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RESEARCH ARTICLE

The Effects of Mild and Moderate Hypothermia on Liver Regeneration, Liver Damage, and Apoptosis in Hepatectomized Rats

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ABSTRACT

Objective: The aim of this study was to investigate the effects of mild and moderate hypothermia on liver damage, apoptosis, and regenerative capacity after hepatectomy and the mechanisms underlying these effects.

Materials and Methods: A total of 34 male Wistar Albino rats were divided into 3 groups. The core temperatures of 10 rats in the normothermia group (Group 1) were maintained at 37°C using an external lamp. In the hypothermia groups, an external ice-cooling method was used. The core temperatures of 12 rats in the mild hypothermia group (Group 2) were reduced to 32–34°C, and those of 12 rats in the moderate hypothermia group (Group 3) were reduced to 26–28°C. Approximately 76% hepatectomy was performed in all rats, and the rats were followed for 48 hours. MDA and GSH (glutathione) levels were measured in liver tissue, and AST, ALT, GSH, and MDA levels were measured in blood samples. Regenerated liver weight was calculated, and liver tissue was evaluated histopathologically.

Results: Tissue GSH levels decreased in Group 2 compared with Group 1, whereas serum MDA levels increased. Tissue GSH levels were significantly higher in Group 3 than in Group 1. There was no difference among the groups in the liver damage index. There was a significant increase in PCNA (proliferating cell nuclear antigen) activity in Groups 2 and 3 compared with Group 1. There was a significant decrease in caspase-9 activity in Groups 2 and 3 compared with Group 1. There was no difference among the groups in regenerated liver weight measurements.

Conclusion: Systemic mild hypothermia applied during hepatectomy increased oxidative stress, whereas moderate hypothermia caused oxidative stress levels to return to normal. Mild and moderate hypothermia had a positive effect by increasing regenerative capacity and suppressing apoptosis after liver resection.

Keywords: Apoptosis, hepatectomy, hypothermia, liver damage, liver regeneration

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INTRODUCTION

Stability of liver function is essential for body homeostasis; for this purpose, multiple regenerative mechanisms have evolved to ensure that adequate liver function is always maintained.[1] In the biological context, the liver is the only internal organ that has the ability to regenerate after partial resection and chemical injury. This property is technically defined as hyperplasia of the remaining hepatocytes to compensate for lost tissue, restore full liver size, and meet the metabolic needs of the organism without recovery.[2]

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Ischemia/reperfusion (I/R) injury is mainly caused by the continuous metabolic demands of a warm ischemic organ and the lack of oxygen to meet these demands. One way to increase the ischemic resistance of the liver is to lower the organ temperature.[3] Hypothermia causes morphological and circulatory changes in the liver. Systemic hypothermia may have a negative effect on liver tissue by causing cellular changes such as a decreased sinusoid-to-hepatocyte volume ratio and *ex vivo* hepatocyte necrosis.[4] On the other hand, studies have also shown that hypothermia has a positive effect on liver damage.[5,6] Experimental studies have demonstrated that mild hypothermia (34°C) decreased liver damage and increased 24-hour survival in rats with I/R injury.[7] Mild external hypothermia (32–35°C) has been shown to decrease plasma aspartate transaminase (AST) levels and histological damage, alleviate apoptosis, and increase survival in mice with acetaminophen (APAP)-induced liver injury.[6]

The aim of this study was to investigate the effects of mild and moderate hypothermia on liver damage, apoptosis, and regenerative capacity after hepatectomy and to compare these effects.

MATERIALS and METHODS

Experimental Protocol

This study was approved by the Ethics Committee of Başkent University (29.04.2008/2008/AP-455). The study was conducted in compliance with the Declaration of Helsinki and local legal regulations. In this study, artificial intelligence tools were used for language editing purposes and to reduce the reference list to 250 words.

A total of 34 adult male Wistar Albino rats weighing 275–375 g were used in the study. Since mortality was observed in 5 of the 34 rats (1 in Group 1, 2 in Group 2, and 2 in Group 3), the study was completed with 29 rats. The animals were housed under a 12-hour light-dark cycle and were given tap water and a standard laboratory rat diet.

The rats were divided into 3 groups (Group 1, n=10; Groups 2 and 3, n=12). Group 1 was the normothermia group. The body temperature of the rats in this group was maintained at 36–37°C using an external heating lamp. Group 2 was the mild hypothermia group. In this group, body temperature was reduced to 32–34°C by spontaneous temperature reduction, and core temperature was kept constant by monitoring without intervention or by applying external ice molds. Group 3 was the moderate hypothermia group. In this group, core temperature was reduced to 26–28°C using an external cooling method and was kept constant using ice molds. Body temperature was monitored using a rectal probe throughout the procedure.

