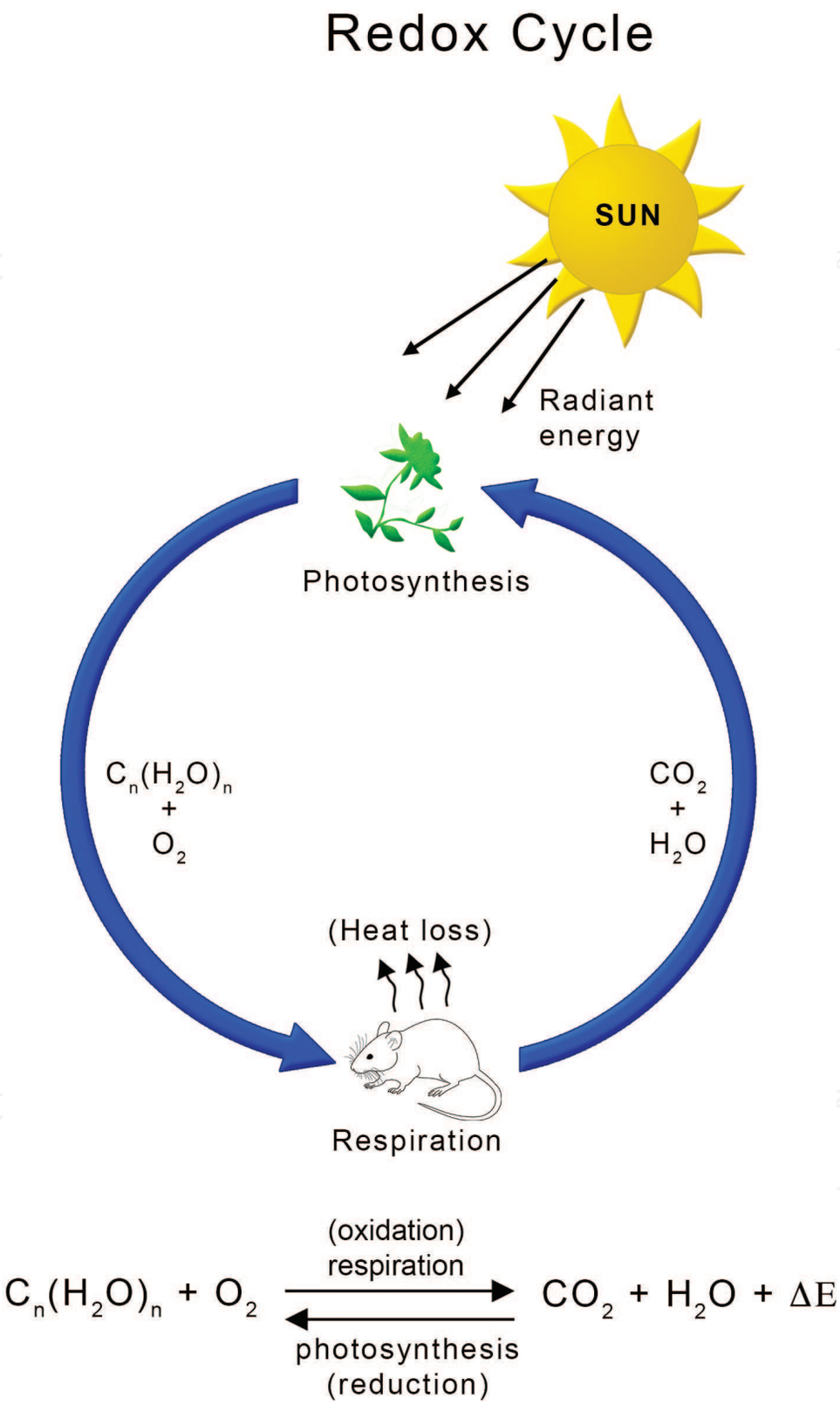

Figure 1. Global energy flux underlying redox cycle between photosynthesis and respiration. Driving by solar energy, autotrophic organisms synthetize biomolecules (carbohydrates for example) and oxygen by photosynthesis. Heterotrophic organisms oxidize carbohydrates to yield carbon dioxide and water by respiration. The cycle is complete when carbon dioxide and water are used again by autotrophic organisms.

reactions are preponderant in mitochondria and peroxisomes whereas reductive reactions are more common in cytosol), (2) available coenzymes (oxidized nicotinamide adenine dinucleotide (NAD^+), reduced nicotinamide adenine dinucleotide (NADH) for catabolism and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide adenine dinucleotide phosphate (NADPH) for anabolism), (3) adenine nucleotides pool (low energy charge, AMP-activated protein kinase (AMPK) activation for catabolism and high energy charge, reduced AMPK activity for anabolism) [7].

2. Redox molecules as metabolic regulators

It was thought that reactive oxygen species (ROS) were damaging molecules that were associated with the main pathological consequences of oxidative stress. However, recently, numerous reports have demonstrated that ROS and reactive nitrogen species (RNS) also play important roles in signaling pathways. In this way, the nowadays perspective of prooxidant reactions in the cellular milieu is that they form part of the physiological response to internal and environmental regulatory factors [8]. Indeed, the liver being one of the major metabolic organs, the signaling consequences of ROS and RNS are very relevant in the hepatic tissue.

It is well known that mitochondrial activity is the major source for ROS production, with consequences in the oxidative phosphorylation coupling affecting the $\Delta\Psi$ (mitochondrial membrane potential) as well as several mitochondrial metabolic networks. Eventually, important processes such as cell proliferation and apoptosis can be triggered by the mitochondrial prooxidant condition [9].

During the electron transfer through the mitochondrial respiratory complexes, O_2 is eventually reduced to water by receiving two electrons, along the creation of a [H^+] gradient that makes possible the formation of ATP. However, some electrons "leak" without completing the pathway until the cytochrome oxidase complex is achieved. In this case, one electron is received by an oxygen molecule forming the anion superoxide (O_2^-). O_2^- is produced in the interphase between sites I and II, as well in site III of the electron transfer chain. Within the mitochondria, O_2^- is transformed to hydrogen peroxide (H_2O_2) by mitochondrial superoxide dismutase (SOD2); when it reaches the cytosolic compartment, the O_2^- is turned into H_2O_2 by SOD1. In cytosol, the mitochondrial O_2^- and H_2O_2 are incorporated to the ROS pool generated mainly by the activity of the family of NADPH oxidases to potentially act as the signaling molecules [8].

