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Liver graft pretreated *in vivo* or *ex vivo* by γ -aminobutyric acid receptor regulation

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Abstract

Background— γ -Aminobutyric acid exists throughout the body, and the brain γ -aminobutyric acid receptor (GABAR) regulation reduces oxidative stress (OS). Effects of GABAR regulation in the liver are unknown. Ischemia or reperfusion injury after orthotopic liver transplantation (OLT) or shear stress after split OLT (SOLT) with a small-for-size graft causes OS-induced graft damage. Here, the strategic potential of graft pretreatment *in vivo* and *ex vivo* by GABAR regulation was investigated.

Materials and methods—Recipient rats were divided into seven groups according to the graft pretreatments and graft types: (1) laparotomy, (2) OLT, (3) GABAR regulation *in vivo* and OLT, (4) GABAR regulation *ex vivo* and OLT, (5) SOLT, (6) GABAR regulation *in vivo* and SOLT, and (7) GABAR regulation *ex vivo* and SOLT. Survival study, biochemical assays, histopathologic or immunohistologic assessments, and Western blotting were performed at 6 h after OLT or SOLT.

Results—Graft pretreatment *in vivo* prolonged survival after SOLT. Histopathologic and biochemical profiles verified that graft pretreatment *in vivo* reduced graft damage after OLT or SOLT. Immunohistologically, graft pretreatment *in vivo* prevented apoptotic inductions after OLT or SOLT. The 4-hydroxynonenal confirmed the OS after OLT or SOLT, and graft pretreatment *in vivo* improved the OS. Graft pretreatment *in vivo* decreased ataxia-telangiectasia–mutated kinase and H2AX after OLT or SOLT. Graft pretreatment *in vivo* increased phosphatidylinositol 3 kinase and Akt after SOLT. In contrast, GABAR regulation *ex vivo* did not work.

Conclusions—Graft pretreatment *in vivo*, not *ex vivo*, prevented the ischemia or reperfusion injury–mediated OS after OLT or SOLT via the ataxia-telangiectasia–mutated kinase/H2AX pathway and the shear stress–mediated OS after SOLT with small-for-size graft via the phosphatidylinositol 3 kinase/Akt pathway.

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Keywords

γ -Aminobutyric acid receptor; Reperfusion injury; Oxidative stress; Shear stress; Small-for-size graft

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are potential hazards with modification of lipids, proteins, and DNA [1–3]. The control of ROS or RNS production plays a physiological role, and oxidative stress (OS) mediated by free radicals is defined as an imbalance between the ROS- or RNS-mediated production and antioxidant capacity [1–3]. γ -Aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in the brain, and almost all researchers have focused on the regulation of the GABA receptor (GABAR) only in the brain [4–8]. Currently, GABA is considered to be a multi-functional molecule throughout the body [9,10]. However, many researchers have verified that GABAR regulation has preventive effects against OS-induced damage only in the field of brain research [5,7,8]. These effects have been explained by the inhibition of the response to DNA damage [5,11,12], the cell survival pathway [13,14], or the free radical scavenging system [15,16]. The liver contains GABA [10], and hepatic GABAR has been detected [17]. However, in the liver, the effects of GABAR regulation have not been documented. We investigated the strategic potential of graft pretreatment *in vivo* or *ex vivo* by GABAR regulation in a rat liver transplantation model.

2. Materials and methods

2.1. Animals

Lewis rats (8–12 wk old) were used as graft donors and recipients. The experimental protocols were approved by the institutional ethical committee (IACUC, A19609).

2.2. GABAR agonist

A dose of 43.56 nmol/g body weight of GABAR agonist (Muscimol, 114.10 g/mol; Fluka, St Louis, MO) was used.

2.3. Surgical procedures and postoperative care

Surgical procedures for orthotopic liver transplantation (OLT) [18] and split OLT (SOLT) with small-for-size graft (SFSG) [19] and postoperative care [18,19] have been previously described. All operative procedures were performed under general anesthesia using isoflurane. Analgesia (buprenorphine, 0.1 mg/kg, intramuscularly) was routinely given. Euthanasia was obtained by CO₂ chamber. A whole-liver graft (100% OLT) and a 40% SFSG with the left median and lateral segments (40% SOLT) were used. The split graft was made at the back table. To avoid any irrelevant signaling, the hepatic artery was reconstructed by ultramicrosurgery [18,19]. In this model, we demonstrated the importance of a short anhepatic phase and the exclusion of unreliable samples based on autopsy findings [18,19]. In this study, the anhepatic phase was kept within 20 min, and no surgical complications were observed at sampling autopsy.