The study was conducted in three phases. In the first phase, the skin of all animals was shaved and sterilized with 10% povidone-iodine. General anesthesia with ketamine and xylazine was administered, and laparotomy was performed under sterile conditions. The desired core temperatures were reached for each group by monitoring rectal temperature and were maintained for 30 minutes. In the second phase, partial hepatectomy involving the caudate lobe, left lateral lobe, and median lobe was performed. The weight of the resected liver tissue was measured. The animals were resuscitated intraperitoneally with 2 mL of normal saline, and the incision was closed. In the third phase, the rats were kept under observation for 48 hours at room temperature. During this period, fentanyl (0.02 mg/kg, SC, twice daily) was administered as an analgesic.

Forty-eight hours postoperatively, the rats were anesthetized with ketamine (200 mg/kg, intramuscularly) and sacrificed by intracardiac blood collection. The abdomen was opened through the previous incision, and the liver tissues were removed and weighed using a precision balance. The tissues were washed with 0.9% NaCl and divided into two equal parts. Half of the samples were wrapped in aluminum foil, immersed in liquid nitrogen for biochemical examinations, and stored at -80°C until analysis. The remaining tissues were fixed in 10% formalin solution for histopathological examination, processed using standard histological tissue-processing methods, and embedded in paraffin blocks. Blood samples were centrifuged at 4000×g for 10 minutes. The obtained plasma was placed in Eppendorf tubes for AST (aspartate aminotransferase) and ALT (alanine aminotransferase) measurements, and the serum was placed in Eppendorf tubes for MDA (malondialdehyde) and GSH (glutathione) determination and stored at -80°C until analysis.

Biochemical Examinations

Homogenization of Liver Tissue Samples

Liver tissue samples were removed from the deep freezer when the biochemical parameters MDA and GSH were to be analyzed. A total of 100 mg of tissue was mixed with saline solution at a 1:10 ratio (w/v) and homogenized in an ice-water bath at 4000 rpm for 1–1.5 minutes. The crude extract (homogenate) was centrifuged in a refrigerated centrifuge at 12,000 rpm for 20 minutes. The resulting supernatants were separated for MDA and GSH determination.

Determination of MDA in Rat Liver Tissue Samples

For MDA determination, 50 µL of tissue supernatant was collected, and 50 µL of 8.1% SDS was added. Then, 375 µL of 0.8% TBA, 375 µL of 20% acetic acid (pH=3.5), and 150 µL of distilled water were added. The resulting mixture was boiled in a water bath at 95°C for 60 minutes. Immediately afterward, the samples were cooled, and 250 µL of distilled water and 1,250 mL of n-butanol/pyridine (15:1, v/v) were added and vortexed. The samples were then centrifuged at 4000 rpm for 10 minutes. A standard curve was obtained using 1,1,3,3-tetramethoxypropane as a standard for MDA, and the amount of MDA in nmol was determined from the absorbance values of the samples. The results were expressed as nmol/g wet tissue.[8]

Serum MDA Determination

For serum MDA determination, 250 µL of serum samples were collected, 1.25 mL of 20% TCA and 0.5 mL of 0.67% TBA were added, and the samples were boiled in a hot-water bath for 30 minutes. They were then immediately cooled, 2 mL of n-butanol was added to all tubes, and the samples were centrifuged at 3000 rpm for 5 minutes. The supernatant was read against n-butanol at 532 nm.

Determination of GSH in Rat Liver Tissue Samples and Serum

Tissue and serum GSH levels were determined by calculating total thiol according to the method described by Ohkawa et al.[7]

For serum total thiol determination, 250 µL of serum samples were collected, and 750 µL of TRIS-EDTA (pH=8.2), 50 µL of DTNB, and 3950 µL of pure methanol were added. To the blank tubes, 250 µL of distilled water, 750 µL of TRIS-EDTA (pH=8.2), 50 µL of DTNB, and 3950 µL of pure methanol were added. The tubes containing the samples and blanks were left in the dark for 15 minutes and vortexed every 5 minutes. At the end of the 15-minute period, the tubes were centrifuged at 3000×g, and the samples were read against a blank at 412 nm. For tissue total thiol determination, 250 µL of the supernatant samples prepared as described above were collected, and 750 µL of TRIS-EDTA (pH=8.2), 50 µL of DTNB, and 3950 µL of pure methanol were added. To the blank tubes, 250 µL of distilled water, 750 µL of TRIS-EDTA (pH=8.2), 50 µL of DTNB, and 3950 µL of pure methanol were added. The tubes containing the samples and blanks were left in the dark for 15 minutes and vortexed every 5 minutes. At the end of the 15-minute period, the tubes were centrifuged at 3000×g, and the samples were read against a blank at 412 nm.

