

MOLECULAR MECHANISMS OF OXIDATION DAMAGE AND LIVER CELL DYSFUNCTION IN PATIENTS WITH METASTATIC COLORECTAL CANCER

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Background: Interaction between tumor cells and tumor microenvironment is critical for homeostasis of normal cells and tumor growth. Tumor cell — stroma interaction represents the potent factor able to initiate cancer and affect tumor progression and disease outcome. The tumors vary by their origin and microenvironment (proportion of stromal cells, their composition and activation state). The surgical stress and tumor microenvironment may potentiate acute hepatic failure in the patients with metastatic colorectal cancer (mCRC). Pathological effect of ischemia-reperfusion (I/R) consists in the increased generation of superoxide radicals (SR) and nitrogen oxide (NO) affecting the postresectional regeneration of liver tissue. Redox state of hepatic tissue in I/R setting upon resection of metastases may trigger the aggressiveness of residual cancer cells and regeneration or degradation of hepatic tissue. *The aim of the study* was to analyze redox state of hepatic tissue following surgery with Pringle maneuver (PM) in the patients with mCRC. *Materials and Methods:* mCRC samples from 145 patients treated at National Cancer Institute, Ministry of Health of Ukraine, were analyzed. The patients obtained chemotherapy according to the approved international and national standards as well as clinical protocols. Two groups of patients were delineated according to the duration of the interruption of blood inflow due to PM, namely ≤ 45 min and > 45 min. The activity of FeS proteins in the electron transport chain (ETC) in mitochondria and lactoferrin (LF) level in the tissues were assessed by EPR (77K). The rates of SR and NO generation were determined with spin traps. The activity of matrix metalloproteinase (MMP)-2 and -9 was measured by gelatin zymography using SDS-polyacrylamide gel electrophoresis. *Results:* In tissue of liver resected in the setting of > 45 min ischemia, ETC function in mitochondria was impaired (decreased activity of FeS protein of N-2 ETC complex I due to interaction with NO). This results in the hypoxia state and glycolysis with uncontrolled SR generation. In addition, the efficiency of detoxification system in hepatocytes is reduced substantially with increase in semiquinone and LF levels as well as MMP-2 and -9 activity as compared with liver without metastatic lesions that was not affected by I/R. *Conclusions:* The ischemic injury of liver in the setting of metastasis resection results from cell response to interruption of blood flow followed by reperfusion. The key factor in the genesis of reperfusion damage is uncontrolled increase of the levels of SR and their metabolites — reactive oxygen species as well as the increased MMP activity. Also, liver tissue affected by I/R contains high levels of xanthine oxidase metabolizing hypoxanthine and monoamine oxidase deaminizing biogenic amines. Both processes are the sources of SR.

Key Words: metastatic colorectal cancer, ischemia/reperfusion, redox state, metastases, liver resection, Pringle maneuver.

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Interaction between tumor cells and tumor microenvironment is critical for homeostasis of normal cells and tumor growth. Tumor cell — stroma interaction represents the potent factor able to initiate cancer and affect tumor progression and disease outcome. This problem is of paramount importance for both understanding the nature of cancer and the search for new treatment modalities. Nowadays, it became clear that tumors vary by their origin and microenvironment (proportion of stromal cells, their composition and activation state). The surgical stress and tumor microenvironment may potentiate acute hepatic failure in the patients with metastatic colorectal cancer (mCRC). The pathological effect of ischemia/reperfusion (I/R) consists in the increased generation of superoxide radicals (SR) and nitrogen

oxide (NO) affecting the postresectional regeneration of liver tissue. Redox state of hepatic tissue in I/R setting upon resection of metastases may trigger the aggressiveness of residual cancer cells and regeneration or degradation of hepatic tissue. The heat ischemia due to limitation and/or interruption of blood inflow/outflow results in the damage of the affected organs/tissues with following acute or chronic dysfunction with accompanying ATP depletion [1, 2]. The damage of tissues affected by heat ischemia may be underlying cause of various pathologies such as arrhythmia, impairment of spinal nerve function, disordered intestinal motility, impairment of vision, proteinuria, hepatic failure, infertility, etc. [3–6]. The cascade of the pathological processes induced by I/R following liver resection or transplantation may be threatening for the functional recovery of the patients [1]. Although pathological effects of I/R are essential in pathogenesis of ever-increasing list of clinical conditions, the pathogenesis of reperfusion-dependent mechanisms is far from being elucidated.