H_2O_2 is itself a metabolic regulator, but at high levels can act as a deleterious factor. H_2O_2 can be converted into H_2O by several antioxidant enzymes: H_2O_2 can be reduced by peroxiredoxins or glutathione peroxidases, which couple reduction of H_2O_2 with oxidation of glutathione (GSH). Oxidized peroxiredoxins can be reduced by thioredoxins. Subsequently, oxidized thioredoxins become reduced by thioredoxin reductase in a NADPH-dependent manner. Oxidized glutathione disulfide (GSSG) is reduced by glutathione reductase again in the presence of NADPH.

H_2O_2 acts as a signaling factor by oxidizing thiol groups (-SH HS-) into disulfide bridges (-S-S-) in cysteine residues of key regulatory proteins (enzymes and receptors). Several years ago, some reports postulated that H_2O_2 showed insulin-like properties in hepatocytes. This

observation was eventually confirmed in the yeast *Saccharomyses cerevisiae* and supported the notion, fully accepted nowadays, that H_2O_2 could be acting as a signaling molecule in many cellular systems [8]. **Figure 2** shows the important role that mitochondria play in the prooxidant reactions of many cellular functions.

2.1. Cellular proliferation

Another system of ROS modulation well characterized in the liver is the control of growth factors by the redox regulation of cysteine residues in tyrosine phosphatases. H_2O_2 downregulates cyclin D1 and cyclin E to inhibit proliferation and upregulates Bcl-2-associated X protein (BAX) to induce apoptosis in hepatocytes and MCF-7 cells (cell line from cancerous mammary gland) [10].

Another example is the modulation of redox-sensitive cysteine residues in the epidermal growth factor (EGF) receptor, which is activated by the action of H_2O_2.

Other protein factors such as erythropoietin (EPO) and vascular endothelial growth factor (VEGF) have been identified as redox regulators of this process.

In response to hypoxic conditions, the hypoxia-inducible transcription factors (HIFs) are upregulated by ROS, especially the ones from mitochondrial source. Paradoxically, in liver

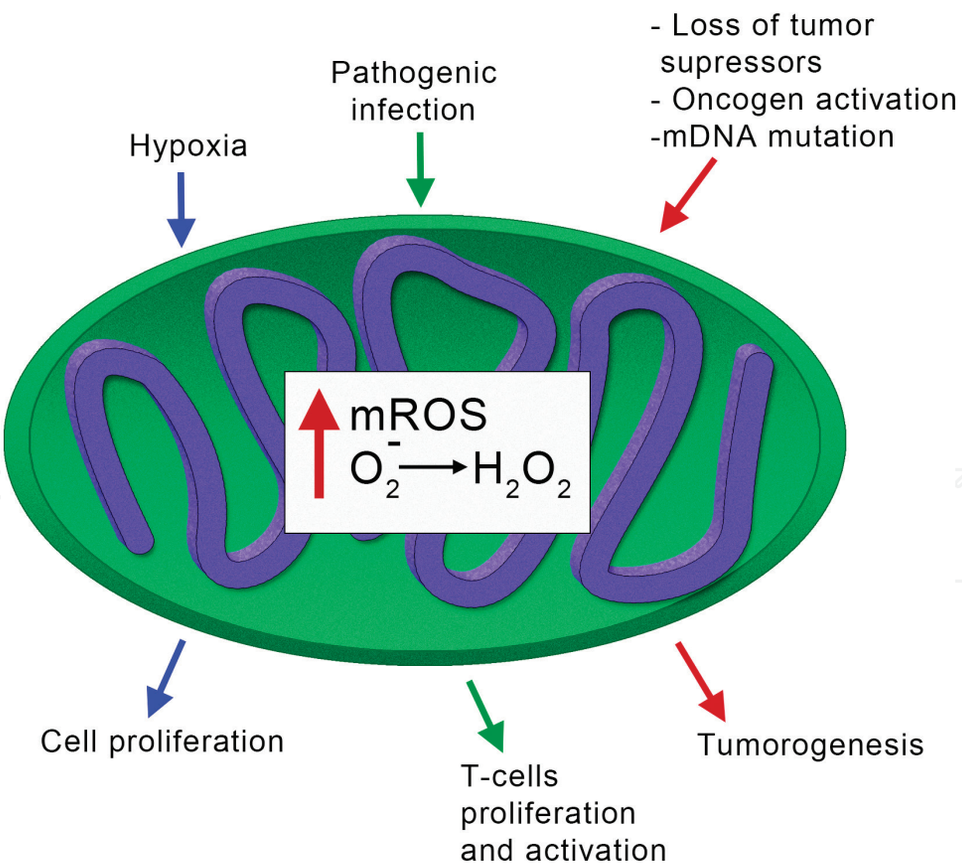

Figure 2. Mitochondrial handling of reactive oxygen species (ROS). Pro-oxidant reactions (synthesis of O_2^- and H_2O_2) are upregulated by a variety of physiological or pathological events. Mitochondrial activation promotes responses such as cell proliferation and activation, as well as cancerous processes.

and vascular smooth muscle cells, in hypoxic condition there is an increase in mitochondrial ROS production from complex III; however, the exact mechanism is not well understood.

In liver and muscle cells, angiotensin II signaling also promotes higher production of mitochondrial ROS, which are necessary to activate downstream responses such as mitogen-activated protein kinases (MAPK) [11].

2.2. Immune system

When an organism is infected primordial T-cells rapidly proliferate, and differentiate into effector T-cells, and mitochondria contribute to this activation through the production of ROS. This has been analyzed adding antioxidants to mice after a viral infection and showing that they exhibited a depressed immune system. Data suggest that ROS play an important role in T-cell activation, proliferation and adaptive immune function [12].

2.3. Cancer

A fundamental characteristic of cancerous cells is their uncontrolled proliferation. It has been observed that they generate high levels of ROS, especially H_2O_2; in addition, elevated levels of antioxidants have been detected, maybe to implement a protective cellular response. The final equilibrium allows a high rate of ROS synthesis concomitant with a transformed functional phenotype.

ROS are responsible of oncogenes activation and/or loss of tumor suppressors enhancing mitogenic signaling.

Mutations in mitochondrial DNA result in ROS increase that has been associated to a great variety of human cancers. One example is the mutation in the gene of NADH dehydrogenase in the mitochondrial complex I, which promotes an elevation of ROS production. The prooxidant stimulus leads to the proliferation of several human and mouse cell lines, as well as tumor formation in rodents. Interestingly, this condition could be rescued by the reconstitution of the wild-type enzymatic activity [13].

3. Anabolism and catabolism. Redox-sensitive enzymes

Prooxidant reactions play an important role in cellular signaling and redox regulation of metabolic processes such as immune defense, growth, and apoptosis among others. The increase in the ROS production requires antioxidant strategies to prevent a potential oxidative damage to cellular components. The imbalance of prooxidants and antioxidants leading to cell damage and tissue injury may cause oxidative stress. Oxidative stress is common in organs and tissues with high metabolic and energy turnover, including skeletal and heart muscle, blood cells, and liver [14].

