The effect of hepatic ischemia in the liver of rats with obstructive jaundice

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OBJECTIVE: *The aim of the current study was to evaluate the effect of ischemia-reperfusion injury on the liver's function and morphology during the establishment and progress of obstructive jaundice.*
MATERIAL AND METHODS: *80 Wistar rats were used for the purposes of the study and were allocated in four groups: JAUNDICE (obstructive jaundice), JAUN-ISC (obstructive jaundice and ischemia reperfusion), CONTROL (laparotomy) and ISCHEMIA (ischemia reperfusion).*
RESULTS: *Obstructive jaundice, and ischemia-reperfusion injury following obstructive jaundice led to increased mortality, while no mortality was noticed in the control and ischemia groups. In the JAUN-ISC group, SGOT was significantly increased on the 10th day and SGPT was significantly increased on the 1st day compared to JAUNDICE group. Moreover, in the JAUN-ISC group, sinusoid dilation was significantly increased on the 5th and 10th days and neutrophil infiltration was significantly increased on the 10th day compared to the JAUNDICE group.*
CONCLUSIONS: *A mild ischemia-reperfusion injury that in the normal liver led only to slight increase of hepatic neutrophil infiltration in the presence of obstructive jaundice led to increased hepatic biochemical markers (SGOT, SGPT) and increased hepatic sinusoid dilatation and enhanced neutrophil infiltration.*

KEY WORDS: Dilatation of sinusoids, Granulocytes infiltration, Oxaloxate, Pyruvate transaminase, Transaminase reperfusion

Introduction

Temporary occlusion of the hepatic circulation is often necessary in various surgical operations on the liver. Surgeons frequently need to stop the blood supply to the liver using the Pringle maneuver [1] in order to facilitate the necessary surgical procedures, such as in hepatic trauma and hepatectomies for the treatment of benign and malignant tumors. The liver is sensitive to this form of ischemia due to the high energy demands of hepatocytes. Cessation of blood supply causes a rapid decrease in cell energy stores, leading to irreversible damage and eventually to cell death [2,3]. Despite the recent progress, there are plenty of unanswered questions concerning the mechanism of ischemia, identification of biomarkers for the viability of ischemic hepatocytes and utilization of substances that could minimize the extent of the lesion. Plenty of techniques and substances have been reported that could safely increase the duration of legation, such as hypothermia, methylprednisolone, chlorpromazine, phenothiazine, fructose and mannitol, relying on reduction or conjugation of free radicals [4,5].

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Moreover, in cases of obstructive jaundice, the capacity of hepatocytes to resist ischemia deteriorates even more. Furthermore, in a cholestatic liver, ischemia and reperfusion may have crucial roles in the recovery of liver functions and the patient's prognosis, as patients operated on in the presence of obstructive jaundice have an increased mortality rate due to postoperative complications, such as renal failure and multi-organ failure [6–8]. While the etiology is unknown, the reduction of blood supply to the kidneys, the direct effect of bile at the intestinal mucosa, bilirubin, bile salts and endotoxins of the gastrointestinal tract may have key roles, and the increased risk of postoperative complications correlates with the level of bilirubin [9]. The aim of the current experimental study was to evaluate the effect of ischemia-reperfusion injury on liver function, as depicted by biochemical markers, and morphology, as depicted by histopathological findings, during the establishment and progress of obstructive jaundice, as the level of bilirubin is rising.

Materials and Methods

Animals' Description

Wistar rats provided by the experimental laboratory of the Theagenio Anticancer Institute of Thessaloniki were used in this experimental study. The experiments were carried out in the experimental operation theater of the Intensive Care Unit of the G. Papanikolaou General Hospital. Storage conditions of the experimental animals complied with everything provided by the number 86/609 Community Directive and P.D. 160/91. 80 male animals, five to eight months old and weighing 250–500kg, were used. Living conditions were the same before and after the procedure. More specifically, lights were on for 12 hours during the day and off the rest of the time. Animals were fed packing 510 of EL.IV.Z for experimental animals. The last 12 hours before the experiment was performed, the rats were fasted, and only water was provided. The study was reviewed and approved by the Faculty of Surgery, Medical School, Aristotle University of Thessaloniki, and all procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the G. Papanikolaou General Regional Hospital .

Anesthesia

Anesthesia was achieved by administration of intraperitoneal injection of 10% pentobarbital sodium 35mg/kg.

Surgical Procedure

After induction of anesthesia, shaving of the abdomen, cleansing with antiseptic solution and placement of the surgical field followed. Median laparotomy was performed in order to expose the abdominal cavity.