SR are of high importance in formation of oxidizing induced damage following I/R. Normally, systemic and multilevel protection against damaging effects of SR exists.

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Abbreviations used: CRC — colorectal cancer; EPR — electron paramagnetic resonance; ETC — electron transport chain; I/R — ischemia/reperfusion; iNOS — inducible NOS; LF — lactoferrin; mCRC — metastatic CRC; ML — metastatic lesion; MMP — matrix metalloproteinase; NO — nitrogen oxide; NOS — NO synthase; PAF — platelet-activation factor; PM — Pringle maneuver; SR — superoxide radicals.

In postischemic tissues, high levels of SR generation and increased content of SR-generating cells are evident. Elevated SR level activates matrix metalloproteinases (MMP)-2 and -9, increases the synthesis of adhesion molecules by endothelial cells and induces the production of the mediators of inflammation [7]. While hemoglobin and myoglobin upon penetration into extracellular matrix may turn into non-enzymatic sources for SR generation [8], most authors agree that the accelerated SR generation in postischemic tissues is a result of the activity of one or several enzymes catalyzing reactions with electron transfer onto molecular oxygen followed by SR formation. In particular, we believe that electron transport chain (ETC) of mitochondria, NADPH oxidases (NOXs), xanthine oxidase, incomplete or damaged NO synthase (NOS) may be involved in the increased SR generation in postischemic tissues.

In this study, we attempted to assess the redox state of hepatic tissue in the setting of surgery on liver utilizing Pringle maneuver (PM) in the patients with mCRC with the aim of clarifying SR sources in hepatic tissue upon I/R.

MATERIALS AND METHODS

145 patients with colorectal cancer (CRC) (pT₁₋₄N₀₋₂M₀ — colon cancer and pT₁₋₃N₀₋₂M₀ — cancer of rectum) with varying extent of metastatic involvement of the liver who were treated in the clinic of the National Cancer Institute (Kyiv, Ukraine) in 2015–2019 were enrolled into the study (Table 1). All patients gave their informed consent allowing for the use of the clinical samples for research purposes. In most patients (77 patients, 53.1%), bilobar metastases were detected. All clinical cases were subjected to the thorough multidisciplinary team with the involvement of the oncologists, surgeons, radiation oncologists and the specialists in chemotherapy. The diagnosis, the stage of the disease and the presence of the metastases have been verified according to the results of the clinical, instrumental and histopathological examinations including fine-needle biopsy of the pathological focus in the liver. Computed tomography with intravenous contrast was performed as routine diagnostic technique. In the complicated cases (suspected canceromatosis or bilobar involvement), the examination was supplemented by magnetic resonance tomography. Positron emission tomography was used only when the metastatic lesions (ML) of other organs were suspected. The tissue of liver biopsy made by diagnostic indications in patients

proved to be free of viral pathology, primary and/or secondary malignant lesions of liver was used as control.

The patients received the adjuvant polychemotherapy according to the international standards (FOLFOX-6/FOLFIRI/XELOX regimen, 4–6 courses). The surgical resection was not performed in patients with progression of the disease in the setting of polychemotherapy. The functional reserves of the liver were assessed by Child — Turcotte — Pugh and MELD scoring. The toxicity of chemotherapy was assessed according to CTCAE 5.0 criteria. Postoperative acute hepatic failure was estimated according to the classification by International Study Group of Liver Surgery (ISGLS).