2.4. Study design

Syngeneic grafts had a cold ischemic time of 4 h at 4° C in normal Ringer solution (100 mL). The liver graft was washed by 10 mL of normal Ringer solution immediately after the graft harvest and before the graft implantation.

As pretreatment *in vivo*, the donor rat received an intravenous injection of GABAR agonist (43.56 nmol/g body weight, 1.0 mL) from the penile vein at 4 h before the graft harvest. As pretreatment *ex vivo*, the harvested graft was washed out again with GABAR agonist (43.56 nmol/g body weight, 1.0 mL) from the portal vein at the back table, and thereafter, the preservation solution included GABAR agonist for 4 h of cold ischemia.

Recipient rats were divided into seven groups according to the pretreatment and graft type: (1) laparotomy only, (2) OLT only, (3) systemic injection of GABAR agonist to the donor and OLT, (4) portal washout with GABAR agonist at the back table and OLT, (5) SOLT only, (6) systemic injection of GABAR agonist to the donor and SOLT, and (7) portal washout with GABAR agonist at the back table and SOLT.

A survival study was performed ($n = 10$, in each). Cell signaling for cell proliferation, differentiation, and apoptosis was confirmed from the early postoperative period after OLT [20–23], and subsequently, progressive necrosis occurred [20–23]. The serum, plasma, and liver samples were collected at 6 h after OLT or SOLT ($n = 3$, in each). All the experiments were repeated twice.

2.5. Biochemical assay and coagulation profile

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), and the international normalized ratio of prothrombin time (PT-INR) were measured.

2.6. Histopathologic and immunohistologic assessments

Morphologic characteristics were assessed after hematoxylin–eosin staining. The graft damage score (point) has been described elsewhere [19]. Scores were counted in 10 fields in each slide and then these scores were averaged.

Apoptotic induction was assessed by terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (ApopTag Peroxidase *In Situ*; Chemicon International, Billerica, MA) and cysteine aspartic acid protease 3 immunostaining (cleaved caspase 3 antibody; Cell Signaling Technology, Danvers, MA). Slides were scanned with an automated high-throughput scanning system (Scan-scope XT; Aperio Technologies, Vista, CA). To quantify the immunohistologic findings, positive-stained nuclei were counted by Aperio Imagescope software (Aperio Technologies). The ratio of positive-stained nuclei to all nuclei was calculated, and the mean ratio per millimeter square was determined.

2.7. Western blotting

The primary antibodies for 4-hydroxynonenal (4-HNE; 4-hydroxynonenal antibody; Abcam, Cambridge, MA), ataxia-telangiectasia–mutated kinase (ATM; Phospho-ATM/ATR Rabbit mAb; Cell Signaling Technology), phosphorylated histone H2AX (H2AX; phospho-histone H2AX antibody; Cell Signaling Technology), phosphatidylinositol 3 kinase (PI3K; phospho-PI3K antibody; Cell Signaling Technology), Akt (phospho-Akt rabbit mAb; Cell Signaling Technology), superoxide dismutase (SOD) 1 (Cu/Zn superoxide dismutase; LifeSpan BioSciences, Seattle, WA), and SOD 2 (Mn superoxide dismutase; LifeSpan BioSciences) were used. Glyceraldehyde-3-phosphate dehydrogenase served as a control. Intensities were quantified by ImageQuant 5.0 software (Molecular Dynamics, Sunnyvale, CA).

2.8. Statistical analysis

Survival curves were constructed by the Kaplan–Meier method (the log-rank test). A Student *t*-test was used for the comparison of unpaired continuous variables. Statistical

calculations were performed using SPSS Software Version 16.0 (SPSS, Chicago, IL). A *P* value <0.05 was considered statistically significant.

3. Results

3.1. Survival curves

Actual survival curves are shown in Fig. 1. Even in the syngeneic graft, the recipients with 40% SOLT showed poor outcomes in comparison with those after laparotomy, although all 100% OLT recipients survived.

In 100% OLT, all recipients who received OLT, OLT with the GABAR agonist *in vivo*, and OLT with the GABAR agonist *ex vivo* survived. In 40% SOLT, there was no significant difference between SOLT and SOLT with the GABAR agonist *ex vivo*, but there was a significant difference between SOLT and SOLT with the GABAR agonist *in vivo*.