Obstructive Jaundice and Hepatic Ischemia and Reperfusion

The common bile duct was isolated, and it was ligated at two sites using silk ligations. In order to achieve hepatic ischemia, the common hepatic artery and the portal vein were clamped for 20 minutes at the base of the porta hepatica with a vessel clamp. Then the clamp was removed and blood supply to the liver restored for 1 hour. The ischemia reperfusion injury took place on the day of sacrifice exactly before the euthenasia of the animals.

Groups

Experimental animals were divided into four groups: JAUNDICE, JAUN-ISC, CONTROL and ISCHEMIA. 30 animals were allocated in group JAUNDICE and subdivided into groups JAUNDICE1, JAUNDICE5 and JAUNDICE10. On the 1st, 5thand 10th postoperative days, animals of J1, J5 and J10 groups, respectively, were sacrificed.
In group JAUN-ISC, 30 animals were exposed to ligation of the bile duct, 20 minutes ischemia of the liver and reperfusion for 1 hour. Animals in group JAUN-ISC1 were sacrificed on the 1st postoperative day, and animals in groups JAUN-ISC5 and JAUN-ISC10 on the 5thand 10thdays, respectively. Groups JAUNDICE and JAUN-ISC were the target groups.
In group CONTROL, including 10 animals, only midline laparotomy was performed.
In group ISCHEMIA, including 10 animals, 20 minutes ischemia of the liver and reperfusion for 1 hour were performed.

Sample Recovery

Blood samples from the heart chamber were taken for biochemical assays.
Liver samples were taken for histological examination.

Euthanasia

All animals were euthanized by intracardiac administration of KCL 10%.

Biochemical Assays

Concentrations of oxalate transaminase SGOT, pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin and direct

bilirubin were measured. Biochemical assays for blood serum were made in the biochemical department of the G. Papanikolaou General Hospital, while the histological examination of liver samples was performed in the Pathological Anatomy Laboratory of Aristotle University in Thessaloniki.

Histological Assays

During sacrifice of the animals, liver samples were taken for histological examination. Bile duct hyperplasia, dilatation of sinusoids, hyperplasia of Kuppfer cells, granulocyte infiltration and vacuolation of hepatocytes were assessed during the histological examination.
In order to quantify the lesions, the grading system of crosses was used. One cross (+) corresponds to zero to five lesions per 10 visual fields in high magnification (x40). Two crosses (++) corresponds to five to ten lesions per 10 visual fields in high magnification (x40).
Three crosses (+++) corresponds to more than ten lesions per 10 visual fields in high magnification (x40).

Statistical Analysis

IBM SPSS software (Version 22) was used for the statistical analysis. Continuous values were expressed in means and standard deviations when normally distributed while in medians and interquartile ranges when not normally distibuted. Categorical variables were expressed with frequencies and percentages. For the values following normal distributions, the ANOVA test and Bonferroni correction for post hoc pairwise t-tests were used. For values that did not follow a normal distribution, the Kruskal-Wallis test and Mann-Whitney test were used. A per protocol analysis was followed. The significance level of statistical hypothesis-testing procedures concerning comparisons of means was set at $p<0.05$ was and two-tailed.

Results

Mortality

No mortality was noticed after 1 day of obstructive jaundice on the JAUNDICE1 group, while after 5 days of obstructive jaundice in the JAUNCDICE5, group mortality was 10%, and after 10 days of obstructive jaundice in the JAUNDICE10 group, mortality was 30%. In the ischemia-reperfusion injury after obstructive jaundice group, mortality was 20%, 20% and 30% for groups JAUN-ISC1, JAUN-ISC5 and JAUN-ISC10 after 1, 5 and 10 days of obstructive jaundice, respectively. No mortality was documented in the CONTROL group or the ischemia-reperfusion injury group.

Biochemical Markers

SGOT
SGOT levels showed statistically significant differences among groups ($p<0.001$). A significant increase in SGOT was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL ($p=0.007$), as well as after ischemia-reperfusion injury and only 1 day of obstructive jaundice in group JAUN-ISC1 compared to CONTROL ($p<0.001$) and compared to ischemia-reperfusion in the normal liver in group ISCHEMIA ($p<0.001$). Furthermore, SGOT was increased after ischemia-reperfusion injury after both 5 and 10 days with obstructive jaundice in group JAUN-ISC5 compared both to CONTROL ($p=0.03$) and to ISCHEMIA ($p=0.04$), and in group JAUN-ISC10 compared both to CONTROL ($p=0.01$) and to ISCHEMIA ($p=0.012$). Also, a significant increase of SGOT after ischemia-reperfusion after 10 days of obstructive jaundice in group JAUN-ISC10 was found compared to 10 days of obstructive jaundice in group JAUNDICE10 ($p=0.049$) (Table I).

