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ORIGINAL ARTICLE

Albumin Cys34 adducted by acrolein as a marker of oxidative stress in ischemia-reperfusion injury during hepatectomy

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ABSTRACT

The aim of this study was to measure and identify the reactive carbonyl species (RCSs) released in the blood of humans subjected to hepatic resection. Pre-anesthesia malondialdehyde (MDA) plasma content (0.36 ± 0.11 nmol/mg protein) remained almost unchanged immediately after anaesthesia, before clamping and at the 10th min after ischemia, while markedly increased (to 0.59 ± 0.07 nmol/mg; $p < 0.01$, Tukey's post test) at the 10th min of reperfusion. A similar trend was observed for the protein carbonyls (PCs), whose pre-anesthesia levels (0.17 ± 0.13 nmol/mg) did not significantly change during ischemia, while increased more than fourfold at the 10th min of reperfusion (0.75 ± 0.17 nmol/mg; $p < 0.01$, Tukey's post test). RCSs were then identified as covalent adducts to the albumin Cys34, which we previously found as the most reactive protein nucleophilic site in plasma. By using a mass spectrometry (MS) approach based on precursor ion scanning, we found that acrolein (ACR) is the main RCS adducted to albumin Cys34. In basal conditions, the adducted albumin was 0.6 ± 0.4% of the native form but it increased by almost fourfold at the 10th min of reperfusion (2.3 ± 0.7%; $p < 0.01$, *t*-test analysis). Since RCSs are damaging molecules, we propose that RCSs, and ACR in particular, are new targets for novel molecular treatments aimed at reducing the ischemia/reperfusion damage by the use of RCS sequestering agents.

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Introduction

Many hepatic surgery procedures demand the maintenance of the liver in an ischemia condition that can last between 15 and 50 min. Although the surgical techniques have concurred to markedly lower the ischemic period, hepatic alterations consequent to partial hepatectomy are still frequent [1]. Similarly, during the several phases of the hepatic transplantation, the hepatic tissues are exposed to more or less extended periods of warm or cold ischemia that can induce its loss of function or temporary malfunction in 4–10% or 20–25% of patients, respectively [2–4]. The main cause of the hepatic damage consequent to partial hepatectomy or to liver transplantation is ischemia/reperfusion (I/R) [4]. The loss of oxygen supply during the ischemic phase determines the de-energization of mitochondria and the reduction of intracellular ATP associated with alterations of the steady state of Na^+, H^+ and Ca^{2+} [5–8]. The re-introduction of the oxygen supply during reperfusion causes an increased production of reactive oxygen species (ROSs) inducing oxidative stress and mitochondrial permeability transition [9]. Moreover, activated Kupffer cells during reperfusion release ROSs, nitric oxide and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), monocyte chemotactic protein-1 (MCP-1), neutrophil-attracting CXC chemokines, and the interleukins (IL)-6, IL-1β, IL-12 and IL-18 [10], which latter inhibits the synthesis of the anti-inflammatory cytokine IL-10 [11]. Overall, the I/R occurring during hepatectomy is a condition of oxidative stress that can induce cell and organ dysfunction. Among the different mechanisms of oxidative stress involved in the I/R damage, those involving the formation of reactive carbonyl species (RCSs), such as lipid peroxidation products, are gaining great interest [12]. RCSs are strongly electrophilic towards endogenous nucleophilic substrates, such as DNA and proteins, and lead to irreversible covalent modifications [13]. Miyata and colleagues have defined

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"carbonyl stress" as the accumulation in the plasma of RCSs derived from lipid, carbohydrate and protein carbonylation [14]. RCSs derived from lipids and carbohydrates possess a longer half-life in respect to free radicals and ROSs. Moreover, while ROSs are generally free radicals and/or ionic species, RCSs are neutral molecules able to diffuse through cellular membranes and therefore to react within sites distant from their production site. For this reason, RCSs are not only direct cytotoxic but can also mediate and propagate oxidative stress and tissue damage as second messengers. RCSs from lipid peroxidation cascade are quite heterogeneous and can be divided into three main classes, namely: (1) the α,β-unsaturated aldehydes, including 4-hydroxy-2-nonenal (HNE) and acrolein (ACR); (2) di-aldehydes, including the well-known lipid peroxidation by product malondialdehyde and glyoxal; (3) keto-aldehydes, such as methylglyoxal [13].

