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Ischemia Effect on Lean and Steatotic Liver in Rats: Evaluating Levels of Lactic Acid and Transaminases

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Abstract

Introduction: Major hepatic surgery includes techniques of controlled ischemia and reperfusion in order to control bleeding and minimize blood loss. Fatty liver disease is considered to be an independent prognostic factor in major hepatic surgery, with negative impact on the outcome of patients. The aim of our experimental study was to evaluate the variance of concentration levels of Lactic Acid (LA) in liver tissue, as well as serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels, after the effect of different periods of liver ischemia followed by reperfusion in rats with lean and in rats with steatotic liver.

Materials and methods: Eighty male Wistar rats aged 12-14 weeks were included. Forty of them were fed a regular laboratory diet, while the other forty were specially fed (choline-free diet) for 12-14 weeks in order to develop severe liver steatosis. Each of the groups was further divided into 4 subgroups: control group (sham-operated), 15, 30 and 45 minutes of surgically induced ischemia. After completion of the ischemic manipulation partial hepatectomy was performed for non-ischemic lobes and reperfusion for ischemic liver lobes. Rats were euthanized and liver tissue was used to measure LA. Blood samples were obtained and used to measure AST and ALT.

Results: LA and aminotransferases levels were higher in steatotic rats than in lean rats in every subgroup (including control groups). Values of LA, AST and ALT increased within the same group (steatotic or lean rats) as the ischemia was prolonged (among different subgroups).

Conclusion: Steatotic rats undergoing ischemia appear to elevate LA, AST and ALT significantly more than lean rats under the same manipulation, related to the time of exposure to hepatic ischemia. This suggests that the steatotic liver is less tolerant to ischemia than the lean one.

Key words: liver, ischemia, steatosis, rat

Introduction

The liver is an important organ for maintaining homeostasis. Any prolonged liver ischemia can cause hepatic dysfunction, leading to severe hepatic failure [1-10]. Liver ischemia is relatively common and can be caused by trauma or during surgical procedures [3,8-10]. The predictive role of a number of biological markers have been studied during ischemic liver damage. The levels of Lactic Acid (LA) and transaminases (aspartate aminotransferase - AST and alanine aminotransferase - ALT) have proven to be useful markers for the evaluation of liver damage caused by ischemia-reperfusion injury (IRI) [4-9].

Hepatic steatosis, also known as fatty liver disease, is the most common liver pathology in the Western world, affecting 15-30% of the population [9-13]. Hence, it is very interesting to study its effect in liver ischemia and reperfusion. Hepatic steatosis has been identified as an independent risk factor in hepatic surgery [8-10,14-16], as fatty liver seems to be more sensitive to ischemia than normal, non-steatotic (lean) liver [17]. A number of possible mechanisms have been proposed, like autophagy or mitochondrial dysfunction [18,19]. Fatty liver's tolerance to ischemia is under intense scrutiny in modern hepatic surgery [20,21].

The aim of our experimental study was to evaluate the variance of concentration levels of LA in liver tissue, as well as serum AST and ALT levels, after the effect of different periods of liver ischemia followed by reperfusion in rats with lean and in rats with steatotic liver.

Materials and Methods

Animals

The study included eighty male Wistar rats, aged 12-14 weeks (weighing 250-300 gr). Animals were provided by the Pasteur Institute of Athens. The study was carried out at the Experimental Research Center of ELPEN (Athens, Greece). The study protocol was approved by the Veterinary Authority of East Attika Prefecture (Protocol reference numbers: 1633 and 2659, directive 609/1986) and was performed

complying with the rules of experimentation and 3Rs (Replace, Reduce & Refine). Animals were provided with free access to water and food, constant ambient temperature and a cycle of alternating light and dark every 12 hours.

The rats were divided in two groups: in the first group (L), 40 rats were fed a regular diet. In the second (S), the other 40 were fed a choline-free diet (Mucedola - PF18770) for 12-14 weeks, so as to induce macrovesicular steatosis, leading to fatty liver disease, as previously described [8,9].

Experimental design

After 12-14 weeks of feeding, experimental manipulations were performed on the experimental groups as described below:

Group LC (sham operated rats with lean liver used as control, n=10) and groups L15, L30 and L45 with rats with lean liver under 15, 30 and 45 minutes of continuous liver ischemia, respectively (each consisting of 10 rats).

