NEW INSIGHTS INTO PHARMACOLOGICAL LIVER PRECONDITIONING STRATEGIES: L-3,3,5-TRIIODOTHYRONINE AND N-3 LONG-CHAIN POLYUNSATURATED FATTY ACIDS

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ABSTRACT

Liver preconditioning (PC) refers to the development of an increased tolerance to injuring stimuli such as ischemia-reperfusion (IR), which is induced by previous maneuvers triggering beneficial molecular and functional changes. Since liver PC is important in human major hepatic resections and liver transplantation, numerous PC strategies have been evaluated in the IR liver injury model. These include gene therapy, surgical maneuvers, and pharmacological approaches, with few of them being transferred to the clinical setting. In recent years, our group has assessed the PC action of thyroid hormone (T3) and n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) that abrogate liver injury due to 1 h ischemia-20 h reperfusion in the rat. The underlying mechanisms involve (i) the T3-induced redox activation of the hepatic transcription factors Nrf2, NF-kB, STAT3, and AP-1 upregulating the expression of protective proteins; and (ii) the n-3 LCPUFA-induced PPAR-α activation and formation of oxidation products with antioxidant and anti-inflammatory responses, PC strategies that have potential clinical application.

Keywords: Ischemia-reperfusion injury, Liver preconditioning, Thyroid hormone (T3), n-3 Long-chain polyunsaturated fatty acids

INTRODUCTION

Due to the multiplicity of processes involved with liver functioning, namely, most pathways of intermediary metabolism, plasma protein synthesis, biotransformation of xenobiotics, excretion and secretion of several metabolites and mediators, an extremely elevated energy requirement is needed under physiological and pathological conditions. This is primarily met by fatty acid (FA) oxidation, with the exception of hyperglycemic conditions, which imposes on the liver an strict dependency on O2 supply and high susceptibility to hypoxic conditions (Videla & Fernández, 2009).

In this respect, liver damage is associated with ischemia-reperfusion (IR) episodes, including hepatic resection, liver transplantation, abdominal surgery requiring hepatic vascular occlusion, and low-blood pressure conditions (Bahde & Spiegel, 2010). From the mechanistic viewpoint, IR liver injury is elicited by shortage of O2 and nutrient supply during ischemia that is exacerbated upon restoration of blood flow, with Kupffer cell activation, neutrophil infiltration, oxidative stress, and pro-inflammatory cytokine signaling as major contributory factors (de Rougemont et al, 2009; Bahde & Spiegel, 2010; Jaeschke, 2011).

Key mediating events in IR liver injury include (i) ischemia-induced ATP depletion with loss of Ca2+, Na+, and H+ homeostasis and concomitant activation of hydrolytic enzymes, impaired cell volume regulation, and cell swelling; (ii) activation of complement in which factor C5a promotes neutrophil recruitment into sinusoids and primes Kupffer cell for reactive oxygen species (ROS) production;
(iii) formation and release of pro-inflammatory cytokines (tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1)) by Kupffer cells; (iv) chemokine generation by neutrophils (cytokine-induced neutrophil chemoattractant-1 (CINC-1)) and Kupffer cells (IL-8); (v) inadequate anti-inflammatory cytokine response (IL-6, IL-10); (vi) lipid mediators such as platelet activating factor (PAF) formed by endothelial cells priming neutrophils for ROS production; (vii) upregulation of intercellular (ICAM-1) and vascular (VCAM-1) adhesion molecules for neutrophil accumulation at the site of inflammation; and (viii) microcirculatory disturbances and non-perfused sinusoids due to insufficient nitric oxide (NO) production and enhanced vasoconstrictors (endothelins) formation (Jaeschke, 2003). Since IR injury to the liver is a major complication in clinical practice, old donors and hepatic steatosis being factors increasing the susceptibility of the liver to IR in organ transplantation, strategies reducing IR liver damage have been addressed extensively (Das & Das, 2008; de Rougemont et al, 2009).