Several studies, both in animal models and in humans, have reported the increase of RCSs such as free aldehydes or protein adducts in the liver under I/R damage [15–19]. The usual analytical methods give an overall measure of RCS/advanced glycation and lipoxidation end-products (AGEs/ALEs) content, but do not identify the specific RCSs or protein substrates involved in the damaging reaction. Such information is of great value not only in the setting-up of methods aimed to identify specific analytics, but also in order to obtain a deep insight into the damaging mechanism and, thereby, to rationally design suitable molecular strategies, which depend on the chemical structure of the RCS involved [20,21].

The aim of this study was to identify by an off-target mass spectrometry (MS) approach the main attacking RCS species formed in the blood of patients subjected to hepatic resection, with particular focus on serum albumin modifications.

For this purpose, we applied a MS strategy for the rapid identification and characterization of albumin Cys34-covalent modifications, as this residue is highly sensitive to formation of various post-translational modifications [22]. This strategy was based on the precursor ion scanning technique, which is able to supply a rapid confirmation of targeted or non-targeted compounds with a common moiety, and has been widely used in drug discovery and development [23] and *in vitro*/*in vivo* drug-metabolism studies [24–26]. Furthermore, we have used this approach also for the rapid identification of free and protein-bound histidine (His) residues modified by RCS in urine from Zucker-obese rats [27].

Methods

Chemicals

Custom-synthesized LQQCPF peptide, with 90% purity was supplied by Sigma–Aldrich (Milan, Italy). ACR was purchased from Fluka (Buchs, Switzerland). Sequence-grade modified trypsin was obtained from Promega (Milan, Italy) and chymotrypsin from Roche Diagnostics S.p.A. (Monza, Italy); Iodoacetamide (IAA), (±)-threo-1,4-dimercapto-2,3-butanediol (DTT) from Sigma-Aldrich (Milan, Italy). LC-grade and analytical-grade organic solvents were from Merck (Bracco, Milan, Italy). LC-grade water (18 mΩ) was prepared with a Milli-Q water purification system (Millipore, Bedford, MA). All other reagents were of analytical grade.

Surgery procedures

During the interventions, the following parameters were routinely monitored: arterial blood pressure (ABP, radial artery catheter), heart rate (HR), arterial oxygen saturation and central venous pressure (CVP). In addition, the radial artery catheter was connected to MostCare® by means of a pressure transducer to monitor the uncalibrated blood pressure wave-based hemodynamic. This monitoring system allows adjunctive measures of cardiac output/index (CO/CI), systemic vascular resistance (SVR/SVRI). The objective of this monitoring is to ensure adequate perfusion of vital organs during all stages of intervention and target therapy. Blood samples for blood gas analysis (BGA) were drawn for the first time 20 min after the induction of anesthesia and then every 15 min. Blood was collected from the catheters inserted in the radial artery in conjunction with the hemodynamic recordings. During the whole surgical time, the fluid infusions (plasma expanders as well as saline solutions) and vasoactive amines (dopamine, 3–6 mcg/kg/min) were administered as needed to maintain a mean SAP above 65 mmHg and autologous red blood cells were administrated in order to compensate intra-operative blood losses and maintain hemoglobin above 10 gr/dL. Metabolic acidosis (pH $<$7.25 and BE $<$8 mmol/L) was corrected with sodium bicarbonate or THAM infusion. Esophageal temperature was kept within a normal range using elect warming pads. Mechanical ventilation was initiated with a continuous flow of 10 L/min, inspired oxygen fraction (FiO2) of 50%, peak inspiratory pressure of 12 cm H_2O, positive end respiratory pressure of 3 cm H_2O, respiratory rate of 10/min.