Histochemical Examinations

Tissue samples obtained from the rats were washed with saline solution, fixed in 10% formalin solution for 72 hours, and embedded in paraffin blocks. Sections 4–5 µm thick were placed on slides, deparaffinized first in an oven and then in a series of xylene solutions, and subsequently rehydrated through a graded alcohol series. The sections were stained with hematoxylin and eosin and evaluated under a light microscope.

To determine liver damage, tissues stained with hematoxylin and eosin were evaluated for degeneration, sinusoidal dilation, sinusoidal congestion, and inflammatory cell infiltration.

The following scoring system was used for the samples, which were evaluated separately for each criterion:

0: No findings

1: Findings in less than 25% of the area

2: Findings in 25–50% of the area

3: Findings in more than 50% of the cross-sectional area.[9]

Immunohistochemical Method

The fixed tissues were processed using conventional light microscopy methods, and paraffin blocks were prepared. Sections 4–5 µm thick were obtained from the prepared paraffin blocks and mounted on polylysine-coated slides. After deparaffinization, the slides were incubated with PCNA (Cat#: MS-106-P, Thermo, Fremont, CA, USA) primary antibody diluted 1:200 and caspase-9 (Cat#: RB-1205-P, Thermo, Fremont, CA, USA) primary antibody diluted 1:100 for 60 minutes at room temperature. After washing the slides with PBS, a biotin-containing secondary antibody (Thermo, USA) was applied for 20 minutes to allow binding to the primary antibody. The slides were then washed with PBS and incubated with the streptavidin-peroxidase enzyme complex (Thermo, USA) for 20 minutes, allowing the enzyme complex to bind to biotin on the slides.

Finally, the chromogen AEC (3-amino-9-ethylcarbazole) (TA-125-HA, Thermo, Fremont, CA, USA) was added and incubated for approximately 10 minutes, allowing the visible reaction product to develop.

Mayer's hematoxylin (0B524092, Merck) was used as the background stain. The stained slides were covered with coverslips using Ultramount and evaluated under a computer-equipped photomicroscope (DM 4000B, Leica, Germany).

To evaluate PCNA, positively stained hepatocytes were counted in 5 randomly selected areas at ×400 magnification.[10]

Table 1. Statistical evaluation of GSH and MDA levels in liver tissue and serum and serum AST and ALT measurements (mean±SEM)

	MDA		GSH		AST (U/L)	ALT (U/L)
	SERUM (nmol/ml)	TISSUE (nmol/gr tissue)	SERUM (µmol/l)	TISSUE (µmol/gr tissue)		
Group 1	8.35±0.26	113.20±21.93	253.77±17.02	17.75±1.13	416.67±48.73	291.00±48.51
Group 2	11.76±0.78[b]	96.24±11.11	260.61±34.19	13.22±0.84[a]	655.20±191.39	466.30±151.29
Group 3	9.27±1.29	115.90±19.26	359.54±65.74	18.50±1.10[c]	683.50±166.28	526.80±154.43

[a]: p: 0.01, mild hypothermia decreased tissue GSH levels; [b]: p: 0.01, serum MDA levels were higher in mild hypothermia group; [c]: p: 0.01, in moderate hypothermia group, tissue GSH levels were increased compared to mild hypothermia group. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GSH: Glutathione; MDA: Malondialdehyde; SEM: Standard error of the mean; U/L: Units per liter; nmol/mL: Nanomoles per milliliter; nmol/g tissue: Nanomoles per gram of tissue; µmol/L: Micromoles per liter; µmol/g tissue: Micromoles per gram of tissue

Table 2. Liver damage index

	Degeneration	Sinozoidal dilatation	Sinozoidal congestion	Cell infiltration	Total score
Group 1	2.22±0.32	2.22±0.28	0.20±0.13	2.22±0.32	5.44±0.65
Group 2	2.10±0.18	2.40±0.22	0.36±0.12	2.22±0.32	6.10±0.38
Group 3	1.60±0.16	1.80±0.20	0.25±0.32	2.22±0.32	5.40±0.30

For caspase-9 evaluation, the entire preparation was scanned, and cells showing positive staining were assessed as follows:

0: No staining

1: Mild staining

2: Moderate staining

3: Strong staining.[11]

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Due to the small sample sizes and non-normal distribution of several parameters, nonparametric tests were employed. Comparisons among the three groups were performed using the Kruskal–Wallis H test, followed by post hoc pairwise comparisons using the Mann–Whitney U test. A Bonferroni correction was applied for multiple comparisons, and the adjusted significance level was set at $p<0.017$. All data are presented as mean±standard error of the mean (SEM). A p-value<0.05 was considered statistically significant.