SGPT
SGPT levels showed statistically significant differences among groups ($p<0.001$). A significant increase in SGPT was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL ($p<0.001$), but SGPT significantly decreased compared to day 1 (group JAUNDICE1) on days 5 and 10 in group JAUNDICE5 ($p=0.004$) and in group JAUNDICE10 ($p=0.005$). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, SGPT was significantly increased in group JAUN-ISC1 compared to CONTROL ($p<0.001$) and compared to ischemia-reperfusion in the normal liver in group ISCHEMIA ($p<0.001$), while after ischemia-reperfusion injury and 5 and 10 days of obstructive jaundice, SGPT significantly decreased in group JAUN-ISC5 compared to day 1 JAUN-ISC1 ($p<0.001$) and in group JAUN-ISC10 compared to day 1 JAUN-ISC1 ($p<0.001$). Also, a significant increase of SGPT after ischemia-reperfusion and 1 day of obstructive jaundice was found in group JAUN-ISC1 compared to 1 day of obstructive jaundice in group JAUNDICE1 ($p=0.016$) (Table I).

LDH

LDH levels showed statistically significant differences among groups ($p<0.001$). A significant increase in LDH was noted on the 1 day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL ($p<0.046$). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, LDH was significantly increased in group JAUN-ISC1 compared to

CONTROL (p<0.001) and compared to ischemia-reperfusion in the normal liver in group ISCHEMIA (p<0.001). Furthermore, LDH was increased after ischemia-reperfusion injury after both 5 and 10 days with obstructive jaundice in group JAUN-ISC5 compared both to CONTROL (p=0.038) and to ISCHEMIA (p=0.042) and in group JAUN-ISC10 compared both to CONTROL (p=0.015) and to ISCHEMIA (p=0.016) (Table I).

ALP

ALP levels showed statistically significant differences among groups (p<0.001). A significant increase in ALP was noted on the 1 day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p<0.001), while ALP significantly decreased compared to day 1 (group JAUNDICE1) on days 5 and 10 in group JAUNDICE5 (p≤0.001) and in group JAUNDICE10 (p≤0.001). However, on day 10 of obstructive jaundice, ALP was significantly higher in group JAUNDICE10 compared to CONTROL (p=0.001). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, ALP was significantly increased in group JAUN-ISC1 compared to CONTROL (p<0.001) and compared to ischemia-reperfusion in the normal liver in group ISCHEMIA (p<0.001), while after ischemia-reperfusion injury and 5 and 10 days of obstructive jaundice, ALP significantly decreased compared to day 1 (JAUNDICE1) in group JAUN-ISC5 (p<0.001) and in group JAUN-ISC10 (p<0.001). However, despite the decrease noted in ALP levels after ischemia-reperfusion injury and 5 and 10 days of obstructive jaundice, ALP remained significantly increased in both groups JAUN-ISC5 and JAUN-ISC10 compared to CONTROL (p=0.01 and 0.044, respectively) (Table I).

Total Bilirubin – Direct Bilirubin

Total bilirubin levels showed statistically significant differences among groups (p<0.001). A significant increase in total bilirubin was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p<0.001), which remained significant also on days 5 and 10 of obstructive jaundice in JAUNDICE5 and JAUNDICE10 compared to CONTROL (p<0.001 in both groups). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, total bilirubin was significantly increased in group JAUN-ISC1 compared to CONTROL (p<0.001) and to ischemia-reperfusion injury ISCHEMIA (p<0.001), and this increase remained significant on days 5 and 10 of obstructive jaundice in JAUN-ISC5 and JAUN-ISC10 compared to CONTROL and ISCHEMIA groups (p<0.001 for all groups). The significant findings are the same for direct bilirubin.
The results are summarized in Table I.