It has been recognized as the interplay between energy metabolism and ROS to make possible the homeostasis in the liver physiology. As it was mentioned above, redox couples as

NADH/NAD$^+$, FADH$_2$/FAD$^+$, and GSH/GSSG are involved in the donation and acceptance of electrons in a variety of reactions. NADPH is a key cofactor for many enzymatic reactions in the metabolism, and it is considered as one of the main regulator of the redox potential (**Figure 3**). Its production is required for the regeneration of GSH in mitochondria for scavenging mitochondrial ROS through glutathione reductase and peroxidase systems [15]. NADP$^+$ is synthesized from NAD$^+$ by NAD$^+$ kinase, whereas NADPH is derived from NADH by three major enzymes in the mitochondrial matrix: NAD(P)$^+$ transhydrogenase, NADP$^+$-dependent isocitrate dehydrogenase (IDH) and malic enzyme and by other three cytosolic enzymes in cytosol: glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase in the pentose phosphate pathway (PPP), and the malic enzyme.

One of the mechanisms for generating cytosolic NADPH from mitochondrial oxidations implies substrates shuttles. This is the case of the shuttle mechanism that involves NADP$^+$-dependent IDHs present in both, mitochondrial and extramitochondrial spaces, whereas NAD$^+$-dependent IDH is solely mitochondrial. IDHs catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate in the tricarboxylic acid cycle (TCA), in a NAD$^+$ or NADP$^+$-dependent manner producing NADH or NADPH, respectively. NADPH may be transported by the isocitrate-2-ketoglutarate shuttle by the cytosolic IDH in which liver has a high enzymatic activity. NADP$^+$-dependent IDH is induced by ROS and controls the mitochondrial redox balance. A decreased expression of NADP$^+$-dependent IDH importantly elevates ROS generation, lipid peroxidation and DNA fragmentation; consequently, a significant reduced ATP level is associated to the mitochondrial damage, whereas overexpression of NADP$^+$-dependent

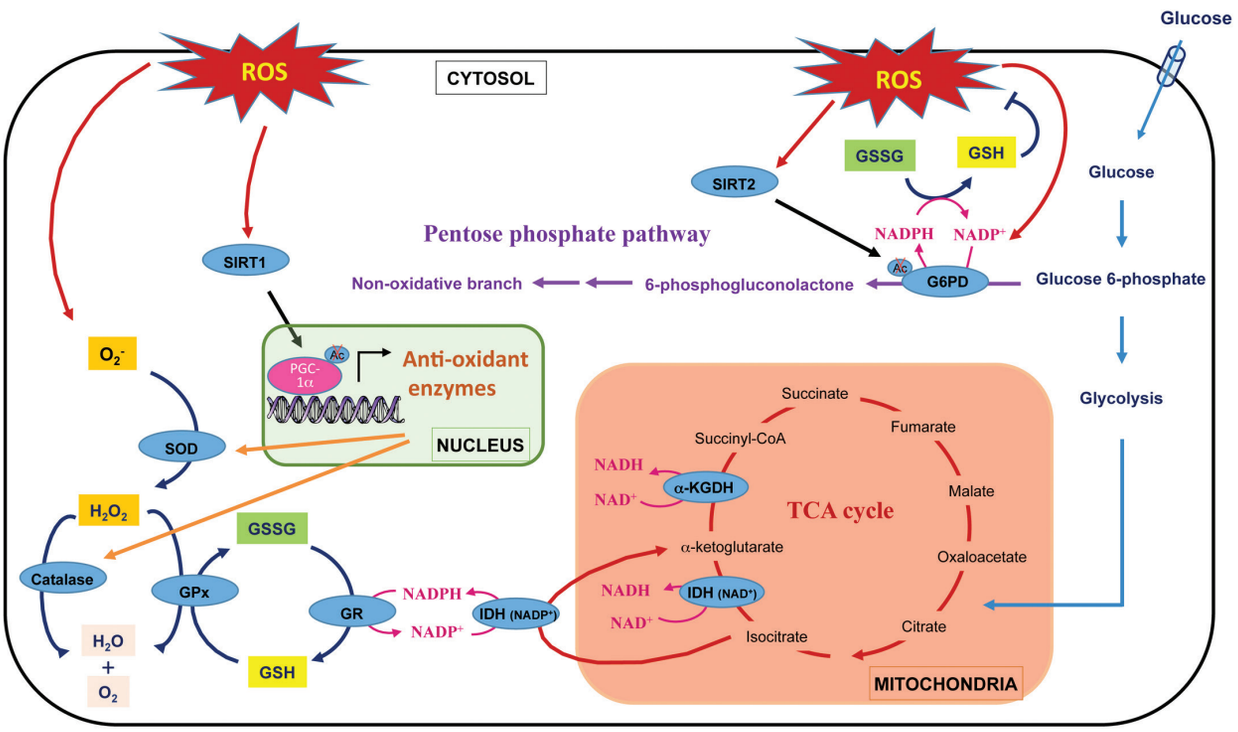

Figure 3. Redox cycles linking metabolic and antioxidant pathways. Glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PD), α-ketoglutarate dehydrogenase (α-KGDH), sirtuin (SIRT), reduced glutathione (GSH), oxidized glutathione (GSSG).

IDH protects from ROS-induced damage [16]. Thus, both cytosolic and mitochondrial IDHs play important roles in cellular defense against oxidative damage by providing NADPH needed for the generation of GSH [17, 18] (**Figure 2**). Likewise, $NADP^+$-dependent IDH is inactivated by GSSG-dependent glutathionylation leading to enzyme inactivation, followed by GSH-dependent reactivation, suggesting an alternative modification to the redox regulation of IDHs [19].

Among enzymes sensitive to redox state is α-ketoglutarate dehydrogenase (α-KGDH). α-KGDH is a mitochondrial enzyme of the TCA that catalyzes the conversion of α-ketoglutarate to succinyl-CoA, producing NADH that supplies electrons for the respiratory chain (**Figure 2**). α-KGDH is considered a component of the mitochondrial antioxidant system and a key sensor of redox status [20]. It is sensitive to ROS and its inhibition impact significantly in the energetic deficit induced by oxidative stress; α-KGDH can also generate ROS by its catalytic action regulated by the $NADH/NAD^+$ ratio [21]. A reversible inhibition of α-KGDH is obtained by glutathionylation. Since α-KGDH controls supply of reducing equivalents generated by the TCA, the redox regulation of α-KGDH would control energy production in response to oxidative stress.