RESULTS

The biochemical findings and liver damage index were analyzed (Tables 1, 2). According to the biochemical findings, no statistically significant differences were found in AST or ALT levels among the groups. There was a statistically significant difference among the groups in tissue GSH levels. When the mild hypothermia group (Group 2) was compared with the normothermia group (Group 1), a statistically significant decrease in tissue GSH levels was observed. Serum MDA levels were significantly higher in the normothermia group (Group 1). When the moderate hypothermia group (Group 3) was compared with the mild hypothermia group (Group 2), a significant increase in tissue GSH levels was observed. When the immunohistochemical findings were analyzed (Table 3), statistically significant differences were found among the groups in terms of caspase-9 and PCNA activity. The following formula was used to calculate regenerated liver weight. No significant difference was found among the groups in terms of regenerated liver weight (Table 4).

Table 3. Immunohistochemical comparison of groups (Mean±SEM)

	CASPASE	PCNA
Group 1	1.51±0.17	44.66±1.13
Group 2	0.64±0.13[a]	59.70±1.41[b]
Group 3	0.79±0.10[a]	68.87±1.24[b,c]

[a]: p=0.01, mild and moderate hypothermia decreases caspase enzyme activity compared to normothermia; [b]: p=0.01, mild and moderate hypothermia increases PCNA number compared to normothermia; [c]: p=0.01, moderate hypothermia increases the number of PCNAs compared to mild hypothermia. PCNA: Proliferating cell nuclear antigen; SEM: Standard error of the mean; p: Probability value (p-value)

Regenerated liver weight=weight of residual lobes/body weight×100

Table 4. Morphological evaluation of liver according to experimental groups

	Rat weight (gr)	Rat liver weight (gr)	Resected liver weight (gr)	Liver weight at autopsy (gr)	Regenerated liver weight (gr)
Group A					
1	250	8.5	6.6	4.9	35.3
2	358	12.2	8.1	5.9	14.7
3	332	11.3	7.4	7.0	27.4
4	220	7.4	5.8	5.5	52.7
5	376	12.8	7.8	7.0	15.6
6	470	16.0	13.8	4.9	16.9
7	400	13.6	11.5	5.3	23.5
8	205	7.0	5.0	3.5	21.4
9	233	7.9	5.3	4.0	17.7
10					
Group B					
1	317	10.8	7.0	5.8	18.5
2	354	12.0	7.5	7.8	27.5
3	306	10.4	8.0	5.7	31.7
4	396	13.5	11.3	4.8	19.3
5	290	9.9	6.5	4.8	14.1
6	333	11.3	9.6	7.3	49.5
7	335	11.4	9.7	4.6	25.4
8	273	9.3	7.9	4.7	35.5
9	215	7.3	5.3	3.8	24.6
10	220	7.5	5.6	3.4	20.0
Group C					
1	337	11.5	7.0	8.0	30.4
2	387	13.2	9.1	7.5	25.7
3	387	13.2	9.3	7.8	29.5
4	222	7.5	5.4	3.6	20.0
5	350	11.9	8.7	5.0	15.1
6	398	13.5	9.6	5.9	14.8
7	390	13.3	103	7.7	45.6
8	230	7.8	5.2	3.7	14.1
9	320	10.9	8.3	4.1	13.7
10	223	7.6	5.1	3.4	11.8

The mild hypothermia (Group 2) and moderate hypothermia (Group 3) groups showed decreased caspase-9 enzyme activity and increased PCNA activity compared with the normothermia group (Group 1). On the other hand, there was no statistically significant difference between the moderate hypothermia (Group 3) and mild hypothermia (Group 2) groups in terms of caspase-9 activity, whereas moderate hypothermia (Group 3) significantly increased PCNA activity compared with the mild hypothermia group (Group 2) (Figs. 1, 2).