LIVER PRECONDITIONING AND POSTCONDITIONING STRATEGIES

Liver preconditioning (PC) refers to the development of an enhanced tolerance to IR injury due to previous maneuvers inducing beneficial molecular and functional changes, a phenomenon originally described in the heart (Murry et al, 1986). In past years, several therapeutic strategies have been developed to attenuate or prevent IR liver injury such as gene therapy, surgical strategies, and pharmacological approaches. In the former case, overexpression of proteins suppressing either (i) ROS production (superoxide dismutase (SOD), catalase, heme-oxygenase-1 (HO-1), (ii) apoptosis (Bcl-2, Bag-1), (iii) pro-inflammatory nuclear factor-κB (NF-κB) activation (inhibitor of κB), or (iv) IL-1 action (IL-1 receptor antagonist) have been tested (Selzner et al, 2003; Coito et al, 2002; Harada et al, 2002; Fan et al, 1999), in addition to downregulation of ICAM-1 expression limiting neutrophil infiltration (Sonnenday et al, 2004). However, problems related to vector toxicity, low transfection efficiency and protein expression in gene therapy limit its use in the clinical setting (Casillas-Ramírez et al, 2006).

Surgical strategies such as ischemic PC (IPC) consisting of a brief period of ischemia prior to a short reperfusion time before a prolonged ischemic stress has also been described in experimental animals, with the autocoids adenosine, NO, bradykinin, and opioids playing crucial protective roles (Peralta et al, 1997). Binding of the above mediators to G-protein coupled receptor increases phospholipase-C activity and diacylglycerol production, with the consequent activation of protein kinase C (PKC), mitogen-activated protein kinase (MAPK), heat shock factor 1, and NF-κB (Selzner et al, 2012). This signaling cascade result in promotion of the nuclear transcription of protective mediators, including inhibitors of apoptosis, SOD, inducible NO synthase, and heat shock proteins (Das & Das, 2008; Selzner et al, 2012). Although IPC is useful in human liver resections and in human liver transplantation, this PC strategy remains controversial (Casillas-Ramírez et al, 2006; Bahde & Spiegel, 2010). In addition to IPC, intermittent clamping consisting of various cycles of short intervals of ischemia (15 min) and reperfusion (5 min) was developed, which represents a preferred technique for prolonged ischemia, pathological liver and elderly patients, whereas IPC seems to be more useful for short ischemia and young patients (Bahde & Spiegel, 2010). Considering that IPC is limited by the timing of the procedure as it has to be initiated before the onset of ischemic injury, the new strategy of ischemic postconditioning (IPostC) was developed, a maneuver consisting of intermittent interruptions of blood flow in the early phase of reperfusion, which has been studied in liver, heart, brain, and kidney (Selzner et al, 2012). Similar to IPC, IPostC involves stimulation of G-protein coupled receptors by mediators such as adenosine, bradykinin, ROS, and others, promoting the activation of kinase signaling pathways and ATP-dependent potassium channels, resulting in inhibition of mitochondrial transition pores with apoptosis prevention (Hauser, 2005). Although liver IPostC is experimental models is an alternate and effective approach against IR injury, no clinical data is available in human liver surgery.

The concept of pharmacological PC is based on protective mechanisms induced by a large number of pharmacologic agents affording defense against IR injury in the liver. The protective mechanisms of action of these agents involve either (i) direct interference of injurious pathways or (ii) indirect induction of a low level of stress triggering cellular defense mechanisms against a subsequent stronger insult, subjecting the liver to PC (Selzner et al, 2003; Bahde & Spiegel, 2010), as shown in Table 1. However, pharmacological approaches often affect only one or a few factors (Table 1) involved in the complex and multifactorial phenomenon of IR. Consequently, most pharmacological PC protocols have not been applied in the clinical setting, with the exception of methylprednisolone that shows promising results in terms of improved clinical outcome (Aldrighetti et al, 2006; Bahde & Spiegel, 2010). Major drawbacks include (i) lack of positive impact on postoperative clinical course (antioxidants, vasodilators); (ii) side effects (dopamine, adenosine); (iii) difficulties in implementation (ozone, hyperbaric oxygen); (iv) agents that do not reach their site of action at the right concentration or time (glutathione ester, TNF-α antibody); (v) changes in dose leading to opposite effects (NO donors); and (vi) lack of effectiveness in the presence of steatosis (Casillas-Ramírez et al, 2006; Bahde & Spiegel, 2010).
According to these considerations, our group has undertaken the study of alternate experimental liver pharmacological PC strategies that might have clinical application, namely, thyroid hormone (L-3,3,5-triiodothyronine, T₃) (Fernández et al, 2007a), n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs) (Zuñiga et al, 2010), or iron (Galleano et al, 2011).