Table I - *Biochemical assays.*

Groups	SGOT P<0.001	SGPT P<0.001	LDH P<0.001	ALP P<0.001	TBIL P<0.001	DBIL P<0.001
JAUNDICE 1	795 ± 270	836 ± 352	2099 ± 1202	1180 ± 371	9.38 ± 2.87	6.98 ± 2.44
JAUNDICE 5	444 ± 234	155 ± 94	1882 ± 895	336 ± 138	10.3 ± 4.38	7.73 ± 3.1
JAUNDICE 10	204 ± 140	110 ± 53	1324 ± 928	594 ± 258	12 ± 5.25	7.57 ± 3.5
JAUN-ISC 1	1393 ± 861	1496 ± 950	3255 ± 2500	1095 ± 442	8 ± 1.42	6.56 ± 1.11
JAUN-ISC 5	788 ± 750	296 ±258	2259 ± 2027	488 ± 191	10.4 ± 2.36	8.7 ± 2.1
JAUN-ISC 10	738 ± 404	194 ± 136	2549 ± 1732	451 ± 105	12.1 ± 4.5	4.4 ± 3.5
CONTROL	30.7 ± 4.1	28.3 ± 6.3	73.6 ± 8.7	59 ± 18.8	0.86 ± 0.14	0.29 ±0.08
ISCHEMIA	48.1 ± 8.4	60.5 ± 15.9	91.3 ± 17.5	137 ± 61.7	0.86 ± 0.14	0.32 ± 0.08
	P Values	**P Values**	**P Values**	**P Values**	**P Values**	**P Values**
	J1 - C 0.007	J1-J5 0.004	JI1*C <0.001	JI-J5 <0.001	J1-C <0.001	J1-C <0.001
	J1-I 0.009	J1-J10 0.005	JI1-I <0.001	J1-J10 <0.001	JI-I <0.001	J1-I <0.001
	JI1-C <0.001	J1-JI1 0.016	JI2-C 0.042	J1-C <0.001	J5-C <0.001	J5-C <0.001
	JI1-I <0.001	J1-C <0.001	JI2-I 0.038	J1-I <0.001	J5-I <0.001	J5-I <0.001
	JI5-C 0.03	J1-I <0.001	JI3-C 0.015	J10-C 0.001	J10-C <0.001	J10-C <0.001
	JI5-I 0.04	JI1-JI5 <0.001	JI3-I 0.016	J10-I 0.008	J10-I<0.001	J10-I <0.001
	JI10-C 0.01	JI1-JI10 <0.001		JI1-JI5<0.001	JI1-C <0.001	JI1-C<0.001
	JI10-I 0.012	JI1-C <0.001		JI1-JI10 <0.001	JI1-I <0.001	JI1-I <0.001
	J10-JI10 0.049	JI1 – I <0.001		JI1-C <0.001	JI5-C <0.001	JI5-C <0.001
				JI1-I <0.001	JI-I <0.001	JI5-I <0.001
				JI5-C 0.01	JI10-C 0.024	JI10-C 0.017
				JI10-C 0.044	JI10-I 0.023	JI10-I 0.019
						JI10-JI5 0.016

Values expressed in mean and standard deviation.

Histopathological Findings

Bile Duct Hyperplasia

Bile duct hyperplasia showed statistically significant differences among groups (p<0.001). A significant increase in bile duct was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p<0.001), and it continued to increase significantly on days B*ile* D*uct* H*yperplasia*
5 and 10 of obstructive jaundice in JAUNDICE5 and JAUNDICE10 compared to CONTROL (p<0.001 in both groups). Also, bile duct hyperplasia was significantly increased on days 5 and 10 compared to day 1 (JAUNDICE1) in group JAUNDICE5 (p<0.001) and in group JAUNDICE10 (p=0.002) (Fig. 4). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, bile duct hyperplasia was significantly increased in group JAUN-ISC1 compared to CONTROL (p<0.001) and to ischemia-reperfusion injury ISCHEMIA (p<0.001), and this increase remained significant on days 5 and 10 of obstructive jaundice in JAUN-ISC5 and JAUN-ISC10 compared to CONTROL and ISCHEMIA groups (p<0.001 for all groups). Also, bile duct hyperplasia was significantly increased on days 5 and 10 compared to day 1 (JAUN-ISC1) in group JAUN-ISC5 (p=0.004) and in group JAUN-ISC10 (p=0.003).

Sinusoid Dilation

Sinusoid dilation showed statistically significant differences among groups (p<0.001). A significant increase in sinusoid dilation was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p=0.001), which slightly decreased on the 5th day but remained significantly greater in group JAUNDICE5 than in CONTROL (p=0.014) (Fig. 6). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, sinusoid dilation was significantly increased in group JAUN-ISC1 compared to CONTROL (p=0.007) and to ischemia-reperfusion injury ISCHEMIA (p=0.007). In addition, sinusoid dilation after ischemia-reperfusion injury and 5 and 10 days of obstructive jaundice in groups JAUN-ISC5 and JAUN-ISC10 was significantly increased compared to 5 and 10 days of obstructive jaundice in groups JAUNDICE5 and JAUNDICE10 (p=0.011 and p=0.037).