Group SC (sham operated rats with steatotic liver used as control, n=10) and groups S15, S30 and S45 with rats with steatotic liver under 15, 30 and 45 minutes of continuous liver ischemia, respectively (each consisting of 10 rats).

Experimental manipulations

All animals were given general anesthesia using a volatile agent (isoflurane), followed by intubation and mechanical ventilation. After proper sterilization of the abdominal wall, a median laparotomy incision was performed. In control groups (sham operated rats) mild maneuvers of liver mobilization and hepatic vascular branch exploration were performed, without ischemic manipulations. On the contrary, in groups L15, L30, L45, S15, S30 and S45, animals underwent segmental hepatic ischemia, according to Rummel's technique [24]. Specifically, a vascular occlusion was performed at the branch providing blood to the middle and left lobes. Blood supply of the right and caudate lobes remained intact. Duration of the ischemia was different in each group, as mentioned above. After vascular occlusion was reversed, a segmental hepatectomy was performed, including the right and caudate lobes. The abdominal wall was closed, and the animals regained consciousness.

Six hours after surgery, animals were euthanized. Liver tissue and blood samples were obtained. Tissue samples were kept in a deep freezer (-86°C) until they were processed. Blood samples were centrifuged at 5000 rounds per minute (rpm) for 10 minutes and an enzymatic method (Advia 1800 Chemistry Analyzer system, Siemens, Munich, Germany) with reagent kits provided by Medicon Hellas (Athens, Greece) (SGOT/1417-0070, SGPT/1417-0080) was used for measurement of AST and ALT serum levels. Tissue samples were rinsed with ice-cold isotonic saline before homogenization with Tris buffer 20 mM (pH 7,40). A volume of 1 ml buffer was used for 0.2 g of tissue. Samples were centrifuged at 4°C at 3000 rpm for 10 minutes using ULTRA-TURRAX (IKA-Labortecnik) [25]. LA Measurements were performed using BioVision Lactate Assay Kit II.

Statistical analysis

Shapiro-Wilk test was used for the assessment of normality of data distribution. Comparisons between two groups were made using Student's t-test. Comparisons among three or more groups were made using Analysis of Variance (ANOVA) or Kruskal-Wallis test, as appropriate. Correlations between quantitative parameters were tested with Spearman's rank correlation coefficient. All tests were two-tailed and results were considered statistically significant if p-value was less than 0.05. The 23nd edition of Statistical Package for Social Sciences (SPSS) (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis [26,27].

Results

Changes in LA values of liver tissue after ischemia and reperfusion

LA values in lean and steatotic rats are shown on Table 1.

Lean rats of groups L15, L30 and L45 showed significant higher values of LA than the non-steatotic rats of control group (LC) ($p<0.0001$). Lean rats of group L15 had significantly lower LA values than those for group L30 ($p<0.0001$) or L45 ($p<0.0001$). However, no significant difference was observed in LA values between groups L30 and L45 ($p=0.9986$). Steatotic rats of groups S15, S30 and S45) showed higher values of LA than the steatotic rats of control group (SC) ($p<0.0001$). Steatotic rats of group S15 had significantly lower LA values than those of group S30 ($p<0.0001$) or S45 ($p<0.0001$). Moreover, LA values were significantly lower in group S30 than in group S45 ($p<0.0001$).

In control groups, LA values were significantly higher in steatotic rats than in lean rats ($p<0.0001$). Steatotic rats of group S15 also showed significantly higher values of LA than lean rats of group L15 ($p<0.0001$). The same result was observed between steatotic and lean rats of groups S30 and L30 as well as S45 and L45, respectively ($p<0.0001$). It is also noticeable that LA values of steatotic rats in group S15 were higher than those of lean rats in group L45 ($p<0.0001$) and L30 ($p<0.0001$).

Changes in AST values after ischemia and reperfusion

AST values in lean and steatotic rats are shown on Table 2.

Lean rats of groups L15, L30 and L45 showed significantly higher values of AST than lean rats of control group LC ($p<0.0001$). Lean rats of group L15 had significantly ($p<0.0001$) lower AST values than those of groups L30 and L45. Also, AST values of group L30 were significantly lower than of group L45 ($p<0.0001$). Steatotic rats of groups S15, S30 and S45 showed significantly higher values of AST than their control group (SC) ($p<0.0001$). Steatotic rats of group S15 had significantly lower AST values than those of groups S30 and S45 ($p<0.0001$). Moreover, AST values were significantly lower in group S30 than group S45 ($p<0.0001$).