The surgical technique for resection of ML in liver provided for the maximal sparing of parenchyma and the adequate safe margin (4–10 mm). In all cases, intraoperative ultrasonic examination was performed for marking the lesions relative to the major hepatic veins and Glisson's structures as well as for detecting non-palpable metastases. The classic and selective PM (20 min ischemia — 5 min reperfusion) was used [8]. For parenchyma transection, “crashclamp” technique was used. For hemostasis of parenchyma after resection, the tissue was sutured with prolene 4.0, 5.0 and LT200, LT300 clips were used.

Two groups of patients were delineated according to the duration of the interruption of blood inflow due to PM, namely ≤ 45 min and > 45 min. Whereas ≤ 45 min group comprised 91 patients (in 52 patients classical PM was used; in 15 patients — modified PM; in 24 patients PM was not applied). > 45 min group comprised 54 patients (in 46 patients classical PM was used; in 8 patients — modified PM). While the groups differed significantly by the number of the resected ML ($n = 4$ (1–17) and $n = 5$ (3–19) for < 45 min and ≥ 45 min; $p = 0.03$), the volumetric analysis of the metastatic tissue proved that the groups were stratified (13.6 ± 5.5 cm³ and 14.1 ± 8.2 cm³; $p = 0.16$).

Activity of FeS proteins of ETC in mitochondria, lactoferrin (LF) and “free” iron content in the tissue were assessed by electron paramagnetic resonance (EPR) at the temperature of 77 °K in the punched samples stored in liquid nitrogen [9]. The rate of SR and NO generation in mitochondria by NADPH oxidase and inducible NOS (iNOS) of neutrophils was determined by EPR with spin traps [10, 11]. The activity of MMP-2 and -9 in tumor samples was measured by gelatin zymography using SDS-polyacrylamide gel electrophoresis [12]. The gelatinase activity was expressed in conventional units (c.u.) referred to the activity of 1 µg of enzyme per 1 g of the tissue [13]; in urine — 1 µg of enzyme per 1 g of creatinine [14].

The data were statistically processed using the software package SPSS 20.0 (IBM, Armonk, NY, USA). The difference was considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

When the effects of I/R on ETC functioning in mitochondria of hepatocytes in the resected tissue of the patients with mCRC were analyzed, the most pronounced impairments have been evident in setting of > 45 min

Table 1. Summary data on patients in the study

Parameter	Value	%
Age	67.3 ± 1.5	
Body mass index	28.3 ± 2.9	
Gender (males/females)	81/64	55.8/44.2
Time of detection of metastatic lesions in liver (synchronous / metachronous), months	13/132	8.9/91.1
Localization of primary tumor (rectum/colon)	44/101	30.4/69.6
Status of regional lymph nodes pN (+/–)	38/107	26.2/73.8
State of patient according to ASA, (I–II/III)	96/49	66.2/33.8
Examinations and treatment:		
CT	145	100.0
MRT	127	87.6
PET-CT	14	9.6
Neoadjuvant chemotherapy	35	24.4
Adjuvant chemotherapy	142	97.3

ischemia as compared to ≤ 45 min ischemia. The EPR signals were recorded in the specimens of the conditionally intact tissue taken at the distance of 5 cm from MLs or adjacent to these lesions. It should be noted that any features of cirrhosis, hepatosis or other concomitant pathology were excluded upon gross examination. It was demonstrated that the activity of FeS protein N-2 of ETC complex I decreased due to its interaction with NO resulting in cell hypoxia and glycolysis with accompanying uncontrolled SR generation (Fig. 1, 2; Table 2). Such a decrease is indicative of the impairment of the coupling between oxidation and phosphorylation in NADH-ubiquinone oxidoreductase complex of ETC. In parallel, the efficacy of detoxifying system was also reduced judging by the decreased activity of cytochrome P450 (CYP 1A2 isoform, EPR signal with $g = 2.25$ and 2.42). Furthermore, the level of semiquinones (free radical state of sex hormones) increased. The effects in the specimens adjacent to MLs were more distinct as compared with specimens taken at the distance of 5 cm (see Table 2).