Collection of blood samples

Samples of peripheral blood were obtained with written consent from patients ($n = 6$) undergoing hepatectomy for hepatic metastases. Samples (5–10 ml) were collected into heparin-coated sterile tubes at the following stages if the surgical intervention: (1) before administration of anesthesia ("anesthesia t0"); (2) before application of clamps ("ischemia t0"); (3) 10 min after application of clamps (ischemia, "ischemia t10"); (4) immediately after release of clamps (reperfusion, "reperfusion t0"); (5) 10 min after start of reperfusion ("reperfusion t10"). Blood samples aliquots were used to prepare cell-free blood serum by centrifugation; the serum samples were immediately frozen and maintained at −80 °C for further analyses. For five patients, a fourth aliquot was collected at anesthesia t0 and reperfusion t10 time points and used to detect covalent modifications of albumin Cys34.

Protein concentration assay

Protein concentration was scored in the samples by the Bradford assay, using a standard curve of bovine serum albumin (0–15 μg protein/200 μl volume) [28].

Protein carbonyl (PC) assay

Oxidative modifications in plasma were assessed based on the carbonyl content using 2-4 dinitrophenylhydrazine (DNPH), as previously reported [29]. Reacting with PC, DPNH forms a Schiff base to produce the corresponding hydrazone, which can be analyzed spectrophotometrically. Briefly, after incubation with DNPH (400 μl), plasma (100 μl) was precipitated with Trichloracetic acid (TCA). The pellet was washed several times with a 1:1 mixture of ethanol/ethyl acetate, resuspended in 500 μl of guanidine hydrochloride and measured at 370 nm. The PC content was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1}, normalized for protein concentration and expressed in nmol/mg.

MDA quantification

Malondialdehyde (MDA) was quantified using the thiobarbituric acid reactive substances (TBARS) assay kit (Oxitek-ZeptoMetrix Corporation, Buffalo, NY), according to the manufacturer protocol. Briefly, 100 μl of plasma were aliquoted in glass tubes (each sample was in triplicate), mixed with reaction buffers and placed in a heat block at 95 °C for 1h. After incubation, samples were quickly cooled on ice and centrifuged at 3000*g* for 15 min to remove debris. The fluorescence emission of the recovered supernatants was measured at an excitation wavelength of 530 nm and an emission wavelength of 550 nm, using a Perkin-Elmer LS55 spectrofluorimeter (Waltham, MA).

Identification of LQQCPF adduct by LC-ESI-MS analysis

LQQCPF-adducts were prepared, as described by Aldini et al. [24], and reduced by incubation with $NaBH_4$ for 60min at 37 °C. Human serum albumin (HSA) was isolated from plasma samples by using the Montage Albumin Deplete Kit (Millipore, Milan, Italy), eluted with 1 M NaCl and then stabilized with $NaBH_4$ (final concentration 5 mM). HSA concentration was measured by absorbance at 279 nm (E 1% 1 cm = 5.31). Samples were then desalted on Microcon YM30 centrifugal filter devices (Millipore, Milan, Italy) by rinsing with distilled water and then lyophilized and stored at −20 °C until analysis. Lyophilized albumin (420 μg) was dissolved in 100 μl of 100 mM Tris-HCl (pH 7.8) solution containing 6 M urea and 10 mM DTT, and heated at 60 °C for 1 h. Cysteinyl thiols were then blocked by the addition of IAA (20 μL of a 200 mM solution) for 60 min in the dark. Residual IAA was quenched by the addition of 20 μL of DTT and incubation for additional 60 min in the dark. The sample was then diluted to 1 mL with 50 mM NH_4HCO_3 (pH 7.8), and a 400 μL aliquot was digested with trypsin at a protease:protein ratio of 1:20 (w/w) at 37 °C overnight. A 200 μL aliquot was then submitted to chymotrypsin digestion at a protease:protein ratio of 1:20 (w/w) for 8 h at 37 °C in the presence of 10 mM $CaCl_2$. To stop the protease activity (for both trypsin and trypsin + chymotrypsin digestion), samples were spiked with TCA (10% final concentration) and centrifuged at 18,000*g* for 10 min.