### Table 1. Pharmacological strategies to prevent hepatic ischemia-reperfusion injury

<table>
<thead>
<tr>
<th>Agent (Reference)</th>
<th>Preconditioning mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α antibody (Colletti et al, 1990) Methylprednisolone (Aldrighetti et al, 2006)</td>
<td>Inhibition of pro-inflammatory TNF-α signaling Anti-inflammatory therapy based on NF-κB and AP-1 downregulation</td>
</tr>
<tr>
<td>Dopamine (Hasselgren, 1987) ATP-MgCl₂ (Chaudry, 1989)</td>
<td>Reduction of microcirculatory disturbances</td>
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<tr>
<td>Cerulenin (Chavin et al, 2004) Carnitine (Tolba et al, 2003)</td>
<td>Downregulation of UCP-2 expression in steatotic livers due to increased ATP availability prior to IR</td>
</tr>
<tr>
<td>Trimetazidine (Kaya et al, 2003)</td>
<td>Anti-ischemic effects by targeting energy metabolism, oxidative stress, and microcirculatory changes</td>
</tr>
<tr>
<td>AICAR (Carrasco-Chaumel et al, 2005)</td>
<td>Enhancement in NO synthesis with reduction of oxidative stress</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme (ACE) Inhibitor Captopril Angiotensin II (Ang-II) type 1 receptor Blocker Losartan (Guo et al, 2004)</td>
<td>Inhibition of ICAM-1 expression and TNF-α synthesis, with stimulation of hepatocellular proliferation</td>
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<tr>
<td>Diazoxide (Tian et al, 2013)</td>
<td>Mitochondrial-Kₑ₅⁷ channel opener</td>
</tr>
<tr>
<td>Pentoxifylline (Rüdiger &amp; Clavien, 2002)</td>
<td>Inhibition of cyclic 3,5'-nucleotide, Phosphodiesterase activity and TNF-α synthesis</td>
</tr>
<tr>
<td>RAP03 (Cursio et al, 2002)</td>
<td>Matrix metalloproteinase inhibitor</td>
</tr>
</tbody>
</table>

**Abbreviations:** AP-1, activating protein 1; AICAR, 5-Amino-4-imidazole carboxamide riboside; ICAM-1, intercellular adhesion molecule-1; n-3 LCPUFAs, n-3 long-chain polyunsaturated fatty acids; NF-κB, nuclear factor-κB; NO, nitric oxide; TNF-α, tumor necrosis factor-α; ROS, reactive oxygen species; UCP-2, uncoupling protein-2. For additional information, see reviews by Selzner et al. (2003), Casillas-Ramírez et al., (2006), and Bahde & Spiegel (2010).
**T₃ LIVER PRECONDITIONING**