Another source of NADPH required for detoxification of free radicals and peroxides is the activity of G6PD, the rate-limiting enzyme of the PPP (**Figure 2**). It has an important role for cell growth by providing NADPH for redox balance [22] and its expression is induced due to oxidative stress [23], whereas the reduction of G6PD sensitizes cells to oxidative stress [24]. In humans, mutants of G6PD alleles are associated with hemolytic anemia, while mutants in mouse embryonic stem cells by targeted homologous recombination have shown that G6PD is essential to protect cells against even mild oxidative stress whereas the null mutant is lethal. A high-carbohydrate, fat-free diet promotes an increase in hepatic G6PD activity [25], but polyunsaturated fatty acids content of the diet decreases its activity. Besides the effect at activity level, gene expression is also altered. Inhibition of G6PD gene expression is caused by polyunsaturated fatty acids but not by saturated or monounsaturated fatty acids [26]. In the liver of young Zucker obese fa/fa rats, G6PD expression and activity are increased prior to the onset of diabetes type 2, which seems to be contributing factors for the induction of oxidative stress [27].

Furthermore, human G6PD is negatively regulated by acetylation on a phylogenetically conserved lysine 403 becoming unable to form active dimers and consequently the loss of activity. The exposure to extracellular oxidative stimuli promotes reduced G6PD acetylation due to deacetylating activity of sirtuins (SIRTs) (**Figure 2**). The inhibition of SIRT2 increases cellular susceptibility to oxidative stress. SIRT2 deletion leads to a higher level of lysine 403 acetylation and impaired activity of G6PD, whereas the addition of SIRT2 rescues the cell death induced by the deletion [28].

In mammals, seven members of SIRT family are known; they have diverse subcellular localization and activity. SIRT1, SIRT6 and SIRT7 are nuclear, SIRT6 is associated with heterochromatic regions and SIRT7 with nucleoli, SIRT2 is in cytosol, and SIRT3–5 are mitochondrial. SIRT1 shows a potent NAD^+-dependent deacetylase activity on lysine 16 of histone H4 that could promote the formation of heterochromatin, as well as on lysine 382 of p53, while SIRT6

and SIRT7 lack deacetylase activity [29]. Several proteins of this family regulate lifespan in diverse organisms and in human cells.

SIRT1 is the homolog of Sir2, a protein with an important role in longevity in yeast, and with an important role in mammalian development, metabolic regulation and modulation of cellular stress response and survival by acting on p53, NF-κB signaling and FoxO transcription factors [29]. SIRT1 is an important metabolic regulator that orchestrates hepatic gluconeogenesis and lipid metabolism, suggesting that it can play an important role in the developing of metabolic and age-related diseases. The effects of SIRT1 are mediated through the induction of antioxidant proteins, SOD2, nuclear respiratory factor 1 (NRF1) [30], catalase, peroxiredoxins 3 and 5, thioredoxin 2, thioredoxin reductase 2, and uncoupling protein 2 (UCP-2) via formation of forkhead box/peroxisome proliferator-activated receptor gamma coactivator 1-α complex (FoxO3a/PGC-1α) [31] (**Figure 3**).

Increasing ROS level modulates activity of SIRT1. Likewise, SIRT1 activity is regulated by AMPK, which is a redox-sensing enzyme, and a metabolic gauge by increasing cellular NAD^+ synthesis [32]. AMPK is activated by high AMP/ATP ratio and is sensitive to glutathionylation by action of H_2O_2 and NO that oxide reactive thiol residues [33]. Oxidative stress also induces S-glutathionylation of SIRT1 and reduces NAD^+ level, thus inhibiting SIRT1 activity [34]. SIRT1 has an important role in the liver glucose metabolism. Ablation of SIRT1 in liver induces lipid accumulation by upregulating lipogenic genes expression and reducing β-oxidation, whereas overexpression of SIRT1 protects against hepatic steatosis induced by high-fat diet [35]. SIRT1 regulates positively β-oxidation through the activation of nuclear receptors peroxisome proliferator-activated receptor α (PPARα) that regulates gene expression of lipid catabolic genes.

Peroxisomes are oxidative subcellular organelles for H_2O_2, fatty acids, and cholesterol. PPARs are lipophilic ligand-activated transcription factors that belong to nuclear hormone receptor superfamily with three subtypes: PPARα, PPARγ, and PPARβ/δ [36]. PPARα is mainly present in the liver, playing a relevant role in the fasting response. Additionally, PPARα contributes to protection from oxidative stress by upregulating expression of genes of the chaperone and proteasome families, with consequences in protein folding and degradation of damaged proteins [37]. In cancerous cells, PPARα leads to increased peroxisome proliferation and production of ROS contributing to DNA damage [38]. Oxidized lipids are produced during oxidative stress and are natural endogenous PPARγ ligands suggesting a role in oxidative stress response. PPARγ upregulates the expression of antioxidant and prooxidant genes such as catalase, SOD2, GPx3, eNOS and mitochondrial uncoupling protein 2 (UCP-2), whereas downregulates cyclooxygenase-2 (COX-2) and iNOS [39].

Handling of cellular oxidative stress involves several metabolic enzymes that are originally described as part of other metabolic pathways. Most of these enzymes reduce free-radical production and protect cell from injury. The loss of control in the events that coordinates redox homeostasis contributes to oxidative damage, metabolic diseases and progression of degenerative diseases associated to aging. Understanding the mechanisms involved in these events will contribute to improve the quality of life during physiological or pathological conditions.

4. Redox alterations in hepatotoxicity

The role played by the cellular redox state in the hepatic pathophysiological mechanisms is not well defined. Indeed, hepatotoxicity has been much linked to the oxidative status and deficiencies in the liver antioxidant system. For instance, in the case of the alcoholic liver disease (ALD) mainly represented by a chronic stage of alcoholic steatohepatitis (ASH), alterations in the cell redox state have been implicated in injured hepatocytes [40]. Ethanol is metabolized via alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system, and by catalase in the liver peroxisomes, although the activity of ADH is responsible for most of the ethanol catabolism. In this pathway, NAD^+ is reduced by a transfer of hydrogen (and one electron, e^-) to NADH, with the concomitant production of acetaldehyde. The $NADP^+$ can be also reduced, and hydrogen equivalents from ethanol, but not NADH, are transferred from the cytosol to the mitochondria via a shuttle mechanism such as the malate shuttle, the fatty acid elongation cycle, and/or the α-glycerophosphate cycle; therefore, mitochondria become more reduced [41, 42].