3.2. Graft parenchymal damage

Actual graft damage scores for hematoxylin–eosin staining are shown in Fig. 2A. In comparison with laparotomy, 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.3. Biochemical and coagulation profiles

Actual values of AST, ALT, T-Bil, and PT-INR are shown in Fig. 2B–E. In AST, both 100% OLT and 40% SOLT showed significant differences in comparison with laparotomy. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

In ALT, both 100% OLT and 40% SOLT showed significant differences in comparison with laparotomy. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

In T-Bil, both 100% OLT and 40% SOLT showed significant differences in comparison with laparotomy. In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

In PT-INR, both 100% OLT and 40% SOLT showed significant differences in comparison with laparotomy. In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.4. Apoptotic induction

Actual ratios of terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling–positive nuclei are shown in Fig. 3A. In comparison with laparotomy, both 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

Actual ratios of cysteine aspartic acid protease 3–positive nuclei in each group are shown in Fig. 3B. In comparison with laparotomy, both 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.5. Lipoperoxidation

Actual values of the normalized 4-HNE are shown in Fig. 4. In comparison with laparotomy, both 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.6. Response to and repair of DNA damage

Actual values of the normalized ATM are shown in Fig. 5A. In comparison with laparotomy, both 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

Actual values of the normalized H2AX are shown in Fig. 5B. In comparison with laparotomy, both 100% OLT and 40% SOLT showed significant differences.

In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist *in vivo* but not in OLT with the GABAR agonist *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.7. Promotion of cell survival

Actual values of the normalized PI3K are shown in Fig. 5C. In comparison with laparotomy, there were no significant differences in 100% OLT, but there was a significant difference in 40% SOLT.

In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and *ex vivo*. In comparison with 40% SOLT, there were significant

differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

Actual values of the normalized Akt are shown in Fig. 5D. In comparison with laparotomy, there were no significant differences in 100% OLT, but there were significant differences in 40% SOLT.

In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and *ex vivo*. In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist *in vivo* but not in SOLT with the GABAR agonist *ex vivo*.

3.8. Activities of antioxidant enzymes

Actual values of the normalized SOD 1 are shown in Fig. 5E. In comparison with laparotomy, there were no significant differences in 100% OLT, but there were significant differences in 40% SOLT.

In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and *ex vivo*. In comparison with 40% SOLT, there were no significant differences in SOLT with the GABAR agonist *in vivo* and *ex vivo*.

Actual values of the normalized SOD 2 are shown in Fig. 5F. In comparison with laparotomy, there were no significant differences in 100% OLT, but there were significant differences in 40% SOLT.

In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist *in vivo* and *ex vivo*. In comparison with 40% SOLT, there were no significant differences in SOLT with the GABAR agonist *in vivo* and *ex vivo*.

4. Discussion

Although our results verified the strategic potential of GABAR regulation *in vivo* as a pretreatment for liver graft, this regulation completely failed to work *ex vivo* in our study. Any pretreatments in a living donor violate ethical policy and spoil donor regulation. Therefore, we initially expected that GABAR regulations *in vivo* and *ex vivo* would work equally. However, we failed to find any effects in the pretreatment *ex vivo*. We describe these results as disappointing. Paradoxically, it may be informative to challenge the clinical translation of graft pretreatment by GABAR regulation.

GABA has various physiological effects throughout the body [9,10], and the liver contains GABA and GABAR [10,17]. In the field of brain research, GABAR regulation has preventative effects against OS-induced damage. Our results from histopathologic, biochemical, and immunohistologic assessments clearly revealed that OLT or SOLT resulted in graft injury and apoptotic induction and that GABAR regulation *in vivo* reduced this damage.

The OS that causes DNA damage and subsequent apoptosis is an imbalance between the free radical-mediated productions and antioxidant defenses [1–3]. From the viewpoint of harmful productions, the ROS or RNS attacks a variety of biological molecules [1–3]. Products of lipid peroxidation can reliably and rapidly reflect sensitive and specific signals of OS, and 4-HNE is an end product of lipoperoxidation [24,25]. The 4-HNE revealed that the OS surely occurred after OLT or SOLT, and GABAR regulation *in vivo* prevented this stress.