**T₃-induced calorigenesis, liver O₂ consumption, and ROS production:** The PC effect of T₃ upon the liver is based on the calorigenic action leading to stimulation of basal thermogenesis (Schwartz & Oppenheimer, 1978) that is carried out through genomic and non-genomic mechanism (Videla & Fernández, 2009). The genomic pathway involves the formation of transcriptionally active complexes of T₃ with thyroid hormone receptors (TRs), which upon recognition by specific T₃ response elements in DNA trigger the expression of respiratory, metabolic, and uncoupling protein genes, with consequent enhancement in the rate of O₂ consumption of the liver (Fig. 1A) (Videla et al. 2007). In addition, a number of effects of T₃ are independent on TRs, show rapid onset action, and employ alternate signaling mechanisms (Davis et al. 2008). Among these non-genomic pathways, significant activation of cytochrome-c-oxidase is elicited by T₃ and 3,5-diodothyronine (3,5-T₂) through binding to its Va subunit that abolishes the allosteric inhibition by ATP, thus enhancing mitochondrial respiration (Arnold et al. 1998). Under these conditions, elevation in the rate of superoxide radical (O₂⁻) and hydrogen peroxide (H₂O₂) production is achieved by T₃ in liver submicrosomal particles, representing a major ROS generating system in hyperthyroid state (Fernández & Videla, 1993). Liver ROS production induced by T₃ is also elicited at microsomal, cytosolic, and peroxisomal levels in hepatocytes, with Kupffer cells playing a main contributory role due to enhancement in their respiratory burst activity associated with enhanced NADPH oxidase function (Fig. 1A) (Videla et al. 2007). The pro-oxidant state induced in the liver by T₃ can be considered as a mild, non-toxic redox alteration, as suggested by the lack of occurrence of morphological changes in liver parenchyma, except for the hyperplasia and hypertrophy of Kupffer cells (Videla et al. 2007). Under conditions of moderate ROS production, redox signaling can be achieved, a process involving site-specific and reversible modifications in proteins participating in signal transduction pathways, such as receptors, kinases, phosphatases, and transcription factors (Leonarduzzi et al. 2011).

**Molecular mechanisms in T₃-induced liver preconditioning:** The administration of a single dose of T₃ (0.1 mg/kg) to fed rats elicited liver PC against 1 h ischemia-20 h reperfusion in association with the development of a transient oxidative stress condition within a time period of 48 h, with concomitant activation of Kupffer cells and lack of hepatotoxicity (Fernández et al. 2007a). Under these conditions, ROS production triggered by T₃ at the Kupffer-cell and hepatocyte levels activates redox-sensitive transcription factors nuclear factor-erythroid 2-related factor 2 (Nrf2), NF-κB, signal transducer and activator of transcription 3 (STAT3), and activating protein 1 (AP-1), as evidenced by the abolishment of their DNA binding capacity by pre-treatment with antioxidants or the Kupffer-cell inactivator gadolinium chloride (GdCl₃) (Fig. 1A) (Romanque et al. 2011; Videla & Fernández, 2009).