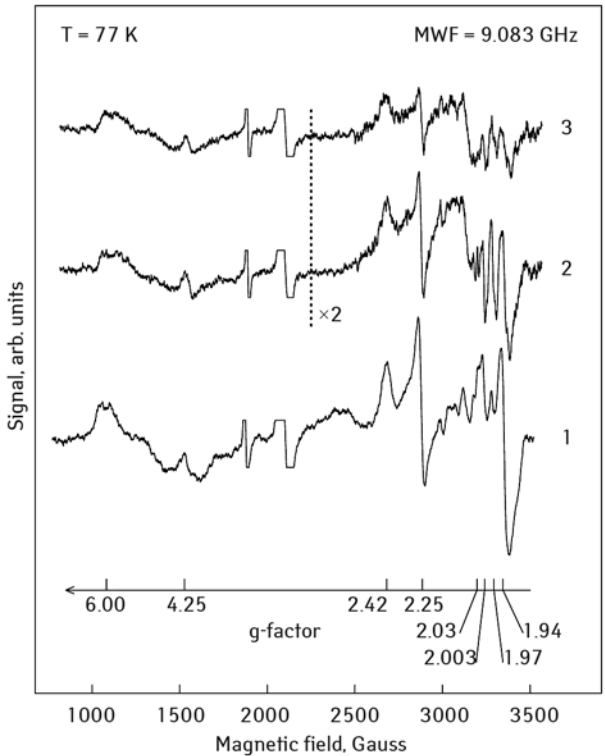


Fig. 1. EPR spectra of liver tissue samples at the distance of less than 5 cm from metastasis after surgical resection using PM: 1 — sample of conditionally intact liver tissue (metastasis-free and without I/R damage); 2 — sample of liver tissue affected by ischemia ≤ 45 min; 3 — sample of liver tissue affected by ischemia > 45 min

Molybdenum-containing enzyme xanthine oxidoreductase hydroxylates xanthine to uric acid. This enzyme exists in forms of xanthine dehydrogenase and xanthine oxidase. The latter uses oxygen molecule as the end-acceptor of electron with SR generation. In fact, xanthine oxidase seems to be a source of SR in I/R in liver and intestine. The ischemic stroke results in depletion of the energy stores in cells, accumulation of hypoxanthine due to ATP catabolism, and conversion of xanthine dehydrogenases into xanthine oxidase. Upon reperfusion following the restoration of blood flow, the partial pressure of the oxygen in tissues increases and xanthine oxidase metabolizes hypoxanthine resulting in the increase of SR and dysfunction of endothelial barrier. During ischemia, redox state of the tissues changes from oxidative to reducing one. IL-1, IFN- γ , IL-6 and TNF- α enhance synthesis of xanthine dehydrogenase and xanthine oxidase in various tissues [11]. The accumulation of inflammation mediators because of macrophage activation in I/R facilitates SR generation.