The ROS or RNS has been suggested as a major contributing factor to DNA damage in the progression of OS. As a DNA damage response, the ATM can be initiated through rapid intermolecular autophosphorylation [12,26], and this DNA damage-inducible kinase activates histone H2AX after DNA damage [5,27,28]. In the field of brain research, many investigators demonstrated that GABAR regulation had beneficial effects against OS via the response to and repair of the DNA pathway [5,11,12]. Our own results for ATM and H2AX clearly revealed that the OS after OLT or SOLT caused the DNA damage signaling and triggered the subsequent DNA repair process and that the GABAR regulation *in vivo* prevented OS after OLT or SOLT via the ATM/H2AX pathway.

Akt also plays a critical role in controlling apoptosis [26,29,30] and promotes cell survival [30–33]. The apoptotic machinery is inhibited by activated Akt [29,34,35]. Akt is positioned as a component of the antiapoptotic process related to the activation of PI3K [14,30]. In brain studies, PI3K or Akt has been documented as the cell survival pathway against OS after GABAR regulation [5,13,14,29]. PI3K-mediated signals are associated with lowering of the liver content of phosphatase and the tensin homologue [36,37], and the PI3K/Akt pathway is also an important signaling pathway for cell survival in the liver [37,38]. Our results suggested that this pathway was strongly disturbed in OS mediated by shear stress after SOLT with SFSG and that the preventive effect of GABAR regulation *in vivo* against SFSG-induced OS depended on this pathway. Note that 100% OLT with GABAR *in vivo* showed no differences in this pathway.

From the viewpoint of antioxidant defenses, the antioxidant enzymes are also important in reducing DNA damage and subsequent apoptosis [2,3]. Normal cells are able to defend themselves against OS through this scavenging system [3]. In brain studies, these effects have been also confirmed for GABAR regulation [15,16]. OS impairs mitochondrial import and the processing of SOD2 [39], and the prevention of DNA damage via SOD2 has been reported in the SFSG [40]. Although our results of SOD2 after SOLT were consistent with these previous reports [40], the scavenging system seemed to not be triggered by GABAR regulation. Basically, our results revealed the decreases of SODs after SOLT with SFSG. One possible explanation for these responses after SOLT may be that this scavenging system failed for some reactive molecules that evaded the detoxification process and damaged potential targets because of drastic damage in the SFSG, although these scavenging enzymes can handle large amounts of ROS or RNS [41].

Brain researchers have documented the DNA damage or repair pathway (ATM/H2AX), cell survival pathway (PI3K/Akt), and antioxidant enzymes (SODs) as the possible and reasonable explanations for the preventative effects of GABAR regulation against OS [5,6,8,13,14]. Our results revealed that GABAR regulation by a specific agonist *in vivo* worked in the liver. This result has beneficial potential, especially for deceased donor liver transplantation. Strategic regulation of GABAR *in vivo* reduced the ischemia or reperfusion injury-induced OS after OLT or SOLT via the ATM/H2AX pathway and the shear stress-induced OS after SOLT with SFSG via the PI3K/Akt pathway.

Proactive strategies through pharmacologic pretreatment to limit graft damage from the ischemia or reperfusion injury after OLT or SOLT and the shear stress after SOLT with SFSG have advantages in overcoming current issues. Previous researches in the brain field demonstrated that GABAR regulation has sure effects against OS-induced damage. Our results suggested that GABAR regulation *in vivo* has a potential for therapeutic strategy in the liver field, and the further studies are needed.

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Fig. 1.

Survival curves. Even in the syngeneic graft, the recipients with 40% SOLT showed poor outcomes in comparison with those after laparotomy ($P < 0.0010$), although all 100% OLT recipients survived. In 100% OLT, all recipients who received OLT, OLT with the GABAR agonist at the donor, and OLT with the GABAR agonist at the back table survived. In 40% SOLT, there were no significant differences between SOLT and SOLT with the GABAR agonist at the back table ($P = 0.8901$), but there were significant differences between SOLT and SOLT with the GABAR agonist at the donor ($P = 0.0447$).

Fig. 2.