Kupffer Cell Hyperplasia

Kupffer cell hyperplasia showed statistically significant differences among groups (p=0.049). A significant increase in Kupffer cell hyperplasia was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p<0.001) which continued on the 5th and 10th days in groups JAUNDICE5 and JAUNDICE10 compared to CONTROL (p<0.001) (see Fig 5). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, Kupffer cell hyperplasia was significantly increased in group JAUN-ISC1 compared to CONTROL (p<0.001), which persisted on the 5th and 10th days in groups JAUN-ISC5 and JAUN-ISC10 compared to CONTROL (p<0.001), but not compared to ischemia-reperfusion injury in the ISCHEMIA group.

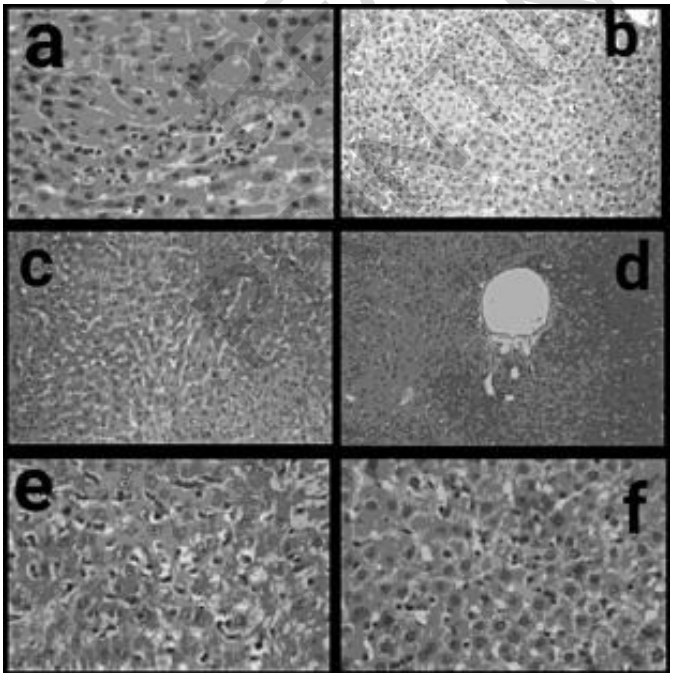

Fig. 1: A) Neutrophil infiltration in sinusoids, grade 3 (+++). Hematoxylin and eosin. Magnification x100; B) Neutrophil infiltration in sinusoids, grade 2 (++). Hematoxylin and eosin. Magnification x25; C) Dilatation and hyperplasia of sinusoids and apoptosis of hepatocytes. Hematoxylin and eosin. Magnification x100; D) Bile duct hyperplasia, grade two (++). Hematoxylin and eosin. Magnification x25; E) Hyperplasia of Kuppfer cells. Hematoxylin and eosin. Magnification x100; F) Neutrophil infiltration in sinusoids, grade 1 (+). Apoptosis of hepatocytes grade1.

Hepatic Cell Apoptosis

Hepatic cell apoptosis showed statistically significant differences among groups (p<0.001). A significant increase in hepatic cell apoptosis was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p=0.003), which persisted on the 5th and 10th days in groups JAUNDICE5 and JAUNDICE10 compared to CONTROL (p<0.001 and p=0.001) (Fig. 6). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice, hepatic cell apoptosis was significantly increased in group JAUN-ISC1 compared to CONTROL (p=0.002) and to ischemia-reperfusion injury ISCHEMIA (p=0.002), which persisted on the 5th and 10th days in groups JAUN-ISC5 and JAUN-ISC10 compared to the CONTROL and ISCHEMIA groups (p=0<0.001 for all comparisons).

Neutrophil Infiltration

Neutrophil infiltration showed statistically significant differences among groups (p<0.001) A significant increase in neutrophil infiltration was noted on the 1st day of obstructive jaundice establishment in group JAUNDICE1 compared to CONTROL (p<0.001), which persisted on the 5th and 10th days in groups JAUNDICE5 and JAUNDICE10 compared to CONTROL (p<0.001 in both groups). Furthermore, neutrophil infiltration was greater after five days in group JAUNDICE5 compared to 1 day of obstructive jaundice in group JAUNDICE1 (p<0.001) (Fig 1). Moreover, after ischemia-reperfusion injury and only 1 day of obstructive jaundice neutrophil infiltration was significantly increased in group JAUN-ISC1 compared to CONTROL (p<0.001) but not compared to ischemia-reperfusion injury ISCHEMIA (p<0.001). However, neutrophil infiltration further increased on the 5th and 10th days in groups JAUN-ISC5 and JAUN-ISC10 compared to CONTROL (p<0.001 for both) and ISCHEMIA groups (p=0.019 and p<0.001 respectively). In addition, neutrophil infiltration after ischemia-reperfusion injury and 10 days of obstructive jaundice in group JAUN-ISC10 was significantly increased compared to 10 days of obstructive jaundice in group JAUNDICE10 (p=0.005) and to ischemia-reperfusion injury and 1 day of obstructive jaundice in group JAUN-ISC1 (p=0.012) Fig 2. Finally, ischemia-reperfusion injury significantly increased neutrophil infiltration in the ISCHEMIA group compared to CONTROL (p<0.001). The results are summarized in Tables II and II and representative histopathological findings are presented in Fig. 1.