Fifty microliter of the supernatants (or of the reduced LQQCPF-adducts mixture) were injected into a quaternary pump HPLC system (Surveyor LC system, ThermoQuest, Milan, Italy) connected by an ESI source to a triple quadrupole TSQ Quantum Ultra (ThermoQuest, Milan, Italy). The ESI source was set in the positive-ion mode, under the following conditions: capillary temperature 270 °C; spray voltage 4.5 kV; capillary voltage 12.5 V. The flow rate of the nebulizer gas (nitrogen) was 5 l min^{-1}. The LC-ESI-MS analyses were done in data-dependent-scan mode with precursor ion scanning as previously reported [22] by selecting the following product ions: *m/z* 242.1, 263.1 and 370.2 (collisional voltage 40 V). A mass unit resolution and scan time of 1 s were used in both quadrupoles Q1 and Q3, and the Q1 scan range was set at *m/z* 300–1500. The

adducts were then characterized in product-ion scan mode in the following conditions: Q1 peak width 0.7, scan time 1 s, collision energy 30 V, scan range 50–1500 *m/z*.

Semiquantitative measurement of LQQC(ACR)PF adduct

The LQQC(ACR)PF adduct content was determined as percentage in respect to the unmodified form detected as carboamidomethylated derivative LQQC(CAM)PF due to the HSA treatment with IAA. The relative content of ACR modified LQQCPF was determined in Multiple Reaction Monitoring mode by setting the following transitions:

$$\text{LQQC(CAM)PF: precursor ion at } m/z \text{ 792.5; product ions at } m/z \text{ 263.1}(y_2 \text{ ion})$$

$$\text{LQQC(ACR)PF: precursor ion at } m/z \text{ 793.5, product ion at 531.1}(b_4 * \text{ ion})$$

These fragments were selected from the MS/MS spectrum obtained by analyzing the sample in product-ion scan mode. In particular, the peak relative to the LQQC(ACR)PF was characterized by a retention time of 54.2 min and by a molecular ion of 793.5 m/z ($[M+H]^+$ of the reduced adduct) [22].

The amount of each analyte was determined by using a calibration curve built by using standards prepared by incubating the native peptide LQQCPF with IAA and ACR until the disappearance of the native peptide [22]. The relative content of [LQQC(ACR)PF] peptide was calculated as it follows:

$$\%\text{LQQC(ACR)PF} = [\text{nmoles LQQC(ACR)PF}/\text{nmoles LQQC(ACR)PF} + \text{nmoles LQQC(CAM)PF}] \times 100$$

Statistical analysis

Data are expressed as mean ± SD unless stated otherwise. For statistical analysis, ANOVA, followed by the Tukey's post-test for multiple group comparison was performed with the GraphPad Prism software 6 (GraphPad Prism Software, Inc., San Diego, CA), with statistical significance set at $p < 0.05$. Student's unpaired *t*-test was used wherever appropriate to compare variations between the groups.

Results

Protein carbonyls and TBARS evaluation

Figure 1 shows the TBARS (upper panel) and protein carbonyl (PC, lower panel) content of plasma at the different stages of the surgical intervention. TBARS levels were of 0.36 ± 0.11 nmol/mg protein before anesthesia (anesthesia t0), remained almost unchanged before and 10 min after clamping (ischemia t0 and ischemia t10) and after 10 min of ischemia (reperfusion t0), but significantly increased to 0.59 ± 0.07 nmol/mg after 10 min of reperfusion (reperfusion t10). A similar trend was observed for the PC content, which basal levels (0.17 ± 0.13 nmol/mg) did not substantially change during ischemia but significantly increased (to 0.75 ± 0.17 nmol/mg) after 10 min of reperfusion.