Lately, the nonalcoholic steatohepatitis (NASH) is becoming a pathological entity gaining a significant public health concern [43]. NASH is a progressive form of nonalcoholic fatty liver disease (NAFLD) and features of NASH include steatosis, inflammation and varying degrees of fibrosis, and seems to follow a 2-hit model, where the "1st Hit" involves excess of lipid accumulation in the liver, which sensitizes the liver to the "2nd Hit". This "2nd Hit" involves inflammation, oxidative stress, liver damage and fibrosis [44]. Here, multiple cellular processes play important roles in maintaining the NAD^+/NADH ratio. For example, during glycolysis, β-oxidation, and the TCA activity, NAD^+ is reduced to NADH. Within the mitochondria, NADH is oxidized by the electron transport chain enzymes during oxidative phosphorylation.

Laboratory animals exposed to high fat diet exhibit impaired oxidative metabolism through reduced electron transport chain activity [45]. Then, it is possible that those animals exposed to high fat during late gestation and early postnatal life present a state of redox imbalance, and although their livers are able to readily reduce NADH during catabolic redox reactions (in response to increased fat intake), the ability to replenish NAD^+ reserves is reduced due to impaired oxidative capacity [46]. High fat feeding is associated with depleted NAD^+ reserves and reduced SIRTs abundance, both established hallmarks of metabolic aging, and supplementation with factors reversing the effects of depleted NAD^+ reserves, and/or SIRT1 and SIRT3 abundance rescue the increased susceptibility to develop severe fatty liver disease in the adult life [47].

Liver steatosis induces a reduced state in cytosol and mitochondria of hepatocytes, as demonstrated by alterations in the NADH/NAD^+ ratio calculated from the β-hydroxybutyrate dehydrogenase and lactate dehydrogenase reactions [48]. The increased formation of reducing equivalents could impair fatty acids oxidation and the TCA cycle [49], but it could also enhance the formation of glycerol-3-phosphate and thus lipogenesis [50]. Saturation of lipids may also modify cellular redox status. Among free fatty acids, monounsaturated fatty acids, such as oleic acids, are less toxic than palmitate, a saturated acid, because the latter increases

the NADH/NAD^+ ratio and promotes uncoupling between glycolysis and TCA cycle fluxes, leading to increased ROS production [51]. The hepatic accumulation of saturated fatty acids can promote redox imbalance and the formation of reactive oxygen intermediates, mainly inducing endoplasmic reticulum (ER) stress and apoptosis [52].

Moreover, the pathogenesis of early-stage NASH is characterized by hyperinsulinemia and *de novo* synthesis of fatty acids and nascent triacylglycerides, which are deposited as lipid droplets within the hepatocytes. Hyperinsulinemia shifts the energy supply from glucose to ketone bodies, and the high ketone body concentration induces the overexpression of cytochrome P450 2E1 (CYP2E1), resulting in unsaturated fatty acids peroxidation and aldehydes production [53]. The NADPH oxidase-derived ROS from arachidonic acid peroxidation can induce the nuclear translocation of *EGR1*, which in turn can stimulate the expression of the downstream genes *ATF3* and *GADD45G*. ATF3 is a transcription factor involved in cell proliferation, apoptosis, and invasion [54]. The increased redox signaling plays a central role in promoting insulin resistance in the liver in early NASH, followed by fibrogenesis through activation of protein kinase R (PKR), protein kinase R-like endoplasmic reticulum kinase (PERK) key stress kinases [55]. There is evidence that activation of the purinergic receptor P2X7 can give rise to NADPH oxidase activation, leading to Kupffer cell activation, a key event in NASH progression [56].

Peroxisomal oxidation of fatty acids is the normal route of metabolism of very long chain fatty acids and dicarboxylic acids, where electrons from $FADH_2$ and NADH are transferred directly to O_2 [57]. Moreover, fatty acids not oxidized by mitochondria are mainly oxidized by CYP2E1; a process that further increases ROS production [58]. Therefore, many cellular systems are important sources of ROS, including the mitochondrial respiratory chain [59], the cytochrome P450s [60], oxidative enzymes (xanthine oxidase, aldehyde oxidase, cyclooxygenase, monoamine oxidase (MAO), and the NADPH oxidase complex) [61]. The uncoupling protein 2 (UCP-2) may also enhance the reoxidation of NADH into NAD^+, which is required for both β-oxidation and the TCA [62]; UCP-2 oxidation might be considered an attempt to prevent steatosis by increasing hepatic fatty acid oxidation [63].

Moreover, it is accepted that in the NAFLD, depletion of hepatic antioxidants may contribute to the progression of steatosis to NASH by increasing oxidative stress that produces lipid peroxidation, inflammation, and fibrosis. Indeed, metabolic adaptations resulting from severe GSH deficiency seem to protect against the development of steatohepatitis [64]. In summary, all the redox alterations seem to be deeply implicated in the onset of NASH and in its progression to liver fibrosis and a putative installation of a cirrhotic process.

However, a possible role of a disturbed cell redox state is much less known in cirrhosis. Nonetheless, it has been suggested that collagen metabolism could be influenced by changes in redox state. It has been postulated that conversion of glutamic acid to proline [65], a decreased NAD^+/NADH ratio [66], as well as impediment of proline transport and oxidation, could increase the liver proline pool, as a fundamental collagen component in the onset of liver fibrosis [67].

In experimental models of rat liver fibrosis/cirrhosis, mitochondrial function and structure show a variety of alterations. ATP synthesis is reduced in rats treated with CCl_4 or thioacetamide, as well as in rats with secondary biliary cirrhosis. These alterations are compensated

by increasing mitochondrial volume per hepatocyte and possible augmentation of extrahepatic ATP production, as an effort for maintaining mitochondrial function in the cirrhotic liver [68]. These reports agree with the statement that in perfused cirrhotic livers, a reduced cytoplasmic and mitochondrial redox states occur accompanied by a diminished activity in the mitochondrial electron-transport chain [69].

5. Redox alterations in experimental portacaval anastomosis

Portacaval anastomosis (PCA) is a pathological condition that usually accompanies the portal hypertension associated to cirrhosis; however, the shunt can occur among a variety of portal and systemic veins [70]. Experimentally, PCA is a surgical procedure that communicates a sectioned porta vein to an oval incision in the inferior cava vein. It results in a straight flow of the full-of-nutrients portal vein from the small intestine directly to the systemic circulation. It has been used for a long time to implement experimental models of hyperammonemia and the consequent hepatic encephalopathy (HE) [71]. However, only few reports exist regarding the metabolic and physiological consequences of the PCA in the hepatic tissue.

Ammonium (NH_4^+)-metabolic handling by the liver involves equilibrium between anabolic (synthesis of proteins, nucleic acids and amination reactions) and catabolic (urea cycle and glutamine synthesis) pathways. Intracellular glutamate plays a key role since high glutamate serves as substrate for the synthesis of N-acetylglutamate, an essential allosteric activator of carbamyl phosphate synthetase I, a key regulatory enzyme in the urea cycle in the periportal hepatocytes. Nitrogen disposal is complemented by glutamine synthesis (glutamate + NH_4^+) in the pericentral hepatocytes [72].