DISCUSSION

In this study, the effects of mild and moderate systemic hypothermia after major hepatectomy on liver damage, oxidative stress, apoptosis, and regenerative capacity were inves-

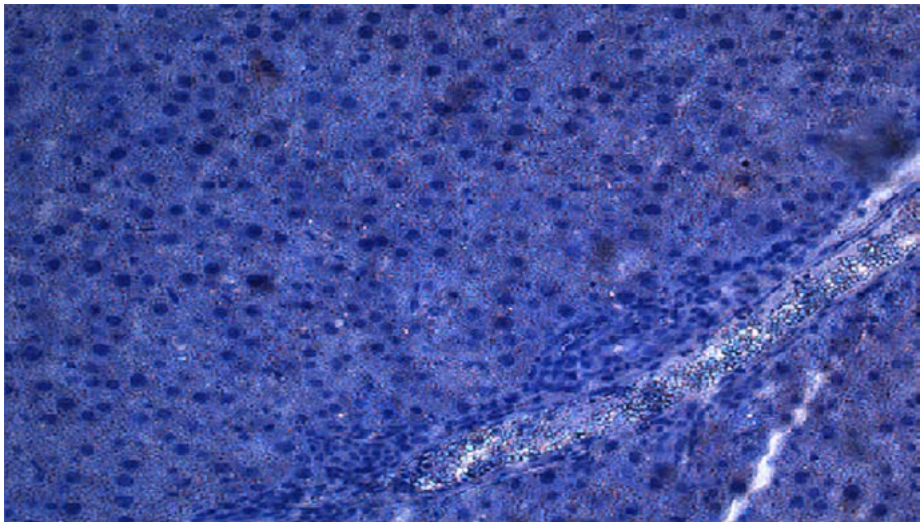

Figure 1. In the picture of the moderate hypothermia group, mild caspase-9 uptake is observed in hepatocytes. (Bar: 2 µm, Anti-caspase-9, immunoperoxidase, X 400)

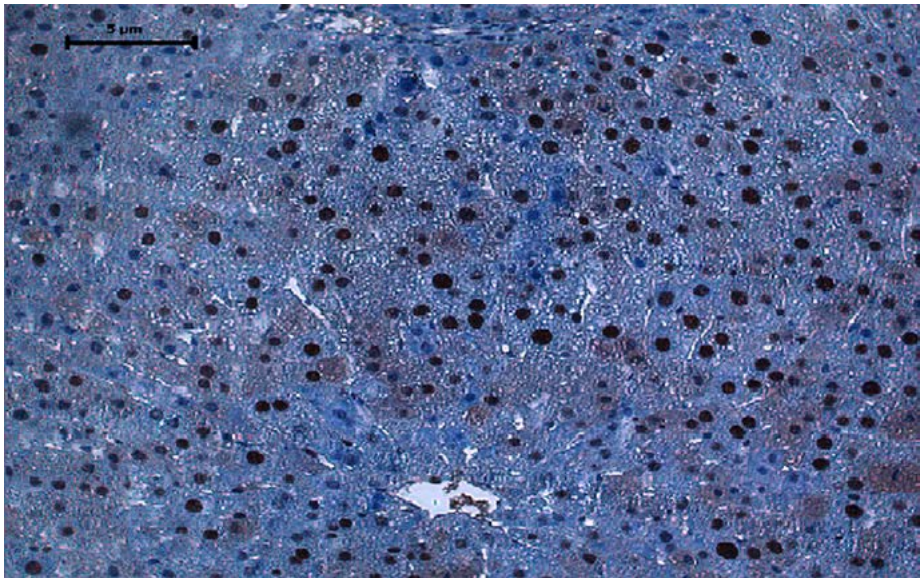

Figure 2. In the picture of the mild hypothermia group, hepatocytes arranged radially around the central vein show moderate to intense uptake (bar: 5µm, PCNA Immunoperoxidase, X 400)

tigated, and it was revealed that the effects of hypothermia differ depending on temperature.

Liver regeneration, which occurs through compensatory hyperplasia and hypertrophy of the residual liver lobes without the involvement of stem cells, depends on neoangiogenesis and tissue proliferation.[12] When a trigger such as hepatectomy or liver damage occurs, almost all (95%) liver cells re-enter the cell cycle (S phase, 12–24 hours), triggering DNA synthesis; DNA synthesis also occurs in nonparenchymal cells. DNA synthesis progresses from the periportal area toward the central vein. Most of the increase in liver mass occurs within the first 3 days after PHx, and parenchymal restoration is completed within 5–7 days.[13] Experimental studies have shown that, in addition to many factors regulating

tissue restoration following hepatocyte necrosis and partial loss caused by severe viral or drug-induced hepatitis, factors such as serum zinc levels also affect liver regeneration.[14]

