Mitochondrial Fusion, Fission, and Biogenesis in Prolonged Critically Ill Patients

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Context: Critical illness induces swelling, enlargement, and dysfunction of mitochondria, which in liver, but not in muscle, is aggravated by excessive hyperglycemia. We previously demonstrated impaired autophagic clearance of damaged mitochondria in fed prolonged critically ill patients. Impaired fusion/fission-mediated repair and/or renewal through biogenesis may further accentuate mitochondrial abnormalities.

Objective: We studied mitochondrial fusion/fission and biogenesis and how these are affected by preventing hyperglycemia with insulin during critical illness.

Design and Setting: Patients admitted to a university hospital surgical/medical intensive-care unit participated in a randomized study.

Patients: We studied adult prolonged critically ill patients vs. controls.

Intervention: Tolerating hyperglycemia up to 215 mg/dl was compared with intensive insulin therapy targeting normoglycemia (80–110 mg/dl).

Main Outcome Measures: In liver and skeletal muscle, we quantified levels of several proteins involved in mitochondrial fusion/fission and biogenesis.

Results: Key players in mitochondrial fusion/fission and biogenesis were up-regulated in postmortem liver (1.4- to 3.7-fold) and rectus abdominis (1.2- to 4.2-fold) but not in in vivo or postmortem vastus lateralis biopsies of critically ill patients. Maintaining normoglycemia with insulin attenuated the hepatic response in the mitochondrial fusion/fission process but did not affect the markers of mitochondrial biogenesis in liver or muscle.

Conclusions: Our observations suggest tissue-dependent attempts of compensatory activation of mitochondrial repair mechanisms during critical illness. Considering the previously observed persistent mitochondrial damage, this activation may be insufficient and contribute to mitochondrial dysfunction. Suppressed activation of fusion/fission when excessive hyperglycemia is prevented with insulin may reflect reduced need for diluting (less) damage during normoglycemia or, alternatively, a suppressive effect of insulin on repair. (J Clin Endocrinol Metab 97: E59–E64, 2012)
We recently demonstrated that activation of autophagy, the pathway responsible for clearance of damaged organelles and potentially toxic protein aggregates, is impaired in liver and muscle of fed prolonged critically ill patients (6). This may have contributed to the persistent mitochondrial structural damage and dysfunction. However, disturbances in two other major mechanisms that repair or compensate for mitochondrial damage may have played a role as well. In this regard, mitochondrial fusion and fission allow exchange of damaged components leading to dilution of molecular damage or bundling of dysfunctional structures in a single, irreversibly damaged organelle that is subsequently targeted for removal by autophagy (7, 8). Mitochondrial biogenesis generates new mitochondria when needed, such as in response to mitochondrial damage and increased energy demand (9). Figure 1 shows a simplified schematic overview of the mitochondrial repair mechanisms.

We hypothesized that impaired mitochondrial fusion or fission and/or compromised activation of mitochondrial biogenesis could contribute to mitochondrial dysfunction in critical illness. We also investigated whether the partial alleviation of such damage by preventing hyperglycemia could in part be mediated via these pathways. We therefore studied several key readouts of mitochondrial dynamics in liver and skeletal muscle biopsies obtained from critically ill patients.

Patients and Methods

Patients

We studied liver and rectus abdominis biopsies taken within 30 ± 20 min after death from 36 randomly selected surgical ICU patients...
patients and vastus lateralis biopsies taken in vivo from 24 randomly selected medical ICU patients included in two randomized, controlled clinical studies on intensive insulin therapy (10, 11). Postmortem vastus lateralis biopsies were available from a subgroup of the surgical ICU patients (n = 14). Patients were optimally matched for baseline characteristics and compared with gender-, age-, and body mass index-matched controls as previously described (6). In each group of critically ill patients, 50% had been assigned to intensive insulin therapy to maintain blood glucose levels between 80–110 mg/dl (4.4–6.1 mmol/liter, normoglycemia), whereas the other 50% received conventional insulin therapy, during which insulin was administered only if glucose levels exceeded 215 mg/dl and stopped when glucose levels fell below 180 mg/dl. As controls, we analyzed liver and rectus abdominis biopsies harvested intraoperatively from 18 patients not suffering from generalized disease and undergoing uncomplicated elective abdominal surgery for restorative rectal resection and vastus lateralis biopsies from five healthy volunteers.

Written informed consent was obtained from volunteers, patients, or closest family member. The Katholieke Universiteit Leuven Institutional Review Board approved study protocols and consent forms (ML1094, ML1820, ML2707).