In control groups, AST values were higher in steatotic liver rats than in lean rats ($p<0.0001$). Steatotic liver rats of group L15 also showed higher values of AST than lean rats of group S15 ($p<0.0001$). The same result was observed between steatotic and lean rats of groups S30 and L30 ($p=0.0014$) and of groups S45 and L45 ($p<0.0001$), respectively.

Table 1: LA values (in μg/mL) in lean and steatotic rats.

Groups	Average	Range	Median	SD
LC	6.14	4.16 - 8.47	6.03	1.32
L15	15.8	13.7 - 18.7	15.4	1.48
L30	24.3	22.7 - 25.9	24.6	1.12
L45	24.7	22.2 - 26.3	24.9	1.21
SC	17.5	13.8 - 20.4	18.5	2.48
S15	28	26.8 - 29.5	27.7	0.917
S30	31.5	29.4 - 32.5	31.8	0.839
S45	35.4	33.6 - 37	35.2	1.19

LC: lean control; L15: lean, 15 min of ischemia; L30: lean, 30 min of ischemia; L45: lean, 45 min of ischemia; SC: steatotic control; S15: steatotic, 15 min of ischemia; S30: steatotic, 30 min of ischemia; S30: steatotic, 30 min of ischemia; S45: steatotic, 45 min of ischemia; SD: standard deviation

Table 2: AST values (in U/L) in lean and steatotic rats.

Groups	Average	Range	Median	SD
LC	51.2	43 - 61	49.5	6.58
L15	71.8	59 - 86	70.5	8.39
L30	142	94 -185	140.0	20.4
L45	602	487 - 705	603	56.7
SC	1109	945 - 1289	1111	113
S15	1337	1004 - 1580	1340	154
S30	2025	1784 - 2319	2012	152
S45	3029	2459 - 3515	3022	269

LC: lean control; L15: lean, 15 min of ischemia; L30: lean, 30 min of ischemia; L45: lean, 45 min of ischemia; SC: steatotic control; S15: steatotic, 15 min of ischemia; S30: steatotic, 30 min of ischemia; S30: steatotic, 30 min of ischemia; S45: steatotic, 45 min of ischemia; SD: standard deviation

Table 3: ALT values (in U/L) in lean and steatotic rats.

Groups	Average	Range	Median	SD
LC	49.6	39 - 59	48.0	6.08
L15	74.4	59 - 89	73.5	8.80
L30	139	103 - 198	124	35.7
L45	491	419 - 571	490	42.1
SC	968	856 - 1229	902	136
S15	1255	916 - 1556	1252	173
S30	1902	1685 - 2109	1906	120
S45	3029	2987 - 3539	3230	196

LC: lean control; L15: lean, 15 min of ischemia; L30: lean, 30 min of ischemia; L45: lean, 45 min of ischemia; SC: steatotic control; S15: steatotic, 15 min of ischemia; S30: steatotic, 30 min of ischemia; S30: steatotic, 30 min of ischemia; S45: steatotic, 45 min of ischemia; SD: standard deviation

Changes in ALT values after ischemia and reperfusion

ALT values in lean and steatotic rats are depicted on Table 3.

Lean rats of groups L15, L30 and L45 showed significantly higher values of ALT than their control group LC ($p<0.0001$). Lean rats of group L15 had significantly lower ALT values than those of groups L30 and L45 ($p<0.0001$). Also, values of group L30 were significantly lower than group L45 ($p<0.0001$). Steatotic rats of groups S15, S30 and S45 showed significantly higher values of ALT than steatotic rats of control group SC ($p<0.0001$). Steatotic rats of group S15 had significantly lower ALT values than those of groups S30 ($p<0.001$) and S45 ($p<0.0001$). Moreover, ALT values were significantly lower in group S30 than in group S45 ($p<0.0001$).

In control groups, ALT values were significantly higher in steatotic rats than in lean rats ($p<0.0001$). Steatotic rats of group L15 also showed significantly higher values of ALT than lean rats of group S15 ($p<0.0001$). The same result was observed between steatotic and lean rats when comparing group S30 with L30 ($p<0.0001$) and S45 group with L45 ($p<0.0001$).

Discussion

Ischemic injury of the liver is a major concern in modern hepatic surgery. The underlying pathophysiologic mechanisms are under constant study [28,29]. Major hepatic surgery includes techniques of controlled ischemia and reperfusion in order to control bleeding and minimize blood loss. Therefore, liver damage caused by IRI is of critical importance in these patients. Fatty liver disease is considered to be an independent prognostic factor in major hepatic surgery and transplantations, with negative impact on the outcome of patients [30-32]. The problem in these patients is believed to be the steatotic liver's higher susceptibility to IRI in comparison to the non-steatotic one [17].