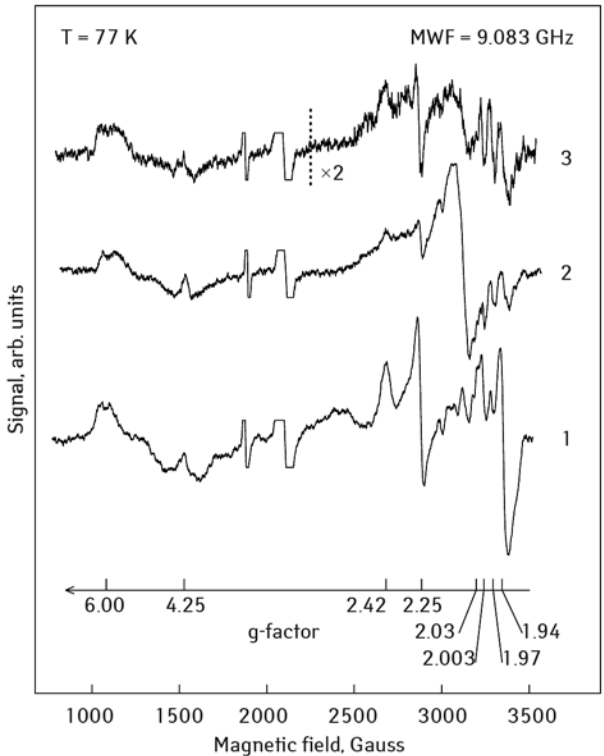


Fig. 2. EPR spectra of liver tissue samples at the distance of more than 5 cm from metastasis after surgical resection using PM: 1 — sample of conditionally intact liver tissue (metastasis-free and without I/R damage); 2 — sample of liver tissue affected by ischemia ≤ 45 min; 3 — sample of liver tissue affected by ischemia > 45 min

Table 2. EPR characteristics of liver tissue following I/R

	Intensity of EPR signals, relative units					
	FeS protein N-2		Semiquinone radicals		Cytochrome P450 (CYP 1A2)	
	Ischemia ≤ 45 min	Ischemia > 45 min	Ischemia ≤ 45 min	Ischemia > 45 min	Ischemia ≤ 45 min	Ischemia > 45 min
Liver tissue, 5 cm from ML	$0.42 \pm 0.04^*$	$0.23 \pm 0.02^*$	$0.54 \pm 0.06^*$	$0.44 \pm 0.07^*$	$0.55 \pm 0.05^*$	$0.48 \pm 0.04^*$
Liver tissue adjacent to ML	$0.23 \pm 0.06^*$	$0.11 \pm 0.03^*$	$0.45 \pm 0.05^*$	$0.31 \pm 0.05^*$	$0.35 \pm 0.05^*$	$0.12 \pm 0.03^*$
Liver tissue, control	1.57 ± 0.07		0.15 ± 0.02		2.46 ± 0.13	

Note: $^*p < 0.05$ compared to control; $^*p < 0.05$ compared to tissue adjacent to ML.

Monoamine oxidase (EPR signal $g = 2.10$, see EPR spectrum 2 in Fig. 1) functions on the mitochondrial membrane and generates SR upon the oxidative deamination of biogenic amines (noradrenalin and dopamine by isoform A and serotonin and histamine by isoform B). Upon interruption of blood flow followed by its recovery in ≤ 45 min activity of monoamine oxidases in liver tissue increases drastically from 0.01 to 1.4 relative units. If blood flow is recovered later than in 45 min, the activity of monoamine oxidases decreases while xanthine oxidase is activated and its activity increases (see Fig. 1).

In fact, we detected activation of xanthine oxidase and increase of its activity (EPR signal with $g = 1.97$) in liver tissue following I/R > 45 min from 0.06 ± 0.08 (normal control) to 0.75 ± 0.07 relative units. In this setting, purines, hypoxanthine, xanthine and succinate accumulate in the liver. Xanthine oxidase may function as nitrate/nitrite reductase catalyzing single-electron reduction of nitrite to NO [12]. Nevertheless, due to the generation of SR and NO, xanthine oxidase may become the potent source of peroxynitrite in postischemic and inflamed liver tissue. Other points of view as to the role of NO synthesizing in ischemic period also exist. In particular, several authors believe that NO may cause vasodilation and reduce inflammation minimizing in such a way the oxidative damage in liver [12]. Upon I/R with SR generation the cells of inflammation, in particular neutrophils are recruited to the focus of inflammation [13]. This process is mediated by platelet-activation factor (PAF), ICAM-1 and P-selectin increasing inflammation in post-ischemic venules of liver [14, 15].