Mild hypothermia is used clinically because of its various metabolic and hemodynamic effects. It has been used as part of the treatment for increased intracranial pressure and has led to a decrease in mortality in an experimental hemorrhagic shock model in rats.[15,16] The purpose of hypothermia in liver surgery is to increase the duration and tolerance of ischemia. The most important cause of mortality after liver resection is operative blood loss. Clamping techniques such as Pringle and TVE are used to prevent this blood loss during partial resection. The main problem with the use of these techniques is that the tissue is exposed to I/R injury. With SOR formation during reperfusion, OS increases and mediates liver damage.[17]

Black et al.[18] demonstrated that systemic hypothermia induced by whole-body cooling decreases I/R injury by prolonging the ischemic period following liver resection but also produces side effects, including cardiac arrhythmia, severe hypotension, metabolic acidosis, and coagulopathy. Dinant et al.[17] used hypothermic perfusion to reduce I/R injury during TVI and decreased the liver core temperature by 10°C. In the normothermic group, TVI caused cellular vacuolization, sinusoidal congestion, neutrophil infiltration, and mitochondrial dysfunction. In the hypothermic perfusion group, liver function indicators such as AST levels and histological damage scores decreased significantly, and survival increased. Fortner et al.[19] performed major hepatic resection for liver tumors with hypothermic perfusion under TVE, with a mortality rate of 10%, whereas Yamanaka et al.[20] performed segmentectomy in cirrhotic livers with hepatocellular carcinoma under mild hypothermic conditions provided by hemihepatic vascular occlusion and topical cooling. They showed that ischemia duration was prolonged, hepatic function was preserved, and complications were reduced with hemihepatic cooling and occlusion compared with the control group. Vaquero et al.[21] showed that, in APAP-induced liver damage in rats, apoptotic cell death and mortality decreased under mild hypothermic conditions compared with normothermia. Grezzana Filho et al.[22] found that selective hepatic hypothermia prevented I/R injury by inhibiting the release of inflammatory cytokines, protected the microcirculation, prevented hepatocellular necrosis and leukocyte infiltration, and allowed preservation of liver architecture.

The effects of hypothermia on liver regeneration are controversial. According to Munoz, hypothermia negatively affects liver regeneration because of decreased cardiac output, mesenteric and hepatic blood flow, changes in genes involved in regeneration, and possible downregulation of biochemical enzymes involved in the regeneration process.[23] In an experimental study investigating the effect of partial liver resection performed under hypothermic conditions on regeneration, Arab et al.[16] divided mice into 2 groups: mild hypothermia and normothermia. The temperature of the hypothermia group was maintained at 32°C, and partial liver resection (2/3) was performed in both groups for 3 hours under standardized conditions, taking the diurnal rhythm into consideration. Although a strong regenerative response was observed in both groups, no difference was found between the groups in morphological and immunohistochemical evaluations. Hypothermia had no effect on regeneration in mice subjected to partial liver resection.

In our study, although there was an increase in the regenerative response evaluated by PCNA staining, which is a standard method, this effect was not observed in morphological measurements in the hypothermia groups. This may be because the increased mitotic activity at 48 hours had not yet resulted in a significant increase in the remaining liver mass. In addition, unlike the study by Arab et al.,[16] our finding of an increased regenerative response with greater hypothermia depth may be related to the fact that the diurnal rhythm was not taken into account during resections or to the duration of hypothermia. While hypothermia may have a positive effect on regeneration over short periods, it may suppress the response because of possible hemodynamic effects when the duration is prolonged. Studies on hypothermia models of different durations and depths, in which possible effects such as diurnal rhythm are standardized, are needed.

Hypothermia, in addition to slowing metabolism and delaying the damage process caused by oxygen and substrate deficiencies, may itself increase cellular damage. This damage is mediated by changes in ion homeostasis and membrane fluidity. The effect of hypothermia on necrosis and apoptosis, which are forms of cellular damage, is controversial, and studies in the literature have shown effects in both directions. Rauen et al.[24] showed that apoptosis occurs in both normal and hypothermic cell cultures, but most apoptotic indicators increase with the duration of hypothermia and especially during rewarming, which they described as cold-induced. The researchers drew attention to the activity of SOR in the mechanism of damage and demonstrated this activity by showing that apoptosis was almost completely suppressed by the addition of antioxidants to the medium. Fu et al.[25] showed that Fas ligand-mediated apoptosis was suppressed

through partial inhibition of signaling events, including mitochondrial damage, cytochrome c release, subsequent apoptosis, and effector caspase activation, in mouse hepatocytes cultured at 32°C. Molodykh et al.[26] showed that hepatocyte necrosis developed as a result of reorganization of stromal and parenchymal cells in rats subjected to whole-body hypothermia. In our study, biochemical and histological indicators of liver damage did not differ among the groups. Hypothermia did not affect liver injury. In the literature, studies in which damage parameters were evaluated after hypothermic liver surgery have primarily involved I/R models.