Nrf2 is sensitive to a rather low oxidative stress status (Gloire et al. 2006) as that induced by the calorigenic action of T₃. Increased cellular ROS production promotes the dissociation of Nrf2 from its negative regulator Kelch-like ECH-associating protein 1 (Keap1) in the cytosol, which favors Nrf2 translocation into the nucleus and its interaction with antioxidant response elements to activate target gene transcription (Zhang, 2006). Liver Nrf2 is transiently upregulated by T₃ administration in a redox- (Romanque et al. 2011) and Kupffer-cell-dependent process (Videla et al. 2012a), with concomitant antioxidant protein expression (Videla et al. 2012b). This is evidenced by the induction of hepatic thioredoxin (Thr) (Romanque et al. 2011), heme-oxygenase-1 (HO-1), and glutamate-cysteine ligase (GCL) (Videla et al. 2012b) (Fig. 1A), a response that is essential to limit oxidative stress associated with injurious phenomena such as IR. In fact, Thr exerts antioxidant protection due to its reducing potential, HO-1 is involved in heme catabolism avoiding its pro-oxidant action, with production of the antioxidant products biliverdin and CO, and ferritin induction by iron release, whereas GCL is the limiting enzyme in the biosynthesis of the main hydrosoluble antioxidant glutathione (GSH) (Romanque et al. 2011; Videla et al. 2012b). Induction of Nrf2-controlled phase-II detoxification enzymes by T₃ represents an additional hepatoprotective mechanism due to their roles in xenobiotic biotransformation of potentially toxic epoxides, quinones, and electrophiles (Fig. 1A). These include hepatic (i) microsomal epoxide hydrolase 1 (Eh1) transforming alkene epoxides and arene oxides into dihydriodiol derivatives, (ii) NADPH-quinone oxidoreductase 1 (NQO1) catalyzing the two electron reduction of quinones into hydroquinones, and (iii) glutathione-S-transferases Ya and Yp (GST) conjugating electrophilic metabolites with GSH (Cornejo et al. 2013). Dihydriodiol derivatives produced by Eh1 and hydroquinones formed by NQO1 are subjected to glucuronidation and/or sulfation, which, in addition to glutathione conjugates, constitute readily excretable forms of reactive metabolites. In this respect, T₃ upregulated the expression of phase-III transporters that is also Nrf2-dependent, namely, sinusoidal multidrug resistance-associated protein 3 (MRP-3) retro-transporting xenobiotic conjugates and bile acids, and canalicular MRP-2 excreting anionic drugs and xenobiotic conjugates (Fig. 1A) (Cornejo et al. 2013). These findings, and the induction of antioxidant proteins and phase-I enzymes by T₃ (Fernández & Videla, 1989), suggest the development of a higher hepatic capacity for xenobiotic processing and elimination in hyperthyroid state, triggering the elimination of toxic.
metabolites arising from oxidative stress and xenobiotic biotransformation processes thus affording PC. ROS generated at Kupffer cell and hepatocyte levels by T₃ trigger specific signaling mechanisms that reinforce each other to achieve significant transcription of cytoprotective genes. Under these conditions, antioxidant-dependent GdCl₃-sensitive activation of NF-κB and AP-1 (Fig. 1A) is associated with enhanced expression and release of TNF-α and IL-6 from Kupffer cells into hepatic sinusoids, which upon interaction with the respective receptors TNF-α receptor 1 and IL-6 receptor/gp130 trigger signaling cascades in hepatocytes that may be also stimulated by hepatocytic ROS (Videla & Fernández, 2009). These include (i) NF-κB activation through inhibitor of κB-α kinase (IKKα) phosphorylation, with the expression of antioxidant, anti-apoptotic, and type I acute-phase proteins (Fernández et al. 2005; Tapia et al. 2006); and (ii) AP-1 activation via clun N-terminal kinase (JNK) phosphorylation that upregulates hepatocyte proliferation (Fig. 1A) (Fernández et al. 2007b). In addition to NF-κB and AP-1 redox activation, T₃-induced Kupffer cell-derived IL-6 activates Janus kinase (JAK)/STAT3 cascade through interaction with IL-6R/gp130, triggering the transcription of Type I and type II acute-phase proteins and cell proliferation (Fig. 1A) (Tapia et al. 2006). Furthermore, recent studies revealed that the protective effect of T₃ against IR liver injury is also associated with downregulation of adhesion molecules ICAM-1 and VCAM-1 expression, as well as that of endothelin-1 (Taki-Eldin et al. 2012).

**Figure 1.**

![Figure 1](image)

**Figure 1.** Molecular mechanisms involved in liver preconditioning induced by thyroid hormone (T₃) (A) and n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) (B). Abbreviations: AP-1, activating protein 1; COX-1, cyclooxygenase 1; Eh1, microsomal epoxide hydrolase 1; GCL, glutamate cysteine ligase; GST, glutathione-S-transferases; HO-1, heme-oxygenase 1; iNOS, inducible nitric oxide synthase; S-LOX, 5-lipoxygenase; MnSOD, manganese superoxide dismutase; MRP-2(3), multidrug resistance protein 2(3); NF-κB, nuclear factor-κB; NQO1, NAD(P)H-quinone oxido-reductase 1; Nrf2, nuclear factor-erythroid 2 related factor 2; PPAR-α, peroxisome proliferator-activated receptor α; QO₁, oxygen consumption rate; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; Thr, thioredoxin.