Mitochondria are the major SR source in the cells affected by I/R in various organs especially those with high metabolic activity (liver, heart, brain). This is quite explainable taking into account that mitochondria generate SR in the course of the oxidative phosphorylation. The rapid translocation of electrons through ETC of the internal mitochondrial membrane may be linked with electron leakage and monovalent reduction of oxygen to SR [16–18]. In addition, mitochondria contain the enzymes capable of generating SR in tricarboxylic acid cycle. In the course of catabolism of carbohydrates (glycolysis), lipids (β -oxidation of fatty acids) and proteins (glucogenic and ketogenic pathways), the reduction equivalents (electrons) are transferred NAD⁺ and FADH producing NADH and FADH₂ that transfer them to ETC consisting of four electron-transfer complexes located on the internal mitochondrial membrane that are bound by the mobile electron carriers (coenzyme Q and cytochrome c). Electron transport complexes are built by the clusters of FeS proteins possessing redox properties. SR are generated in ETC at several points. And especially at the points of complexes I and III, the possibility of premature electron leakage to oxygen exists resulting in SR generation. The rate of SR generation depends on the level of membrane potential ψ . Several electron transport complexes may be combined as “supercomplexes” or “respirosomes”. The formation of such supercomplexes and their integrity depends on the availability of cardiolipin in the

inner mitochondrial membrane. One may suppose that such supercomplexes provide structural and functional interconnections between separate complexes facilitating electron transport with minimal losses. The oxidative damage of cardiolipin due to I/R may result in changing the composition of the said complexes increasing the probability of SR generation. The intense SR generation by one of ETC complexes may trigger the damage of the adjacent complexes extending ETC dysfunction with auto-amplification of SR generation [19–24]. In ischemia, ETC complexes are dephosphorylated by phosphatases (priming) while upon reperfusion, the primed complexes generate SR. In such setting, the tissues accumulate succinate. This fact is explained by the altered activity of succinate dehydrogenase when mitochondria switch over to anaerobic metabolism with intensification of SR generation and tissue damage [25–29]. Although some other sources of SR generation exist in mitochondria in I/R setting, the rate of SR generated by them is less by several orders of magnitude. Among such sources are NOX4 and p66^{Shc}. The latter represents the isoform of proapoptotic adaptor ShcA protein expressed in cytoplasmic membrane, endoplasmic reticulum and mitochondria. The mitochondrial pool of p66^{Shc} increases in response to I/R resulting in the electron leakage from ETC with SR generation [30]. Pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and other dehydrogenases are also capable of generating SR under certain conditions. Dehydrogenases that function with NAD contribute much into SR generation by mitochondria in postischemic tissues due to the increased NADH/NAD⁺ ratio. Nevertheless, the role of ETC in cell death in the I/R setting is predominant one.

The cells damaged due to I/R release the mediators that activate NADPH oxidases in neutrophils and macrophages. Phospholipase A2 in the setting of I/R initiates the production of PAF and the synthesis of arachidonic acid and its metabolism to thromboxane and leukotrienes, the latter activate SR generation by NADPH oxidase [31, 32]. In addition, the complement system is activated that also contributes to the increased NADPH oxidase activity in postischemic tissue. Also, cytokines (TNF- α , IL-1 β) released from macrophages and mast cells following reperfusion increase NADPH oxidase activity, while cytokine blockade decreases SR generation rate in the cells [33]. Angiotensin II also stimulates SR generating activity of NADPH oxidase. In the blood of the patients under study, we observed high levels of superoxide- and NO-generating activity of neutrophils. The data on the NOX-2 and iNOS activities in the neutrophils of the patients are presented in Fig. 3.