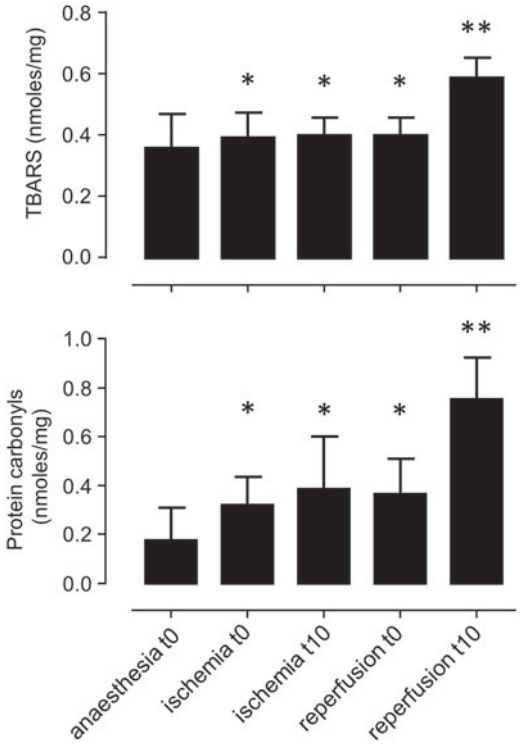

Figure 1. TBARS (upper panel) and protein carbonyl levels (lower panel) at the different stages of hepatectomy. X-axis indicates the following stages of the surgical intervention: before anaesthesia (anaesthesia t0); before application of clamps (ischemia t0); 10 min after application of clamps (ischemia t10); immediately after release of clamps (reperfusion t0); 10 min after start of reperfusion (reperfusion t10). Statistical analysis: ANOVA, followed by the Tukey's post-test. *ischemia t0, ischemia t10 and reperfusion t0 versus reperfusion t10 ($p < 0.01$); **reperfusion t10 versus anaesthesia t0 ($p < 0.05$).

Identification of albumin Cys34 covalent modification by an MS precursor ion scan approach

The oxidized isoforms of albumin, isolated by affinity chromatography from blood samples withdrawn before anesthesia and after 10 min of reperfusion, were detected using an off-target approach based on an MS precursor ion scan method, as previously reported [22]. This method is based on the isolation and enzymatic digestion of HSA, to generate suitable-length peptides containing the Cys34 residue (LQQCPF) and the corresponding adducted derivatives, which are identified by LC-ESI-MS/MS in precursor ion scan mode. As already reported, three ions of the LQQCPF sequence at y_2 (*m/z* 263.1), b_2 (*m/z* 242.1) and b_3 (*m/z* 370.2) were employed in precursor ion scan mode to identify and characterize the covalent modifications of Cys34. These b (where charge is retained on the N terminal fragment) and y (where charge is retained on the C terminal fragment) ions were selected for their abundance, stability and diagnostic value both for the native and adducted LQQCPF, as well as for the independence of their formation from the moiety linked to the Cys34 residue.

Figure 2 shows a representative precursor ion scanning of digested HSA isolated from the blood sample of the subject 2 after 10 min of reperfusion. The precursor ion scan current, recorded with the product ion set at *m/z* 242.1 (panel A), contains not only a peak eluting at 20.9 min relative to the carboamidomethylated LQQCPF peptide [LQQC(CAM)PF] (precursor ion at *m/z* 792.5) but several other peaks. The product ions at *m/z* 263.1 and 370.2 (panels B and C of Figure 2) gave similar results. To increase selectivity, the data for the precursor ion scans were analyzed using the Boolean logic algorithm, as already reported [22]. Briefly, for each time point, the data for the ion current of a selected precursor ion scan was kept and summed with the data of the subsequent precursor ion scan only when their value were higher than a certain threshold. The output data are reconstituted as a virtual current trace (VCT) where the displayed peaks are the sum of those present in all three precursor ion scan traces, while those lacking at least one trace are automatically discarded. The VCT of HSA (panel D), isolated from subject 2 after 10 min of reperfusion, displayed a main peak at *m/z* 792.5 with a retention time of 20.7 min and a minor peak at *m/z* 793.4 with an increased retention time at 21.2 min. Similar results were obtained for the other subjects (data not shown). No other adducted LQQCPF ions were unequivocally detected.