Steatosis does not affect the liver's regenerating capacity after extensive hepatectomy [14,16]; it is the prolonged ischemic manipulation that leads to decreased regenerating capacity of the steatotic liver [16,33]. As a result, liver impairment is rapid, extensive and irreversible despite reperfusion, leading to acute hepatic failure and increased morbidity and mortality rates [29,32,33]. Steatotic liver rats exposed at liver ischemia exceeding 45-60 minutes have a poor survival, limiting available study time. Thirty-minute ischemia followed by reperfusion in rats with fatty liver disease also leads to poor survival, not exceeding 29 hours [17,34-37]. Experimental models studying the steatotic liver's effect on excessive liver impairment and hepatic failure due to IRI are still a work in progress [19,30].

This experimental study was conducted so as to explore how manipulations of hepatic ischemia, followed by reperfusion, would affect LA and aminotransferase values in steatotic liver rats. LA was the parameter studied in liver tissue. A significant rise in LA values in liver tissue was observed after IRI manipulations, both in lean and in steatotic liver rats ($p<0.0001$). Furthermore, LA rise was significantly higher (ranging from 7.2 to 12.2 μg/mL, depending on the ischemia) in steatotic liver rats than those with a non-steatotic liver after ischemia manipulations of the same duration ($p<0.0001$). An interesting finding was that lean rats enduring a 45-minute ischemia showed lower LA levels than steatotic liver rats whose ischemia manipulations lasted only one third of that time (15 minutes) ($p<0.0001$).

Aminotransferase (AST and ALT) levels studied in blood serum provided similar findings. Values of AST and ALT were significantly higher after IRI manipulations, both in lean and steatotic liver rats ($p<0.0001$). Furthermore, AST and ALT blood values in steatotic liver rats were significantly higher (ranging from 20.6 to 1004 and from 24.8 to 1328 U/L, respectively) than in lean rats that endured ischemia manipulations of the same duration ($p<0.0017$). These findings are in accordance with previous studies where LA levels in steatotic liver rats have been found to exceed those of lean rats after transient ischemia manipulations [42-45]. This suggests that steatotic liver is more susceptible to IRI than the non-steatotic liver [1,2,4]. Furthermore, our study explores the rise in LA values after longer periods of ischemia than usually found in literature. Moreover, it allows to make comparisons between lean and steatotic liver's susceptibility to ischemia, adjusted for the duration of the ischemia. Measurement of AST and ALT, as established markers of hepatocellular death, allowed us to assess the extent of liver IRI and correlate this to the LA rise that we documented.

Study limitations

We recognize a number of limitations in this study. Although surgical manipulations were performed similarly to all animals, steatotic rats presented with more visceral fat. This could cause manipulations to be more strenuous in this group, leading to increased tissue damage. Surgical technique was particularly meticulous, in order to avoid this possibility.

Furthermore, increased LA values could be attributed to ischemia of other organs apart from the liver, e.g. the intestine. Vascular

occlusion was therefore performed selectively, at the distant arterial branch providing blood to the middle and left lobes. Blood supply of the right and caudate lobes remained intact, in order to avoid occluding intestinal arterial branches arising in the vicinity. By that technique, intestinal ischemia was avoided.

Finally, the right and caudate lobes were excised after the ischemic manipulation, preventing non-ischemic liver tissue from compensating for the functional decline of the ischemic lobes. This was necessary in order to study the IRI effect without the confounding factor of non-ischemic liver function. However, it was also a maneuver leading to additional liver injury.

Conclusions

The results of this experimental study suggest that steatotic liver is more susceptible to ischemia than non-steatotic liver, and the effect IRI has on the liver parenchyma is time related. In other words, hepatocellular damage is proportional to the duration of ischemia, and is more extensive in steatotic liver than in non-steatotic liver. Experimental models of liver IRI comparing steatotic and non-steatotic liver can contribute to the understanding of steatotic liver's higher susceptibility to IRI in comparison to the non-steatotic liver. In clinical practice, this could lead to a more targeted use of surgical manipulations in patients with fatty liver disease who undergo major hepatic surgery. However, more studies are required in order to achieve a more quantified view on this critical matter.

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