Graft parenchymal damage and biochemical and coagulation profiles. (A) Graft damage score (points): In comparison with laparotomy (0.0 ± 0.0), 100% OLT (4.3 ± 0.1 , $P = 0.0003$) and 40% SOLT (6.5 ± 0.9 , $P < 0.0001$) showed significant differences, respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (3.0 ± 0.2 , $P = 0.0006$) but not in OLT with the GABAR agonist at the back table (4.1 ± 0.4 , $P = 0.9158$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (3.5 ± 0.7 , $P = 0.0125$) but not in SOLT with the GABAR agonist at the back table (6.4 ± 0.4 , $P = 0.4676$). (B) AST (U/L): Both 100% OLT (297.8 ± 3.1 , $P < 0.0001$) and 40% SOLT (404.6 ± 39.6 , $P < 0.0001$) showed significant differences in comparison with laparotomy (36.6 ± 3.7), respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (178.6 ± 26.9 , $P = 0.0016$) but not in OLT with the GABAR agonist at the back table (278.3 ± 19.7 , $P = 0.1642$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (274.1 ± 8.8 , $P = 0.0051$) but not in SOLT with the GABAR agonist at the back table (392.8 ± 31.8 , $P = 0.7079$). (C) ALT (U/L): Both 100% OLT (327.6 ± 41.2 , $P = 0.0003$) and 40% SOLT (404.6 ± 39.6 , $P = 0.0001$) showed significant differences in comparison with laparotomy (53.9 ± 2.5), respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (242.8 ± 21.4 , $P = 0.0341$) but not in OLT with the GABAR agonist at the back table (353.3 ± 20.7 , $P = 0.3880$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (290.1 ± 26.8 , $P = 0.0143$) but not in SOLT with the GABAR agonist at the back table (406.0 ± 64.5 , $P = 0.9754$). (D) T-Bil (mg/dL): Both 100% OLT (0.69 ± 0.08 , $P = 0.0015$) and 40% SOLT (1.54 ± 0.21 , $P = 0.0005$) showed significant differences in comparison with laparotomy (0.26 ± 0.05). In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist at the donor (0.52 ± 0.15 , $P = 0.1609$) and

back table (0.70 ± 0.09 , $P = 0.8151$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (1.03 ± 0.09 , $P = 0.0150$) but not in SOLT with the GABAR agonist at the back table (1.43 ± 0.18 , $P = 0.4484$). (E) PT-INR: Both 100% OLT (1.14 ± 0.04 , $P = 0.0024$) and 40% SOLT (1.17 ± 0.03 , $P < 0.0001$) showed significant differences in comparison with laparotomy (0.96 ± 0.02), respectively. In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist at the donor (1.09 ± 0.03 , $P = 0.1404$) and back table (1.17 ± 0.03 , $P = 0.3931$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (1.10 ± 0.05 , $P = 0.0071$) but not in SOLT with the GABAR agonist at the back table (1.22 ± 0.10 , $P = 0.6251$). *In comparison with the laparotomy, $P < 0.05$. †In comparison with OLT or SOLT, $P < 0.05$. NS = not significant.

Fig. 3.

Apoptotic induction (TUNEL and caspase 3). (A) TUNEL: In comparison with laparoscopy (0.004 ± 0.005), both 100% OLT (0.114 ± 0.008 , $P < 0.0001$) and 40% SOLT (0.157 ± 0.012 , $P < 0.0001$) showed significant differences, respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (0.052 ± 0.011 , $P = 0.0014$) but not in OLT with the GABAR agonist at the back table (0.112 ± 0.021 , $P = 0.8761$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (0.070 ± 0.020 , $P = 0.0029$) but not in SOLT with the GABAR agonist at the back table (0.163 ± 0.045 , $P = 0.8163$). (B) Caspase 3: In comparison with laparoscopy (0.000 ± 0.000), both 100% OLT (0.061 ± 0.003 , $P < 0.0001$) and 40% SOLT (0.127 ± 0.013 , $P < 0.0001$) showed significant differences, respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (0.036 ± 0.007 , $P = 0.0051$) but not in OLT with the GABAR agonist at the back table (0.064 ± 0.011 , $P = 0.6839$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (0.066 ± 0.018 , $P = 0.0087$) but not in SOLT with the GABAR agonist at the back table (0.117 ± 0.025 , $P = 0.5683$). *In comparison with the laparoscopy, $P < 0.05$. †In comparison with OLT or SOLT, $P < 0.05$. Caspase = cysteine aspartic acid protease; NS = not significant; TUNEL = terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.

Fig. 4.

Lipoperoxidation (4-HNE). In comparison with laparotomy (1.00 ± 0.01), both 100% OLT (1.60 ± 0.17 , $P = 0.0035$) and 40% SOLT (1.58 ± 0.14 , $P = 0.0023$) showed significant differences, respectively (* $P < 0.05$). In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (1.11 ± 0.04 , $P = 0.0078$) but not in OLT with the GABAR agonist at the back table (1.55 ± 0.15 , $P = 0.7263$, $P < 0.05$; NS, not significant). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (1.03 ± 0.18 , $P = 0.0153$) but not in SOLT with the GABAR agonist at the back table (1.58 ± 0.21 , $P = 0.9887$, $P < 0.05$; NS, not significant). GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Fig. 5.