Discussion

The aim of this study was to investigate the effect of prolonged jaundice and prolonged jaundice in combination with ischemia and reperfusion in the liver tissue. The great progress in liver operations that has been achieved in recent years is closely linked to the temporary occlusion of liver circulation. As a result, research has

Table II - *Histological assays.*

GROUPS	Hyperplasia p<0.001	Sinusoids p<0.001	Kuppfer p=0,05	Apoptosis p<0.001	Granulocytes p<0.001
JAUNDICE (J1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
JAUNDICE (J5)	2 (2)	0.5 (1)	2 (1)	1 (0)	2 (1)
JAUNDICE (J10)	3 (2)	0 (1)	1 (1)	1 (1)	1 (1)
JAUN-ISC (JI1)	1 (0)	1 (1)	1 (1)	1 (1)	1 (1)
JAUN-ISC (JI5)	2 (2)	1 (1)	1 (1)	1 (1)	1,5 (1)
JAUN-ISC (JI10)	3 (2)	1 (1)	1 (1)	1 (0)	2 (1)
CONTROL(C)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ISCHEMIA(I)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
	P Values	**P Values**	**P Values**	**P Values**	**P Values**
	J1-J5 <0.001	J1-I 0.001	J1-C <0.001	J1-JI1 0.027	J1-J5 <0.001
	J1-J10 0.002	J5-JI5 <0.001	J5-C<0.001	J1-I <0.001	J5-I <0.001
	J1-JI5 0.002	J5-I 0.014	J10-C<0.001	J5-I <0.001	J10-I 0.001
	J1-JI10 0.001	JI1-JI5 0.024	JI1-C<0.001	J10-JI10 0.005	JI5-C <0.001
	J1-I 0.004	JI1-I 0.007	JI5-C<0.001	J10-I <0.001	JI10-C <0.001
	J5-I <0.001	JI5-JI10 0.023	JI10-C<0.001	JI5-I <0.001	JI1-JI10 0.012
	J10-I <0.001	JI10-I 0.007			JI1-I <0.001
	JI1-JI5 0.004	J10-JI10 0.037			JI10-I <0.001
	JI1-JI10 0.003				
	JI1-I <0.001				
	JI5-I <0.001				
	JI10-I <0.001				

Values expressed in median (interquartiles)

focused on hepatic resistance to ischemia and revascularization. A factor that plays a key role in the viability of the liver tissue is the duration of ischemia. Prolonged ischemia in a normal liver for intervention is not a serious problem, in contrast to increased mortality and morbidity observed in the presence of chronic liver disease.

A normal liver can tolerate ischemia for about 65 minutes [10,11]. Hepatic hypoxia due to cardiogenic or hypovolemic shock may be well tolerated if there is an early treatment of the underlying cause. However, up to 30 minutes of ischemia in a cirrhotic liver is not dangerous and is not accompanied by postoperative complications [12,13]. Direct hepatic lesions and distant organs' failure are common findings in icteric patients postoperatively [14-16]. Preoperative external drainage appears not to fully restore liver function, while at the same time, ischemia and reperfusion in an operation on a cholestatic liver have an aggravating effect on the recovery of liver functions and the patient's prognosis [13,17,18]. The objective of the current experimental study was on the 1 hand to compare the effect of ischemia-reperfusion injury on the icteric liver compared to ischemia-reperfusion injury to the normal liver and on the other hand to evaluate the additional damage caused by ischemia-reperfusion injury in an already icteric liver. Also, the study aimed to evaluate these effects on liver function, as depicted by biochemical markers, and morphology, as depicted by histopathological findings, during the establishment and progress of obstructive jaundice on days 1, 5 and 10 after ligation of the common bile duct.