n-3 LPCUFA LIVER PRECONDITIONING

The n-3 LPCUFAs eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA) are crucial structural components of cellular lipids and substrates for the biosynthesis of physiological mediators, which are synthesized from α-linolenic acid (C18:3n-3). EPA and DHA are related to several positive health effects, allowing the proposal for their use in the prevention of numerous non-transmissible chronic diseases (Fetterman & Zdanowicz, 2009; Valenzuela & Videla, 2011) and against the injury caused to heart (Rodrigo et al. 2008) and liver (Zúñiga et al. 2010) by IR episodes. In fact, n-3 LPCUFA administration to rats for seven days prior to IR led to enhancement in the liver n-3 LPCUFA content, with diminution in n-6/n-3 LPCUFA ratios and prevention of IR-induced liver injury. This protective response is associated with abrogation of hepatic oxidative stress and recovery of both pro-inflammatory cytokine homeostasis and NF-κB functionality lost during IR (Zúñiga et al. 2010). Furthermore, significant reduction in both portal pressure during reperfusion and in the time for Trypan Blue to distribute evenly in the liver was observed in n-3 LPCUFA-fed rats over controls, concomitantly with improvement in bile production, suggesting that n-3 LPCUFA minimized IR injury by improving the hepatic microcirculation (Zhong & Thurman, 1995). Similar changes are achieved by n-3 LPCUFA supplementation in animals subjected to a high fat diet, which also exhibited reduction in hepatic liver lipid content and insulin sensitivity improvement (Valenzuela et al. 2012).

n-3 LPCUFA-INDUCED ANTIOXIDANT RESPONSES

Antioxidant responses associated with in vivo n-3 LPCUFA provision are related to their high susceptibility to undergo spontaneous lipid peroxidation with formation of cyclopentenone-containing J-ring isoprostanes (J3-isoprostanes), which promote Nrf2 activation (Fig. 1B) (Gao et al. 2007). This is achieved by reaction of n-3 LPCUFA-derived J3-isoprostanes with Keap1, initiating Keap1 dissociation from Culin3 and nuclear Nrf2 translocation, thereby inducing Nrf2-directed gene expression (Gao et al. 2007). In agreement with these findings, n-3 LPCUFA oxidation products enhanced the expression of the Nrf2-controlled antioxidant enzymes HO-1 and GLC in HepG2 cells (Gao et al. 2007) and glutathione peroxidase, glutathione reductase, GST, and catalase in mouse liver (Demoz et al. 1992). The enhancement in the cellular antioxidant potential by n-3 LPCUFA results in increased liver GSH content and diminished lipid peroxidation extent in mice (Gao et al. 2007), as well as in rats subjected to IR (Zúñiga et al. 2010) or mice given a high-fat diet (Valenzuela et al. 2012).

n-3 LPCUFA-INDUCED ANTI-INFLAMMATORY RESPONSES

Dietary n-3 LPCUFA supplementation can diminish the production of inflammatory cytokines from activated immune cells, a process associated with arachidonic acid (C20:4n-6; ARA) release from cell membranes at early stages of inflammation through phospholipase A2 activation, leading to the synthesis of bioactive eicosanoids (Fetterman & Zdanowicz, 2009). The well recognized anti-inflammatory action of n-3 LPCUFA has been related to (i) the increased proportion of EPA and DHA in the membranes of inflammatory cells, with reduction in ARA availability for conversion into pro-inflammatory mediators (prostaglandin E2, leukotriene B4, thromboxane A2); (ii) production of n-3 LPCUFA derivatives (prostaglandins I3 and E3, leukotriene B3, thromboxane A3) that are less potent inflammation stimulators and/or effective antagonists toward classic pro-inflammatory mediators; (iii) inhibition of pro-inflammatory transcription factors NF-kB and AP-1 by n-3 LPCUFA-dependent peroxisome proliferator-activated receptor α (PPAR-α) activation (Fig. 1B); and (iv) enzymatic transformation of n-3 LPCUFA into resolvins, protectins, and epoxide derivatives (Fig. 1B) (Fetterman & Zdanowicz, 2009; Zúñiga et al. 2011).