Structural characterization of the adducted LQQCPF peptide

In order to record the MS/MS fragment ions of the precursor ion at *m/z* 793.4, samples were then analyzed in product ion scan mode. Figure 3(A) shows the selected ion chromatogram (SIC) of digested HSA isolated from the subject 2 after 10 min of reperfusion and setting the filter ion at *m/z* 793.4. The well-defined peak detectable at 21.2 min is also present in the other subjects (data not shown). Figure 3(B) shows the MS/MS spectrum of the ion at *m/z* 793.4. Since it is characterized by a set of characteristic ions of the LQQCPF peptide (not containing the Cys34 residue), namely y_1 (*m/z* 166.1), y_2 (*m/z* 263.1), b_2 (*m/z* 242.1), b_2-NH_3 (*m/z* 225.1), b_3 (*m/z* 370.2), b_3-NH_3 (*m/z* 353.2), this is definitively attributed to an

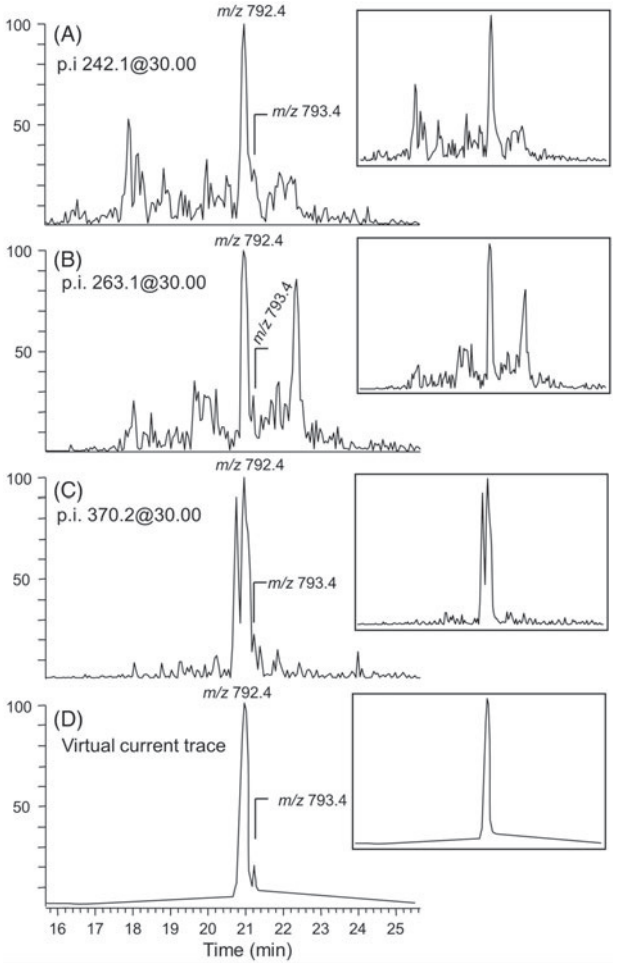

Figure 2. LC-ESI-MS/MS analysis (precursor ion scanning) of trypsin/chymotrypsin digested HSA isolated after 10 min of reperfusion and before anesthesia (insert). Panels A, B and C: precursor ion scanning currents recorded setting the product ions at *m/z* 242.1, 263.1, 370.2, respectively; the precursor ion at *m/z* 792.5 is attributed to the carboamidomethylated derivative [LQQC(CAM)PF]); panel D shows the VCT identifying two peaks, attributed to LQQC(CAM)PF and to LQQCPF adduct at *m/z* 793.5.