In human adults, approximately 1 mol (about 17 g) of NH_4^+ is produced daily in the liver. Part is reutilized in biosynthesis, while the rest is a metabolic disposal with the potential of being neurotoxic. Its normal concentration in the portal blood varies from 300 to 600 μM, but in the blood leaving the liver the concentration is clearly reduced to 20–60 μM [73]. Other organs such as the brain, muscle and kidney play a role in regulating the NH_4^+ levels. Insult to the liver, whether acute or chronic in nature, reduces its capacity to metabolize NH_4^+ with the consequence to promote in hyperammonemic state, with up to five times elevation of circulating NH_4^+ [72]. Although the brain is partially protected by the blood-brain barrier from toxic agents such as ammonia, excessive amounts of NH_4^+ can pass into the brain, constituting the principal factor in the onset of HE.

PCA in the rat results in liver atrophy, sustained hyperammonemia, and subtle neurological symptoms of HE including abnormal locomotor activity, altered sleep patterns, and modifications of neuromuscular coordination. Feeding NH_4^+ salts or resins to the shunted rats leads to more severe signs, eventually progressing to coma. Neuropathological examination of these rats reveals Alzheimer type II astrocytosis, the histological characteristic of chronic hyperammonemic syndromes [74].

Oxidative stress is believed to play a role in the pathogenesis of HE because acute doses of NH_4^+ are prooxidant [75]. ROS include molecules, such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and the hydroxyl radical ($OH^\cdot$). Indeed, there is a physiological role for ROS including cellular proliferation, differentiation and signaling. However, a nonphysiological increase in ROS or a decrease in the antioxidant capacity of the organism can lead to an oxidative stress condition [76]. NH_4^+ promotes oxidative stress by increasing ROS [77]. In this context, the brain is susceptible to oxidative stress due to high content of unsaturated fatty acids prone to peroxidation, high O_2 consumption, elevated Fe^{2+}/Fe^{3+}, and low antioxidant systems [78]. However, a polemic issue has arisen since recent report in a model of hyperammonemia using a four-week PCA rat model did not express any signs of oxidative stress in the frontal cortex and in arterial plasma by 4-hydroxy-nonenal (4-HNE)-linked proteins and detection of carbonyl moieties [70].

Liver is by excellence the main metabolic organ, and shows an extensive handling of prooxidant reactions; especially during the biochemical transformation of nutrients and the processing of xenobiotics [79]. However, no information has been reported characterizing putative prooxidant reactions during the experimental PCA.

5.1. Lipid peroxidation

Oxygen is needed for proper energetic metabolism and correct mitochondrial function, but at the same time, it promotes the formation of ROS and, in consequence, oxidation of biomolecules. Lipid peroxidation is a suitable assay to estimate prooxidant reactions. By measuring the presence of conjugated dienes, it is possible to infer the rate of peroxidative activity under "*in vivo*" conditions, and it is also feasible to deduce the balance between prooxidant reactions and antioxidant defenses using the thiobarbituric acid reactive substances (TBARS) assay. When the TBARS test is done with Fe^{2+} supplementation, it offers another set of information: Because it enhances the breakdown of hydroperoxides, the Fe^{2+}-induced lipid peroxidation is maximum and gives an idea about the total antioxidant mechanisms and the presence of global unsaturated fatty acids present in the studied membrane [80].

Initial observations in hepatic subcellular fractions, liver homogenate and serum from sham (n = 10) and PCA (n = 23) operated rats after 8 to 13 weeks of surgery were used to test lipid peroxidative activity (data not published). Conjugated dienes and TBARS were quantified by standard techniques [81]. Strikingly, rats with PCA showed reduced TBARS levels in the liver homogenate and most of the subcellular fractions (**Figure 4**), whereas conjugated dienes showed no changes with lower levels in the mitochondrial fractions (**Figure 5**). The reduction of TBARS was also observed when the assay was supplemented with Fe^{2+} (**Figure 6**).

PCA is an experimental protocol to generate a hypofunctional liver condition. The abovementioned information strongly suggests that redox equilibrium within the liver under PCA surgery shows a reduction in the prooxidant reactions and/or increase in antioxidant defense. More focused experiments are needed to elucidate the underlying mechanism(s), but it is interesting to consider the biochemical consequences that the alteration in the portal blood flow can promote within the hepatocytes' redox equilibrium.

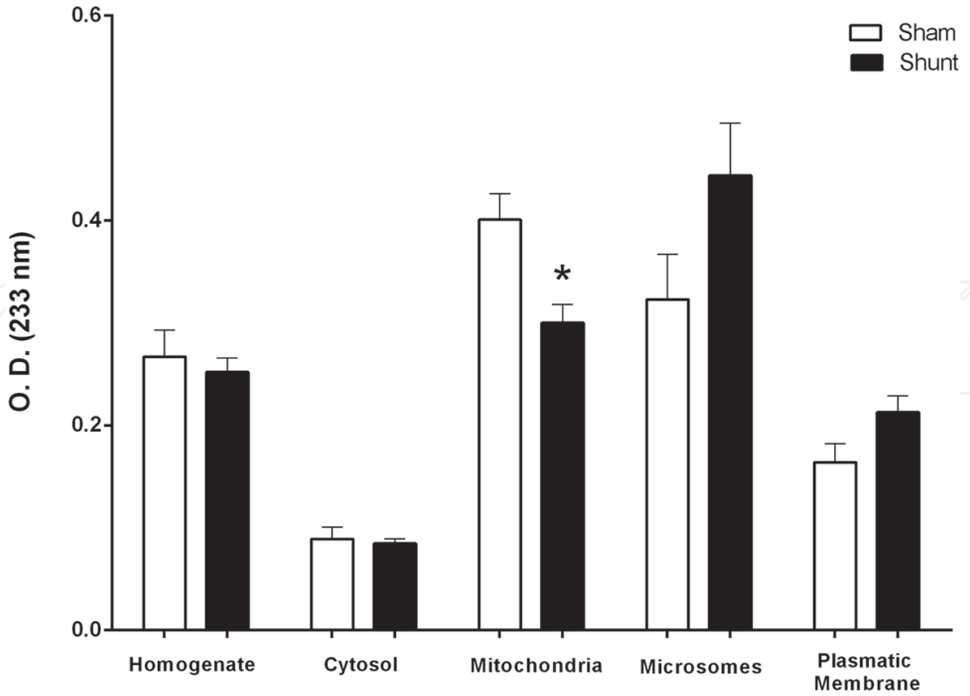

Figure 4. Effect of portacaval anastomosis on thiobarbituric acid reactive substances (TBARS) assay of liver homogenate, subcellular fractions and serum. Sham, false-operated rats (white bars); shunt, rats with portacaval anastomosis for 6-8 weeks (black bars). Data are average ± SEM from at least eight independent observations. *Significant statistical difference by *t*-student test, $p < 0.05$.