Previous reports by our group indicate that suppression of early IR-induced liver injury and NF-κB activation (Zúñiga et al. 2010) was achieved by n-3 LPCUFA supplementation, which was paralleled by enhanced mRNA expression of the PPAR-α-controlled enzymes carnitine-palmitoyl-CoA transferase-I and acyl-CoA oxidase (Zúñiga et al. 2011), thus supporting PPAR-α activation by n-3 LPCUFA. This contention is sustained by the formation of PPAR-α/NFκBp65 transcriptionally inactive nuclear complexes and by the decreased cytosolic IkB-α phosphorylation found, which contribute to reduce NF-κB DNA binding activity and pro-inflammatory cytokine signaling (Fig. 1B) (Zúñiga et al. 2011). Besides, activated PPAR-α also interacts with the cJun component of AP-1 forming PPAR-α/AP-1cJun inactive complexes that hinder transactivation of inflammatory genes (Delerive et al. 1999). Interestingly, the anti-inflammatory effect of n-3 LPCUFA may occur via PPAR-α-dependent mechanisms regulated by oxidized EPA and DHA, which readily undergo oxidation due to their polyunsaturated structure (Sethi et al. 2002; Archana et al., 2004). N-3 LPCUFA oxidation products include (i) EPA- and DHA-derived E-series and D-series of resolvins that are synthesized by the cyclo-oxygenase-1 (COX-1) and 5-lipoxygenase (5-LOX) pathway, respectively; and (ii) DHA-derived D protectins formed by 5-LOX activity (Fig. 1B) (de Roos et al. 2009). In addition to PPAR-α activation dependent anti-inflammatory signaling, n-3 LPCUFA oxidation products may signal through alternate mechanisms as shown for resolving E1, which upon binding to the G-protein-coupled receptor (GPCR) ChemR23 and leukotriene B4 receptor attenuates the pro-inflammatory
action of NF-κB and leukotriene B4 (Arita et al. 2007). Furthermore, EPA and DHA may be transformed into epoxygenated fatty acids by cytochrome P450 NADPH-dependent epoxygenases, such as 17(R), 18(S)-epoxygenosauatraenoic acid and epoxydocosapentaenoic acids (de Roos et al. 2009) that attenuate inflammation in animal models (Wagner et al. 2011), although their mechanisms of action remain to be studied. In the context of oxidized products of n-3 LCPUFA, these derivatives can react with lysine to produce Ne-(propanoyl)lysine, which was proposed as a biomarker of oxidative stress in humans, as evidenced by the increased urinary levels found in diabetic patients compared to healthy subjects (Hisaka et al. 2009).

CONCLUSIONS AND PERSPECTIVES

Genomic, redox-independent actions of T3 on the liver activate the transcription of respiratory genes with enhancement in the rates of O2 consumption and ROS production at several subcellular sites, as the primary mechanism triggering energy metabolism. Consequently, T3-induced ROS generation leads to the redox activation of Nrf2, NF-κB, STAT3, and AP-1 upregulating the transcription of protective genes (Fig. 1A), thus representing a non-genomic secondary mechanism of T3 action that affords protection against IR liver injury (Videla et al. 2007; Fernández et al. 2007a). Besides these liver PC effects, stimulation of hepatocyte proliferation in rats subjected to 70% and 90% partial hepatectomy (Columbano et al. 2008) or to 50% partial liver transplantation (Taki-Eldin et al. 2011) is achieved by T3, a primary hepatic mitogen upregulating the expression of cyclin D1, cyclin A (Taki-Eldin et al. 2011), and cyclin kinase-2 (Fernández et al. 2007b). In line with these views, T3 accelerates the differentiation of hepatic progenitor cells into hepatocytes in vivo, which may have a substantial clinical impact (László et al. 2008). PC by thyroid hormone is not restricted to the liver, considering that long-term L-thyroxine (T4) administration is known to exert cardioprotection against IR, as assessed by the enhancement in postischemic recovery function (Pantos et al. 2002) or reduction of myocardial infarct size (Kumar et al. 2012). Under these conditions, underlying protective mechanisms include significant diminution of p38 MAPK activation (Pantos et al. 2002) or inhibition of the mitochondrial permeability transition pore opening during reperfusion (Kumar et al. 2012). T3 administration also preconditioned the kidney against IR injury and malfunction, which is related to HO-1 induction (Li et al. 2011) and lower expression of poly (ADP ribose) polymerase 1 (PARP-1) (Ferreira et al. 2009), with reduced proteinuria and serum urea and creatinine levels, and improved oxidative stress-related parameters compared to non-preconditioned animals. Furthermore, T3 was recently reported to protect the brain against cerebral IR injury, which was related to down-regulation of pro-apoptotic Bax and up-regulation of both anti-apoptotic Bcl2 and neurotrophic factors (Genovese et al. 2013). In addition to IR injury, thyroid hormones may also play a critical role for the repair in several tissues and organs subjected to different types of injury, including mechanical injury, nerve transaction, chemotherapy-induced toxicity, hyperoxia injury, serum starvation, or wound (for specific references see Mourouzis et al. 2013).