The rate of SR generation in neutrophils of patients operated without PM is 0.51 ± 0.12 nmol/10⁵ cells $\cdot$ min. In cases where PM was used the rate of SR generation in neutrophils is 1.03 ± 0.16 nmol/10⁵ cells $\cdot$ min. Therefore, NOX-2 activity increases two-fold. Similar data were obtained for iNOS activity in neutrophils (rate of NO generation is 0.93 ± 0.09 nmol/10⁵ cells $\cdot$ min in cases without ischemia of liver tissue and 1.68 ± 0.06 nmol/10⁵ cells $\cdot$ min in cases with ischemia of liver).

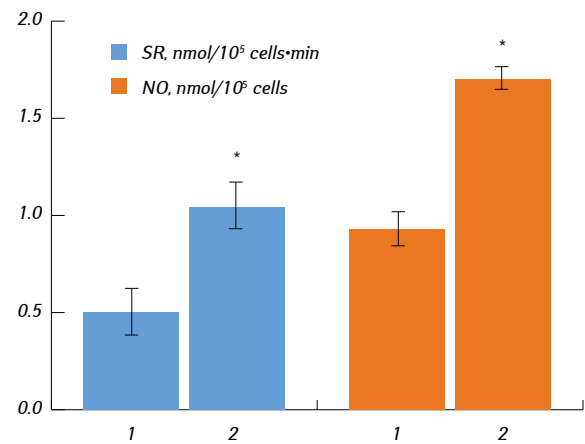


Fig. 3. NOX-2 and iNOS activities in neutrophils of mCRC patients with liver metastases: 1 — ischemia ≤ 45 min; 2 — ischemia > 45 min; **p* < 0.05 as compared with values for ischemia ≤ 45 min

The data obtained confirm that NADPH oxidases in cells of innate immunity (NOXs) comprise the principal source for SR generated upon I/R in various pathological conditions [34–36]. NOX-2 or gp91phox responsible for the ability of phagocytosing cells (neutrophils) to generate SR are detected in the cells of the vascular walls. In non-phagocytosing leukocytes NOX-2 activity is lower. Neutrophils are associated with reperfusion damage of different tissues of the body (heart, kidneys, intestine, stomach, lungs, brain, liver) as well as systemic organ impairments following hemorrhagic shock. The involvement of neutrophils is confirmed by the data demonstrating that the recruitment of the inflammation cells in the organ coincides with the increased levels of SR generation. All NOX normally are non-active constitutionally and require cell stimulation for SR being generated. At rest, the protein subunits (p47^{phox}, p40^{phox} and p67^{phox}) and small GTPase Rac1/2 are located in cytosol spatially separated from large catalytic subunits incorporated into cell membrane. Upon cell activation, the regulatory subunits transfer from cytosol to cell membrane where they combine with membrane components of NOX. Only assembled enzyme is capable of generating SR through single-electron reduction of the molecular oxygen using cytoplasmic NADPH as electron donor.

Several human NOS isoforms are known: neuronal NOS (nNOS), endothelial NOS (eNOS), iNOS, and mitochondrial (mtNOS). iNOS is induced in the immune cells. It is generally accepted that NO in I/R is a protective factor due to its anti-inflammatory properties (inhibition of neutrophil adhesion/migration).

NO plays an important role in angiogenesis/lymphangiogenesis [2, 37]. In particular, NO generated by iNOS in neutrophils is mediator for VEGF-induced lymphangiogenesis. Therefore, NO is involved in early stages of metastasizing and increased NO levels are associated with tumor progression. According to our

data, NO levels in liver tissue following I/R correlate inversely with I/R duration (*r* = 0.62; *p* = 0.05) (Table 3).

All three isoforms of NOS are expressed in cancers of gastrointestinal tract. In particular, high levels of iNOS and eNOS expression are detected in CRC. The tumor-induced angiogenesis is of paramount importance for tumor progression and metastasizing and facilitate cell proliferation via the development of blood vessels through VEGF activation [38].