The reason why liver injury parameters were not positively or negatively affected by hypothermia in our study may be the absence of an ischemic period. Since continuous hemodynamic monitoring was not performed during our study, it is not possible to draw a clear conclusion on this issue. Previous studies have shown that hypothermia increases oxidative stress in both liver tissue and other organs.[18,24] In our study, hypothermia was also found to increase oxidative stress. There is strong evidence of a correlation between oxidative stress levels and inflammatory cytokines.[27] In our study, systemic mild hypothermia was also shown to increase oxidative stress after hepatectomy while suppressing apoptosis. Moderate hypothermia caused oxidative stress levels to return to normal. Despite the decrease in oxidative stress, no change was observed in the apoptosis-suppressing effect of moderate systemic hypothermia compared with mild hypothermia.

Hypothermia may exert its apoptosis-suppressing and hepatocyte-protective effects by decreasing proinflammatory cytokines.

In our study, mild hypothermia increased oxidative stress, as reported in a previous study.[28] However, unlike the findings of that study, mild hypothermia in our study favorably contributed to regeneration by increasing hyperplasia. In addition, mild hypothermia was shown to suppress apoptosis without affecting liver damage. Experimental studies using appropriate protocols are needed to understand the mechanisms underlying this chain of events. Qin et al.[29] showed that mild hypothermia preconditioning can increase liver cell viability, including cell proliferation, LDH regulation, and glycogen synthesis capacity. In addition, the researchers suggested that mild hypothermia preconditioning maintained its protective effect on liver cells in a cold environment. Ohashi et al.[30] emphasized that hypothermia may be a useful clinical marker for the early detection of liver failure after hepatectomy.

When moderate hypothermia was applied, the level of oxidative stress was similar to that observed under normothermic conditions, in contrast to mild hypothermia. Apoptosis was suppressed to a similar extent as with mild hypothermia. However, the increase in liver regeneration was more pronounced than that observed with both normothermia and mild hypothermia. This may be due to the favorable effect of moderate hypothermia without inducing severe oxidative stress.

CONCLUSION

In this study, hypothermia was shown to favorably affect liver regeneration, with a more pronounced effect under moderate hypothermia. The oxidative stress level, which increased with mild hypothermia, was similar to that observed under normothermia with moderate hypothermia. Histological and biochemical parameters showed that hypothermia did not affect liver damage. Hypothermia suppressed apoptosis regardless of its degree.

Disclosures

Ethics Committee Approval: The study was approved by the Başkent University Ethics Committee (No: 2008/AP-455, Date: 29/04/2008).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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REFERENCES