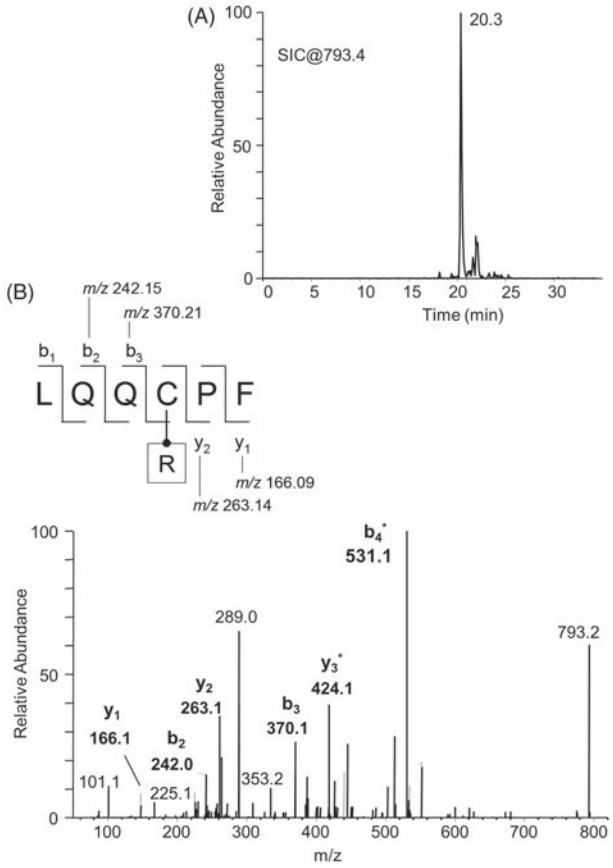

Figure 3. LQQC(ACR)PF adduct characterization. Panel A: LC-ESI-MS/MS of digested HSA isolated after 10 min of reperfusion in product ion scan mode; the ion at *m/z* 793.5 was set as parent ion. Panel B: MS/MS spectrum relative to the ion at m/z 793.5, attributed to the Michael-adducted peptide LQQC(ACR)PF. The asterisk indicated the adducted aa.

LQQCPF backbone. Based on the molecular weight (MW) of the precursor ion shifted by 58 Da in respect to the native peptide (*m/z* 735.4), the adducted moiety was clearly attributed to the acrolein Michael adduct reduced by the treatment with $NaBH_4$, used as reducing and stabilizing agent. The Cys34 modification site was then unequivocally identified by the presence of the diagnostic y and b adducted ions, and in particular by the ions at *m/z* 531.1 (base peak) and 424.1 referring to b_4* and y_3*, respectively. Final confirmation was achieved by comparing the MW, retention time (RT) and MS/MS fragment ions of the identified peak with those relative to a genuine standard of LQQC(ACR)PF adduct.

Semi-quantitative analysis of LQQC(ACR)PF adduct by MRM analysis

The relative content of ACR modified LQQCPF in respect to the unmodified form (detected as carboamidomethylated derivative [LQQC(CAM)PF] was determined in Multiple Reaction Monitoring (MMR) mode, by setting the diagnostic and transitions as reported in Methods.

The amount of each analyte was determined by a calibration curve built with peculiar standards synthetized in our laboratory by incubating the native peptide LQQCPF with IAA and ACR until the disappearance of the native peptide.

As shown in Figure 4, the relative amount of LQQC(ACR)PF peptide in respect to the LQQC(CAM)PF, which was of 0.6 ± 0.4% in the subjects before anesthesia, increased by almost fourfold (2.3 ± 0.7%) after 10 min of reperfusion ($p < 0.01$; *t*-test analysis).

Discussion

The first step of this study was aimed to quantify RCS species during the different stages of liver hepatectomy, and in particular before anesthesia and clamping, during ischemia and after reperfusion. Protein carbonyl (PC) and MDA levels did not significantly change during ischemia and immediately after reperfusion respect to pre-anesthesia levels but significantly increased after 10 min of reperfusion (by 4.4- and 1.6-folds, respectively). This trend well agrees with the well-known burst of oxidative stress that occurs during the reperfusion stage [30]. In particular, the oxidative damage and the consequent formation of lipid peroxidation products occur during reperfusion [31]. Our data confirm the results recently reported by Tsai et al. which found a significant MDA increase within a reperfusion period between 3 and 30 min in patients undergoing liver transplantation [32]. The increase of RCSs determined by the DNPH assay was higher respect to that of MDA, which suggested that other carbonyl species are generated. These assays were performed despite their lack in specificity, because our experience demonstrated that using appropriate positive and negative controls, they represent a very effective and sensitive tool for measuring overall oxidative stress-induced damage.