Tissue analyses

Antibodies for Western blot were purchased from Abcam (Cambridge, UK) [mitofusin-2, optic atrophy-1 (OPA1), dynamin-related protein-1 (Drp1), fission-1 (Fis1), receptor-inhibitory protein-140 (RIP-140), mitochondrial DNA (mtDNA) polymerase-y (Pol–y), mitochondrial transcription factor-A (TFAM), and cytochrome-18], Molecular Probes (Eugene, OR) (ND6 and NDUFA9), or DakoCytomation (Glostrup, Denmark) (horseradish peroxidase-conjugated goat antirabbit or rabbit antimouse antibodies). Cytokeratin-18 (liver) and actin levels (muscle) were measured to control for equal loading. Protein levels were normalized to median control levels. After DNA extraction and ribonuclease treatment (GenElute Mammalian Genomic DNA Miniprep kit; Sigma-Aldrich, Bornem, Belgium), mtDNA content was determined (12) by amplification of a mtDNA-encoded (ND6) complex I subunit (Hs02596878_g1; Applied Biosystems, Foster City, CA) and a nuclear DNA (nDNA)-encoded cystic fibrosis transmembrane conductance regulator fragment (5'-TGGCACATTTAAGAAATATCATCT-3' forward primer, 5'-GAAGGGTTCATATGCATAATCAAAA-3' reverse primer, and 5'-TAGATACAGAAGCCTCATAAACGCA-3' probe; Eurogentec, Seraing, Belgium). All analyses were performed blinded for group allocation.

Statistical analyses

Differences analyzed by Kruskal-Wallis and Mann-Whitney U test were considered statistically significant when two-sided P values were <0.05 (StatView version 5.0.1; SAS Institute, Cary, NC).

Results

Blood glucose and insulin infusion

Mean morning blood glucose levels during the ICU stay of surgical critically ill patients were 178 ± 16 mg/dl (mean ± SD) in the conventional and 101 ± 7 mg/dl in the intensive insulin therapy group (P < 0.0001). Patients received a median (interquartile range) insulin dose of 14 (2–34) and 44 (23–87) IU/d, respectively (P = 0.005). The mean morning blood glucose levels of medical critically ill patients were 156 ± 26 mg/dl in the conventional and 99 ± 12 mg/dl in the intensive insulin therapy group (P < 0.0001), with insulin doses of 12 (3–32) and 77 (57–98) IU/d, respectively (P = 0.0002).

Mitochondrial fusion and fission

Protein levels of the mitochondrial fusion mediators mitofusin-2 and OPA1 and the mitochondrial fission mediator Drp1, but not Fis1, were increased above controls in liver of conventionally treated patients (Fig. 2A). Patients who received intensive insulin therapy had comparable levels of mitofusin-2, Drp1, and Fis1 as controls, whereas OPA1 remained elevated. In postmortem rectus abdominis biopsies, critical illness had up-regulated the four proteins, irrespective of insulin treatment group (Fig. 2A). In contrast, vastus lateralis showed no up-regulation, whether taken in vivo (Fig. 2A, without a difference between eventual survivors or nonsurvivors, data not shown) or postmortem (data not shown).

Mitochondrial biogenesis

Protein levels of nDNA-encoded (NDUFA9) and mtDNA-encoded (ND6) complex I subunits were up-regulated in liver and rectus abdominis, but not in vivo (Fig. 2B) or postmortem (data not shown) vastus lateralis biopsies, of conventionally treated critically ill patients. Similar results, including also an increase in in vivo vastus lateralis muscle, were obtained for TFAM. Pol–y was up-regulated only in liver. Protein levels of the mitochondrial biogenesis inhibitor RIP-140 were always comparable to controls. All tissues failed to up-regulate mtDNA levels. For all tissues, the patients in the normoglycemic, intensive insulin therapy group responded similarly as the hyperglycemic conventional group. Markers in in vivo vastus lateralis were comparable for eventual survivors and nonsurvivors (data not shown).

Discussion

Critical illness is associated with mitochondrial damage and impaired cellular energy metabolism, which have been implicated in organ dysfunction and adverse outcome. We demonstrated an up-regulation of several key mediators of mitochondrial fusion/fission and biogenesis in postmortem liver and rectus abdominis, but not in in vivo or postmortem vastus lateralis of the critically ill. Overall, maintenance of normoglycemia with insulin did not affect this.
response, except for a partial attenuation of mitochondrial fusion and fission markers in liver. We previously observed mitochondrial abnormalities, more pronounced in hyperglycemic than in normoglycemic livers (3) and, regardless of glycemic control, also impaired autophagy (6). Together, our observations suggest that mitochondrial protection brought about by glycemic control is mediated by prevention of direct glucose toxicity, rather than via an effect on mitochondrial repair.