The formation of oxidation products and PPAR-α activation by n-3 LCPUFA represent major mechanisms associated with liver PC against IR injury, triggering antioxidant and anti-inflammatory responses (Fig. 1B). Although the role of n-3 LCPUFA-induced PPAR-α activation in liver PC is supported by studies using PPAR-α agonist WY-14643 in normal (Xu et al. 2008) and steatotic (Teoh et al. 2010) livers from experimental animals subjected to IR, its therapeutic potential in liver surgery in man has not been addressed, while beneficial effects of n-3 LCPUFA supplementation on liver steatosis and hepatic injury are reported in non-alcoholic fatty liver disease patients (Elias-Miró et al. 2012).

T3 and n-3 LCPUFA are hormetic agents with a dose-response phenomenon characterized by beneficial effects in the low dose range (organ PC) and harmful responses at high doses (thyrotoxicosis or gastrointestinal upset/increased bleeding time, respectively), whose underlying mechanisms of action must be fully understood before transfer to clinical application (Videla, 2010). Considering (i) that IR liver injury is a multi-step phenomenon; and (ii) that T3 and n-3 LCPUFA afford liver PC through similar and distinct molecular mechanisms (Fig. 1), combination of protective therapies is advisable. This aspect was recently addressed by our group by reducing single T3 dosage from 0.1 mg/kg (Fernández et al. 2007a) to 0.05 mg/kg and shortening n-3 LCPUFA supplementation (300 mg/kg/day) from 7 to 3 days, which are non-PC protocols when applied separately, but afford protection against IR injury upon combined administration, thus suggesting a reinforcing behavior (Mardones et al. 2012). Besides, the combination of IPC and IPostC protocols reduce IR liver injury more effectively by increasing the activity of ROS scavengers and antioxidants, with utilization of proanthocyanidins from grape seed improving the oxidation resistance in combined IPC and IPostC groups (Song et al. 2012). Another important issue to be considered regarding protective mechanisms in liver PC is energy availability. As indicated in the Introduction, the liver exhibits a rather high energy requirement to perform metabolic, secretory, and excretory functions under physiological conditions, which is further increased to effectively support PC mechanisms. These include ATP demands for the expression of antioxidant, anti-apoptotic, anti-inflammatory, and acute-phase response proteins,
oxidized biomolecules repair (phospholipids, DNA) or resynthesis (proteins) mechanisms, and promotion of hepatocyte and Kupffer-cell proliferation, which are needed to cope with the damaging processes set in by IR. These considerations led to the proposal that upregulation of AMP-activated protein kinase (AMPK) could constitute the metabolic basis for T₃ liver PC, a downstream component of a protein kinase cascade sensing energy dynamics by reducing anabolic pathways, to prevent excessive ATP utilization, and stimulating catabolic processes, to enhance ATP production (VIDELA et al. 2012c). This aspect is currently under study in our laboratory.

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