6. Redox alterations in liver cancer

6.1. Redox and carcinogenesis

Hepatocellular carcinoma (HCC) is a relevant problem of public health since it is in the sixth place in incidence, and the second in mortality at worldwide level [82]. The main risk factors conducing to HCC are viral hepatitis, steatosis and cirrhosis [83]. HCC could be considered the final stage of chronic liver disease (CLD), characterized by a persistent presence of inflammation and oxidative stress [84].

One of the best-characterized effects of redox changes is related to hepatic carcinogenesis [85]. The accumulation of ROS in early stages of hepatic damage produces lipid peroxidation of the cellular membranes; the reaction of ROS with the double bounds of polyunsaturated fatty acids results in the generation of aldehydes as 4-hydroxynonenal (4-HNE), acrolein, crotonaldehyde, and malondialdehyde, all with the capability of forming DNA adducts and genetic instability [86, 87]. 4-HNE forms exocyclic guanine adducts (4-HNE-dG) and consequently induces carcinogenic mutations [85]. These mutations affect loci of oncogenes or tumor suppressors conduce to malignant transformation. An example is the demonstration that 4-HNE induce G-C to A-T changes in the codon 243 of the p53 locus, an extensively studied tumor suppressor [88], promoting phenotypic loss [89, 90].

In this context, mechanisms activated by oxidative stress have the capability to influence a variety of proteins whose function impact in cancer, by the ability to oxidize thiol residues in

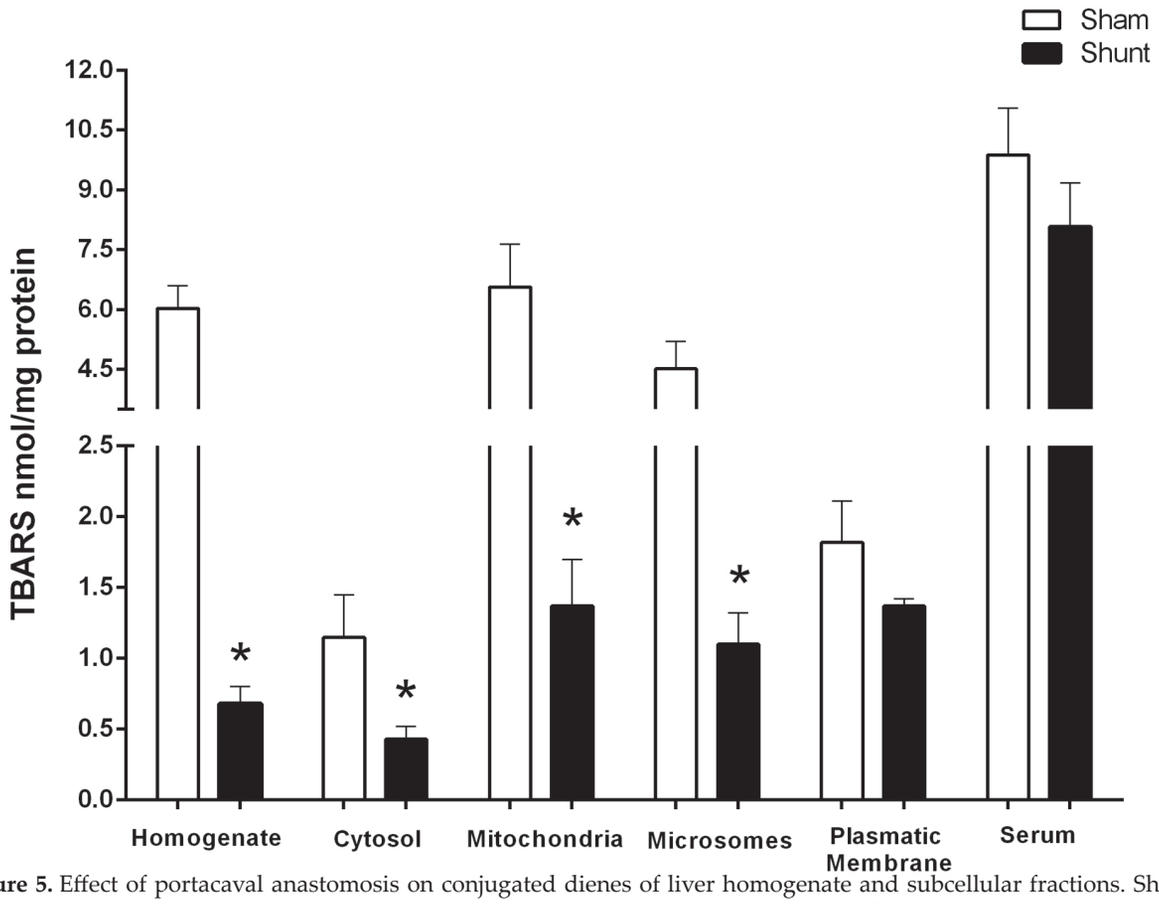

Figure 5. Effect of portacaval anastomosis on conjugated dienes of liver homogenate and subcellular fractions. Sham, false-operated rats (white bars); shunt, rats with portacaval anastomosis for 6-8 weeks (black bars). Data are average ± SEM from at least eight independent observations. *Significant statistical difference by *t*-student test, $p < 0.05$.

a process known as "redox priming" [91]. Examples of these redox sensors are the factor nf-κB [92], oncogenes or tumor suppressors as p53 [93] and Src tyrosine kinase [94].

Interestingly, accumulated evidence suggests that antioxidant systems activated in response to oxidative stress, improve the proliferation rate and protect cancer cells from a hostile environment [95, 96]. For example, it was demonstrated that prostaglandin 1 reductase-1 (Ptgr1), an oxidoreductase involved in the catabolism of eicosanoids and LPO-derived compound as 4-HNE, whose expression is regulated by the transcription factor (erythroid-derived-2)-like-2 (NRF2) [97], is overexpressed in human HCC biopsies and in samples from experimental animals. It was documented that Ptgr-1 regulates positively the proliferation rate of cells and improves their survival in two models of experimental HCC [97], suggesting that this antioxidant response plays a protumoral role in HCC.