Mitochondria constantly undergo regulated fusion and fission, which determine mitochondrial morphology, organelle number, shape, size, content, distribution, and function (13, 14). Fusion of nonfunctional, damaged mitochondria with healthy, fully functional mitochondria provides a mechanism to regain essential components and dilute molecular damage after subsequent fission (13). However, giant mitochondria that accumulate within aged or diseased cells are unable to fuse and exchange contents with other mitochondria (7). Recent findings put forward fission followed by selective fusion as quality control mechanism to segregate irreparable, dysfunctional mitochondria and target them for removal by autophagy.

**FIG. 2.** Mitochondrial fusion/fission and biogenesis in critically ill patients. Relative protein expression levels of several key mediators of mitochondrial fusion and fission (A) and mitochondrial biogenesis (B) were normalized to levels of cytokeratin-18 (liver) or to actin (skeletal muscle) and to levels of controls. These data as well as mtDNA levels (normalized to levels of controls, B) are shown as box plots, with medians, interquartile ranges and 10th and 90th percentiles. *, P ≤ 0.05; (*), 0.05 < P < 0.1 vs. healthy reference; #, P ≤ 0.05; (#), 0.05 < P < 0.1 between critically ill patients receiving conventional insulin therapy (CIT) or intensive insulin therapy (IIT). Representative blots are shown of, from left to right, three samples from controls and CIT and IIT patients each for liver and rectus abdominis and two controls followed by three to four CIT and IIT patients for vastus lateralis.
(8). Mitochondrial fission thus can generate two asymmetrical daughter organelles, one of which contains the defects. These fusion-incompetent mitochondria are then eliminated by autophagy. Importantly, increased fusion or reduced fission compromises autophagy (8) and may spare mitochondria from degradation (15). Data on impact of critical injury on these processes are scarce and available only from experimental models. These suggest protection by mitofusin-2, whereas extensive fission may be detrimental by triggering apoptosis (16, 17). Importantly, the two processes need to be appropriately balanced, because both unopposed fusion and unopposed fission are detrimental for cellular function. Proteins involved in fusion and fission were up-regulated in liver and rectus abdominis of our patients, but not in vastus lateralis. In liver, however, Fis1 was not increased. Although relative protein levels as such preclude conclusions on the balance between the processes, it could be speculated that fusion would prevail over fission, because Fis1 may be rate limiting (14). This could further contribute to impaired targeting for mitophagy (8, 15). The hepatic responses were partially attenuated with insulin therapy, either suggesting a reduced need for repair of mitochondrial damage in line with better-preserved structure and function (3) or, alternatively, a negative effect of insulin on mitochondrial repair.

In animal models, severe sepsis induces oxidative mitochondrial damage, with decreased mtDNA copy number, mitochondrial transcription, and oxidative phosphorylation. Mitochondrial biogenesis is activated to restore the damage (9, 18, 19). This response allows metabolic recovery and was put forward as a powerful pro-survival mechanism (9). Indeed, skeletal muscle taken on d 1–2 in the ICU from critically ill patients showed a mitochondrial biogenesis response in survivors but not in nonsurvivors (20). However, partial activation of this pathway failed to maintain mitochondrial function in skeletal muscle of patients with sepsis (21). Likewise, activation of key regulators of mitochondrial biogenesis observed in our study did not affect mtDNA content and appeared insufficient to restore the functional mitochondrial damage (3). Vastus lateralis did not even show a clear response, in contrast to the increased marker protein levels in postmortem liver and rectus abdominis. This may suggest that muscle type (with rectus abdominis being more active along with respiration than the immobilized vastus lateralis) explains the different responses in both muscles, rather than survival status.

In conclusion, activation of the mitochondrial repair systems appeared insufficient in skeletal muscle and particularly in liver considering the persistence of severe morphological and functional mitochondrial abnormalities (3). Preventing excessive hyperglycemia with insulin did not affect biogenesis and suppressed activation of fusion/fission, which may reflect either a reduced need for diluting (less) damage during normoglycemia or, alternatively, a suppressive effect of insulin on repair. Together with the recently observed impaired activation of autophagy, these data are compatible with the hypothesis that the major driving force for the better preservation of mitochondrial ultrastructure and function with this therapy (3, 4) is a reduction in the damage directly inflicted by pronounced hyperglycemia on the mitochondria during critical illness, rather than an effect on the repair mechanisms. Because incomplete clearance of damaged mitochondria in vital organ systems, either by their repair or removal, as well as inappropriate replacement by new healthy mitochondria could contribute to organ dysfunction, the current observations open perspectives for therapies that effectively activate these mitochondrial repair mechanisms during critical illness.

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