Response to and repair of DNA damage (ATM/H2AX), promotion of cell survival (PI3K/Akt), and activities of antioxidant enzymes (SODs 1 and 2). (A) ATM: In comparison with laparotomy (1.00 ± 0.04), both 100% OLT (1.35 ± 0.10 , $P = 0.0046$) and 40% SOLT (1.27 ± 0.10 , $P = 0.0076$) showed significant differences, respectively. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (0.99 ± 0.05 , $P = 0.0058$) but not in OLT with the GABAR agonist at the back table (1.47 ± 0.25 , $P = 0.4854$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (0.72 ± 0.17 , $P = 0.0071$) but not in SOLT with the GABAR agonist at the back table (1.24 ± 0.12 , $P = 0.7122$). (B) H2AX: In comparison with laparotomy (1.00 ± 0.15), both 100% OLT (1.91 ± 0.22 , $P = 0.0039$) and 40% SOLT (2.97 ± 0.55 , $P = 0.0038$) showed significant differences. In comparison with 100% OLT, there were significant differences in OLT with the GABAR agonist at the donor (0.89 ± 0.11 , $P = 0.0020$) but not in OLT with the GABAR agonist at the back table (2.01 ± 0.29 , $P = 0.6775$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (1.21 ± 0.23 , $P = 0.0068$) but not in SOLT with the GABAR agonist at the back table (3.14 ± 0.19 , $P = 0.6363$). (C) PI3K: In comparison with laparotomy (1.00 ± 0.06), there were no significant differences in 100% OLT (0.87 ± 0.06 , $P = 0.0578$), but there were significant differences in 40% SOLT (0.44 ± 0.27 , $P = 0.0232$). In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist at the donor (1.01 ± 0.09 , $P = 0.0748$) and back table (0.84 ± 0.05 , $P = 0.4837$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (0.90 ± 0.11 , $P = 0.0496$) but not in SOLT with the GABAR agonist at the back table (0.41 ± 0.31 , $P = 0.9317$). (D) Akt: In comparison with laparotomy (1.00 ± 0.04), there were no significant differences in 100% OLT (0.76 ± 0.14 , $P = 0.0495$), but there were significant differences in 40% SOLT (0.20 ± 0.17 , $P = 0.0012$). In comparison with 100% OLT, there were no significant differences in OLT with the

GABAR agonist at the donor (0.94 ± 0.20 , $P = 0.2903$) and back table (0.68 ± 0.20 , $P = 0.5946$). In comparison with 40% SOLT, there were significant differences in SOLT with the GABAR agonist at the donor (1.28 ± 0.18 , $P = 0.0015$) but not in SOLT with the GABAR agonist at the back table (0.25 ± 0.12 , $P = 0.7096$). (E) SOD 1: In comparison with laparotomy (1.00 ± 0.13), there were no significant differences in 100% OLT (0.92 ± 0.15 , $P = 0.5612$), but there were significant differences in 40% SOLT (0.64 ± 0.16 , $P = 0.0387$). In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist at the donor (0.97 ± 0.17 , $P = 0.7745$) and in OLT with the GABAR agonist at the back table (0.94 ± 0.13 , $P = 0.8893$). In comparison with 40% SOLT, there were no significant differences in SOLT with the GABAR agonist at the donor (0.83 ± 0.15 , $P = 0.2079$) and back table (0.70 ± 0.20 , $P = 0.7028$). (F) SOD 2: In comparison with laparotomy (1.00 ± 0.15), there were no significant differences in 100% OLT (0.83 ± 0.11 , $P = 0.1850$), but there were significant differences in 40% SOLT (0.61 ± 0.15 , $P = 0.0346$). In comparison with 100% OLT, there were no significant differences in OLT with the GABAR agonist at the donor (0.82 ± 0.04 , $P = 0.8744$) and back table (0.92 ± 0.18 , $P = 0.4725$). In comparison with 40% SOLT, there were no significant differences in SOLT with the GABAR agonist at the donor (0.74 ± 0.11 , $P = 0.3071$) and back table (0.59 ± 0.20 , $P = 0.9007$). *In comparison with the laparotomy, $P < 0.05$. †In comparison with OLT or SOLT, $P < 0.05$. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; NS = not significant; PI3K = phosphatidylinositol 3 kinase; SOD = superoxide dismutase.