6.2. Redox and hepatocellular carcinoma

A role for redox reactions has been detected during the HCC, from the proliferation of initial cancer cells to the dissemination process [96]. At the beginning of CLD, cells have a high proliferation rate, since the tumor growth demands nutrient supply and metabolic conditioning; in consequence, important architectural changes occur including the formation of new

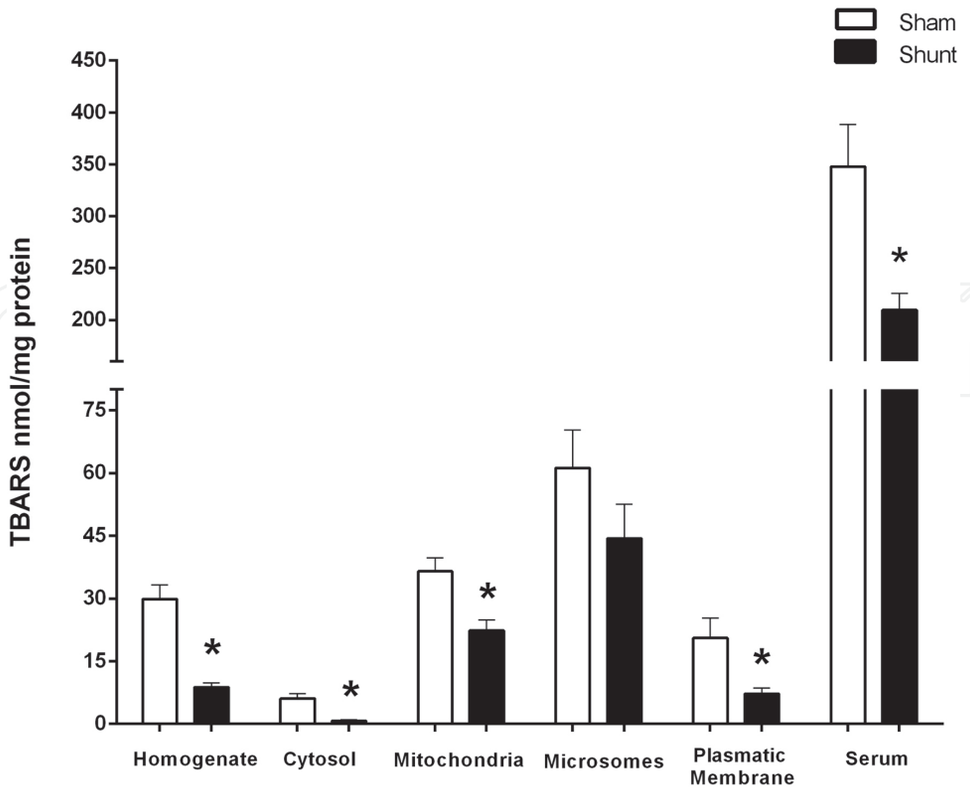

Figure 6. Effect of portacaval anastomosis on thiobarbituric acid reactive substances (TBARS) assay supplemented with FeSO4 50 (μM) of liver homogenate, subcellular fractions and serum. Sham, false-operated rats (white bars); shunt, rats with portacaval anastomosis for 6-8 weeks (black bars). Data are average ± SEM from at least eight independent observations. *Significant statistical difference by *t*-student test, $p < 0.05$.

blood vessels and the establishment of specific microenvironment within the tumor. These adaptations make the tumor cells adapt to highly variable O_2 concentration environment with successive lapses of hypoxia-reperfusion [98].

From the metabolic point of view, lack of O_2 favors the onset of aerobic glycolysis, by the Warburg's effect [99]; hypoxia also induces adaptive responses as the expression of specialized proteins named hypoxia-inducible factors (HIFs) [100]. HIF-1 is a transcription factor formed by two subunits (HIF-1α and HIF-1β). Although HIF-1 is constitutively expressed in normoxic conditions, HIF-α is constantly degraded by prolyl-hydroxylases; hydroxylated residues serve as docking site for von Hippel-Lindau tumor suppressor protein that is a constituent of an E3 ubiquitin ligase complex. The ubiquitinated HIF-1α suffers proteasomal degradation [101]. Low oxygen tension and some proinflammatory cytokines stabilize HIF-1α and allow its nuclear translocation to regulate key genes for the hypoxic response [100]. HIF-1α mediates the expression of genes that supports tumor growth such as NADH dehydrogenase (ubiquinone) 1α subcomplex, 4-like 2 (NDUFA4L2), a protein that attenuates the activity of the mitochondrial complex I, reducing the ROS production in low O_2 conditions [102].

It was shown that the kinase inhibitor sorafenib, an antineoplasic extensively used in oncology with potent antiangiogenic effects, induces intratumor oxidative stress that favor drug resistance in HCC; the insensibility to the drug requires the action of HIF-1α regulating the

expression of specific genes. For example, Nf-κB induces the expression of the antiapoptotic protein Bcl-2 and vascular endothelium growth factor (VEGF) is a potent inductor of angiogenesis [103]. The resultant evasion of apoptosis and genesis of new blood vessels makes evident that protective cellular mechanisms are exploited during the progression of cancerous cells.

A role for ROS has also been described in the invasive process particularly in the epithelium to mesenchymal transition (EMT). EMT is a differentiation process consisting of the change from epithelial to mesenchymal characteristics. Epithelial cells are coupled through specialized structures as adherens junctions and exhibit apicobasal polarity, whereas in the mesenchymal phenotype the cells lose the coupling with neighbors and acquire migratory abilities [104]. EMT is considered the fundamental process in metastasis, being the cytokine TGF-β an effective EMT inductor as well as the growth factors EGF and PDGF [105]. These factors regulate the activity of the transcription factor SNAIL [106]. It has been described that in renal tubular epithelial cells, TGF-β induces synthesis of H_2O_2 and the onset to the EMT [107]. In hepatocarcinoma-derived cells HepG2, the stimulation with the phorbol-ester TPA, favors the activation of protein kinase C (PKC), phosphorylation of ERK and accumulation of ROS, resulting in the induction of EMT and cellular migration. These effects were prevented by ROS scavengers, suggesting a key role for these molecules in the cell migration promoted by TPA [108].

Recently, it was shown that ROS regulate EMT and cell migration induction through the activity of eukaryotic translation initiation factor *eIF5A2*; the expression pattern in the HCC-derived cell line SUN449 correlated with those modified by the knock down of *eIF5A2*, strongly suggesting that eIF5A2 is an effector of ROS signaling [109].

Hepatocellular tumoral cells show a variety of adaptive mechanism in extreme environmental conditions (hypoxia, low nutrients) to continue their growth and progression. Thus, understanding carcinoma cells biology requires yet exhaustive research and integrative efforts of the available data and an intimate knowledge of redox regulation.

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