Obstructive jaundice caused by obstruction of bile circulation due to intrahepatic or extrahepatic obstruction leads to stagnation of bile, which has various effects on the liver and other organs. In the liver, it causes enlargement of the organ, greenish discoloration and node formation. Prolonged bile stasis causes a decrease in albumin levels, prolongation of prothrombin time, reduction of mitochondrial enzyme activity and ketogenesis [19]. In the current experimental study, bile duct ligation led to establishment of obstructive jaundice on the 1 day with severe impairment of liver function as the levels of SGOT, SGPT, LDH, ALP and bilirubin were significantly increased. However, in the next days, despite the fact that bilirubin slightly increased, liver functions improved, as shown by the decrease in the levels of SGOT, SGPT, LDH and ALP on the 5th and 10th days

Table III – *Histological findings in percentages.*

GROUPS	Score	Hyperplasia	Sinusoids	Kuppfer	Apoptosis	Granulocytes
JAUNDICE 1	1					
	0	40%	30%	30%	30%	0
	1	60%	70%	70%	60%	100%
	2	0	0	0	10%	0
JAUNDICE 5						
	0	0	50%	37,5%	0	0
	1	0	50%	62,5%	87,5%	25%
	2	100%	0	0	12,5%	75%
JAUNDICE 10						
	0	0	71,5%	28,5%	25%	0
	1	28,5%	28,5%	43%	75%	71,5%
	2	14%	0	28,5%	0	28,5%
	3	57%	0	0	0	0
JAUN-ISC 1						
	0	15%	43%	43%	28,5%	0
	1	85%	43%	57%	71,5%	57%
	2	0	14%	0	0	43%
JAUN-ISC 5						
	0	0	0	33%	0	0
	1	17%	57%	66%	100%	50%
	2	83%	43%	0	0	33,3%
	3	0	0	0	0	16,6%
JAUN-ISC 10						
	0	0	43%	14%	0	0
	1	14%	57%	86%	87,5%	0
	2	29%	0	0	12,5%	75%
	3	57%	0	0	0	25%
CONTROL						
	0	100%	100%	100%	100%	100%
ISCHEMIA						
	0	100%	100%	70%	100%	10%
	1	0	0	30%	0	90%

compared to the 1st. This decrease was significant only for SGPT and ALP on days 5 and 10 compared to day 1, but not for SGOT or LDH.

In the initial stages of cholestasis, hepatic lesions include dilatation of sinusoids, hyperplasia of Kuppfer cells, granulocyte infiltration and vacuolation of hepatocytes. Centrilobular cholestasis, appearance of cholesterol granules in the cytoplasm of liver cells and Kupffer cells, and bile plugs in central bile canaliculi are common findings as well. Also, "bile infiltrations" are formed focally, and some liver cells present characteristic degeneration, while others undergo necrosis [20]. In the current experimental study, histopathological findings on the 1st day after the common bile duct ligation included mild bile duct hyperplasia, mild dilation of sinusoids, mild Kupffer cell hyperplasia, mild apoptosis and degeneration and mild neutrophil infiltration. Obstructive jaundice may lead to Gram-negative–induced sepsis. In this case, the hepatic immune system shifts to overproduction of Kupffer cells, which release tumor necrosis factor a (TNF-a) [21]. TNF-a is a major element of the inflammation reaction [22]. Agents that reduce the activity and the number of Kupffer cells reduce complications of obstructive jaundice as well [23]. During the establishment of obstructive jaundice from day 1 to day 10, the significant differences noted were the increased bile duct hyperplasia on days 5 and 10 compared to day 1 and the increased neutrophil infiltration on day 5 compared to day 1.

Systemic effects of cholestasis appear with jaundice and pruritus. Cardiovascular system complications are most often subclinical, while in other systems they are particularly severe, such as renal failure, sepsis, poor healing and gastrointestinal hemorrhage. The etiology of the abovementioned disorders, while not fully elucidated, seems to be multifactorial. Four important factors that are implicated are bilirubin, bile acids, lipids and endotoxins [24]. The mechanism mentioned above is the cause of increased postoperative infections, mortality and morbidity in patients with obstructive jaundice and is probably the cause of increased mortality noted in our experiments in the groups with obstructive jaundice.

During hepatic ischemia, decreased oxygen and nutrient supply to the liver and accumulation of intermediate metabolic products lead to reduction in energy production capacity, destruction of cell membranes, mitochondrial dysfunction [25] and disruption of protein synthesis [26]. Reduction in energy production capacity is caused by decreased oxygen supply to the liver cells, and disruption of oxidative phosphorylation causes rapid reduction in ATP levels. Hepatocytes shift to glycogenolysis [27] for energy production, leading to increased lactic acid production and dropping intracellular pH. Prolonged ischemia exhausts glycogen stores, ceases energy production and leads to cell death [28]. Mitochondrial dysfunction during ischemia and revascularization is the leading cause of cell death [29,30]. Decreased or absent adenine–ribosyl transferase activity leads to inability to resynthesize nucleotides (ATP, ADP, AMP) [28]. In addition, during ischemia, there is increased membrane permeability, causing influx of Na+ and Ca++ and efflux of K+ and Mg++. Calcium cations activate specific enzymes (phospholipases and proteinases) which destruct mitochondrial and cellular membranes [31,32]. Also, decreased pH during ischemia leads to induction of phospholipases, which decompose phospholipids of the cell membranes, leading to altered membrane permeability and disruption of cell homeostasis. Liver lesions during reperfusion are caused by free radicals. Free radicals normally get inactivated by mitochondria; however, during ischemia, there is mitochondrial malfunction. Thus, free radicals cannot be inactivated [33-36] .The lesions mentioned above include direct destruction of hepatocyte organelles [37], alteration of their shape [38,39], increased adhesion of white blood cells [40,41] and increased adhesion and coagulation of platelets [42]. Hepatocellular lesions due to revascularization include direct cell destruction because of free radicals [43,44] Furthermore, increased adhesion of white blood cells is observed, especially in the sinusoids, following revascularization [45]. Production of proteases and oxygen metabolites cause the increased adhesion. Formation of ischemic emboli and production of cytokines and platelet activation factor (PAF) are the results of increased adhesion of white blood cells [46,47].