1. Michalopoulos GK, Bharat B. Liver regeneration: biological and pathological mechanisms and implications. Nat Rev Gastroenterol Hepatol 2021;18:40–55. [CrossRef]
2. Abu Rmilah A, Zhou W, Nelson E, Lin L, Amiot B, Nyberg SL. Understanding the marvels behind liver regeneration. Wiley Interdiscip Rev Dev Biol 2019;8:e340. [CrossRef]
3. Olthof PB, Reiniers MJ, Dirkes MC, Gulik TMV, Golen RFV. Protective mechanisms of hypothermia in liver surgery and transplantation. Mol Med 2016;21:833–46. [CrossRef]
4. Mito M, Tamaki A, Kon T, Ohira S, Mikami J. Experimental studies on differential hypothermia of the liver. J Surg Res 1965;5:207–12. [CrossRef]
5. Yamaoka Y, Ozawa K, Kumada K, Shimahara Y, Tanaka K, Mori K, et al. Total vascular exclusion for hepatic resection in cirrhotic patients. Application of venovenous bypass. Arch Surg 1992;127:276–80. [CrossRef]
6. Hansen TN, Dawson PE, Brockbank KG. Effects of hypothermia upon endothelial cells: mechanisms and clinical importance. Cryobiology 1994;31:101–6. [CrossRef]
7. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8. [CrossRef]
8. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872–9. [CrossRef]
9. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol 1979;135:372–6. [CrossRef]
10. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192–205. [CrossRef]
11. Erpek S, Bilgin MD, Doger FK. Elektromanyetik alanın (50HZ, 6mT) sıçan karaciğer ve böbreğine etkileri. ADU Tıp Fak Derg 2007;8:5–11. [Article in Turkish]
12. Andersen KJ, Knudsen AR, Kannerup AS, Sasanuma H, Nyengaard JR, Hamilton-Dutoit S, et al. The natural history of liver regeneration in rats: description of an animal model for liver regeneration studies. Int J Surg 2013;11:903–8. [CrossRef]
13. Moris D, Vernadakis S, Papalampros A, Vailas M, Dimitrokallis N, Petrou A, et al. Mechanistic insights of rapid liver regeneration after associating liver partition and portal vein ligation for stage hepatectomy. World J Gastroenterol 2016;22:7613–24. [CrossRef]
14. Takahashi Y, Oyama H, Nakamura A, Minegishi Y, Tanaka K. Zinc supports liver regeneration after partial resection. Turk J Surg 2023;39:344–53. [CrossRef]
15. Choi S, Noh J, Hirose R, Ferell L, Bedolli M, Roberts JP, et al. Mild hypothermia provides significant protection against ischemia/reperfusion injury in livers of obese and lean rats. Ann Surg 2005;241:470–6. [CrossRef]
16. Arab JP, Pizarro M, Solis N, Sun H, Thevananther S, Arrese M. Mild hypothermia does not affect liver regeneration after partial hepatectomy in mice. Liver Int 2009;29:344–8. [CrossRef]
17. Dinant S, van Veen SQ, Roseboom HJ, van Vliet AK, van Gulik TM. Liver protection by hypothermic perfusion at different temperatures during total vascular exclusion. Liver Int 2006;26:486–93. [CrossRef]
18. Black PR, van Devanter S, Cohn LH. Effects of hypothermia on systemic and organ system metabolism and function. J Surg Res 1976;20:49–63. [CrossRef]
19. Fortner JG, Shiu MH, Kinne DW, Kim DK, Castro EB, Watson RC, et al. Major hepatic resection using vascular isolation and hypothermic perfusion. Ann Surg 1974;180:644–52. [CrossRef]
20. Yamanaka N, Furukawa K, Tanaka T, Tanaka W, Yamanaka J, Imakita M, et al. Topical cooling-assisted hepatic segmentectomy for cirrhotic liver with hepatocellular carcinoma. J Am Coll Surg 1997;184:290–6.
21. Vaquero J, Bélanger M, James L, Herrero R, Desjardins P, Côté J, et al. Mild hypothermia attenuates liver injury and improves survival in mice with acetaminophen toxicity. Gastroenterology 2007;132:372–83. [CrossRef]
22. Grezzana Filho TJM, Longo L, Santos JLD, Gabiatti G, Boffil C, Santos EBD, et al. Induction of selective liver hypothermia prevents significant ischemia/reperfusion injury in Wistar rats after 24 hours. Acta Cir Bras 2020;35:e202000205. [CrossRef]
23. Munoz SJ. Hypothermia may impair hepatic regeneration in acute liver failure. Gastroenterology 2005;128:1143–4; author reply 1144–5. [CrossRef]
24. Rauen U, Polzar B, Stephan H, Mannherz HG, de Groot H. Cold-induced apoptosis in cultured hepatocytes and liver endothelial cells: mediation by reactive oxygen species. FASEB J 1999;13:155–68. [CrossRef]
25. Fu T, Blei AT, Takamura N, Lin T, Guo D, Li H, et al. Hypothermia inhibits Fas-mediated apoptosis of primary mouse hepatocytes in culture. Cell Transplant 2004;13:667–76. [CrossRef]
26. Molodykh OP, Klinnikova MG, Lushnikova EL, Nepomnyashchikh LM. Tissue and intracellular reorganization of rat liver during total body hypothermia. Bull Exp Biol Med 2005;139:741–6. [CrossRef]
27. Yamanaka N, Okamoto E, Furukawa K, Oriyama T, Fujimoto J, Kanno H, et al et al. Hepatic resection under in situ hemihepatic hypothermic perfusion with hepatoprotective agents. Hepatogastroenterology 1995;42:1–4.
28. Cosgrove BD, Cheng C, Pritchard JR, Stolz DB, Lauffenburger DA, Griffith LG. An inducible autocrine cascade regulates rat hepatocyte proliferation and apoptosis responses to tumor necrosis factor-alpha. Hepatology 2008;48:276–88. [CrossRef]
29. Qin J, Mai Y, Li Y, Jiang Z, Gao Y. Effect of mild hypothermia preconditioning against low temperature (4°C) induced rat liver cell injury *in vitro*. PLoS One 2017;12:e0176652. [CrossRef]
30. Ohashi N, Hori T, Uemoto S, Jermanus S, Chen F, Nakao A, et al. Hypothermia predicts hepatic failure after extensive hepatectomy in mice. World J Hepatol 2013;5:170–81. [CrossRef]