These results prompted us to identify the main RCSs generated during the reperfusion. Several MS-based approaches to identify RCSs in free or adducted forms have been reported [33,34]. We have previously found that free plasma RCSs rapidly disappear (by almost 70% within 5 min) during reperfusion, since they form covalent adducts with plasma proteins [22]. By proteomic studies, we identified albumin as their main protein target and Cys34 as the most reactive site, whose high nucleophilic reactivity has been clarified by molecular modeling studies [35]. Based on these results, we aimed at identifying the chemical structures of RCSs adducted to Cys34. For this purpose, an off-target method, based on precursor ion scanning was used, as already reported

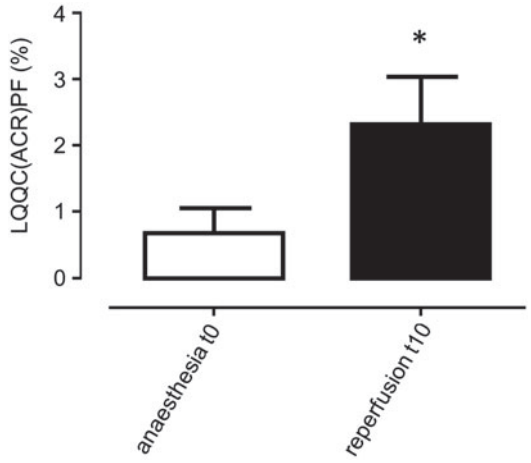

Figure 4. LQQC(ACR)PF adduct increases after reperfusion. Relative amount of LQQC(ACR)PF peptide in respect to the LQQC(CAM)PF before anesthesia and after 10 min of reperfusion. *$p < 0.01$, *t*-test analysis.

[22]. Unequivocally, we identified acrolein (ACR) as the main Cys34 RCS adduct. By using a semiquantitative approach able to establish the relative content of ACR-Cys34 adduct in respect to the unmodified form, we found that the ACR-Cys34 Michael adduct was detectable already before clamping and significantly rose during the reperfusion period. These results prompted us to ascertain whether the ACR-Cys34 Michael adduct is normally present in healthy subjects or is characteristic of hepatic dysfunctions. ACR is a toxic and highly reactive α,β-unsaturated aldehyde widely distributed in the environment as a common pollutant and generated endogenously mainly by lipoxidation reactions. The biological effects of ACR are due to its ability to react with the nucleophilic sites of proteins, to form covalently modified biomolecules, which are thought to be involved in the onset and progression of many pathological conditions, such as cardiovascular and neurodegenerative diseases [36].

Others have demonstrated that ACR is released after I/R damage in the heart [37] and in the retina of rats [38]. Here, we first demonstrated, to our knowledge, that ACR is the main RCS formed during liver reperfusion in humans. Most likely, albumin acts as an endogenous neutralizing agent of RCSs and hence as a defense system against the damaging by-products released during the oxidative stress. The amount of ACR that escapes from the neutralizing reactions mediated by albumin and most likely by liver glutathione (GSH), thus acting as a damage signal in the liver, remains to be evaluated.

Recently, it has been reported that 2-acetylcyclopentanone (2-ACP) and other 1,3-dicarbonyl compounds provide direct protection against electrophile ACR-induced injury [39]. These compounds ionize in aqueous solutions to form nucleophilic enolate anions, which can form 1,4-Michael adducts with ACR, HNE and other electrophilic unsaturated aldehydes involved in the oxidative stress-induced injury. Recently, it has been found that 2-ACP reduces the liver I/R damage in dose-dependent manner by sequestering electrophilic species and in particular ACR, as indicated by the normalization of the I/R-altered liver histologic and biochemical parameters [40]. Hence, our results demonstrate that ACR is produced also in the liver I/R damage and, although partially neutralized by albumin and endogenous detoxificants, it can induce a damaging action.

In conclusion, our results clearly demonstrated that the I/R occurring during hepatectomy induces the release of MDA and RCSs in the blood and that ACR is the main RCS adducted to albumin Cys34. By considering that ACR is a damaging molecule, these results offer new target for novel molecular treatments aimed at reducing I/R damage by agents able to sequester RCSs and in particular α,β-unsaturated aldehydes such as ACR.

Disclosure statement

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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