In our experimental study, ischemia-reperfusion injury was established by 20 minutes' occlusion of the hepatic circulation, followed by 1 hour of reperfusion before the sacrifice of the rats. The effect on the function and morphology of the normal liver caused by this duration of ischemia-reperfusion injury seems not to be significant compared to control, as only a slight elevation of biochemical liver tests was noted, while regarding histopathological changes, the only finding was a mild increase in neutrophil infiltration. Regarding the effect of this duration of ischemia-reperfusion injury on the icteric liver, however, we noted a significant increase of SGOT on day 10 after bile duct ligation, a significant increase on the levels of SGPT on day 1 after bile duct ligation (which however didn't remain on the next days), a significant increased dilation of sinusoids on days 5 and 10 and increased neutrophil infiltration on day 10 after bile duct ligation and ischemia-reperfusion injury on the icteric liver compared with the presence of only obstructive jaundice. Sinusoidal congestion and dilatation are common findings in hepatic ischemia that have been pinpointed by many authors [48]. In addition, according to the literature, agents with hepatoprotective properties ameliorate the dilatation of sinusoids [49,50]. Neutrophils have an essential role in changes caused in various tissues due to ischemia and reperfusion, as they mediate immune response through the production of various cytokines and by activating other components of the immune system [51-53]. Previous studies have shown that monoclonal antibodies acting against neutrophils ameliorate liver function during ischemia [51,54,55].

Conclusions

In conclusion, our study shows that biochemical markers, except SGOT and SGPT, are not affected by mild ischemia and reperfusion when obstructive jaundice has already been established, possibly because their levels are already high, and the ischemia-reperfusion injury is considered mild and because it has no significant effect on the normal liver's function. The same applies for the histological markers on the 1st day after the establishment of obstructive jaundice. However, even this mild ischemia and reperfusion injury that only increased neutrophil infiltration of the normal liver led, after increased duration of obstructive jaundice, to more severe histological changes and, more specifically, to increased sinusoid dilatation and enhanced neutrophil infiltration.

Riassunto

Con questa ricerca sperimentale si è voluto valutare l'effetto della lesione da ischemia-riperfusione sulla funzione e sulla morfologia del fegato durante l'instaurazione e il progresso di un ittero ostruttivo.
Per la ricerca sono stati utilizzati 80 ratti Wistar, suddivisi in quattro gruppi: JAUNDICE (ittero ostruttivo), JAUN-ISC (ittero ostruttivo e riperfusione di ischemia), CONTROL (laparotomia) e ISCHEMIA (riperfusione di ischemia).
Risultati: l'ittero ostruttivo e il danno da ischemia-riperfusione nell'ittero ostruttivo hanno portato ad un aumento della mortalità, mentre non è stata osservata mortalità nei gruppi di controllo e ischemia. Nel gruppo JAUN-ISC, la SGOT è aumentata significativamente al decimo giorno e la SGPT è aumentata significativamente al primo giorno rispetto al gruppo JAUNDICE. Inoltre, nel gruppo JAUN-ISC, la dilatazione dei sinusoidi è aumentata significativamente il quinto e il decimo giorno e l'infiltrazione di neutrofili è aumentata significativamente il decimo giorno rispetto al gruppo JAUNDICE.
Conclusione: Una lieve lesione da ischemia-riperfusione, che nel fegato normale ha portato solo a un leggero aumento dell'infiltrazione epatica dei neutrofili, in presenza di ittero ostruttivo ha portato ad un aumento dei marcatori biochimici epatici (SGOT, SGPT) e ad un aumento della dilatazione epatica sinusoide e ad una maggiore infiltrazione di neutrofili.

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