We have assessed the activity of MMP-2 and -9 in the tissue of MLs and in liver tissue adjacent to MLs and at the distance of 5 cm from MLs as well in blood serum and urine of the patients with mCRC following liver ischemia (≤ 45 min or > 45 min) in the course of surgery. The activity of MMP-2 and -9 is detectable in all analyzed samples. Nevertheless, the gelatinase activities in the tissue adjacent to ML are 1.3–2.0-fold higher than the corresponding activities in the samples taken at the distance of 5 cm from ML (both for PM ≤ 45 min and > 45 min). MMP-2 and -9 activities upon ischemia > 45 min are superior to that upon ischemia ≤ 45 min in all samples under study. The high indices of extracellular matrix destruction in the tissue adjacent to ML prove the important role of gelatinases in providing the metastatic microenvironment facilitating ML growth and extension by proteolytic cleavage of the components of extracellular matrix. More intensive hypoxia in the setting of long-term ischemia in the course of ML resection results in the significant increase in the activity of hypoxia-dependent enzymes and intense destruction of liver tissue and probably other tissues of the body. This fact is corroborated by high gelatinase activities in blood serum and urea of the patients (Table 4).

The results of the treatments of the patients under observation in corresponding groups are given in Table 5.

The radicality of the surgery was satisfactory in both groups of the patients under study. R0 resections were recorded in 89 and 40 cases for < 45 min and ≥ 45 min, respectively. 24 cases required peri- and postoperative hemotransfusions depending on the PM duration. It should be noted that the group differ significantly by the rate of the acute hepatic failure grade A. Longer PM duration results in the impairment of the functional capacity of the cell stroma in liver that is corroborated by several changes in molecular biological parameters. The treatment outcomes in our study conform to that reported by other authors [39]. The increase in the complications ≥ grade III (4 patients in < 45 min group and 10 patients in ≥ 45 min) should also be noticed.

Acute phase of liver injury in course of resection of metastases triggers the series of the inflammatory cascades associated with SR generation that could activate the signal pathways involved in invasion and migration of cancer cells. On the other hand, generated SR may be involved in mobilization of circulating immune cells and modulation of intercellular redox state facilitating the recurrence and metastasizing. The liver damaged by oxygen radicals may represent the favorable microenvironment for the growth of cancer cells, their migration and invasion due to disordered function

Table 3. NO level in liver tissue depending on ischemia duration

Ischemia duration (PM)	NO, nmol / g wet tissue
≤ 45 min (n = 11)	1.19 ± 0.18
> 45 min (n = 14)	0.44 ± 0.20
Normal tissue (without ischemia)	1.48 ± 0.08

Table 4. Activities of MMP-2 and -9 in liver, ML, blood and serum of mCRC patients following liver ischemia in the course of the surgery

Samples	MMP-2, conditional units		MMP-9, conditional units	
	ischemia ≤ 45 min	ischemia > 45 min	ischemia ≤ 45 min	ischemia > 45 min
Liver tissue, 5 cm from ML	0.42 ± 0.04*	0.23 ± 0.02*	0.54 ± 0.06*	0.44 ± 0.07*
Liver tissue adjacent to ML	0.23 ± 0.06*	0.11 ± 0.03*	0.45 ± 0.05*	0.31 ± 0.05*
ML	3.9 ± 0.9	4.7 ± 1.3*	8.4 ± 1.7*	15.3 ± 2.1*
Blood serum	0.71 ± 0.09	1.2 ± 0.18*	0.98 ± 0.12*	1.66 ± 0.21*
Urea	274 ± 33	378 ± 47*	364 ± 51*	488 ± 62*

Note: * $p < 0.05$ as compared to ML; * $p < 0.05$ as compared to ischemia ≤ 45 min.

Table 5. Immediate results of the surgical treatment

Variables	Total, n (%)	Ischemia < 45 min, n (%)	Ischemia ≥ 45 min, n (%)	<i>p</i>
Patients subjected to surgery	145	91 (62.7)	54 (37.3)	
Number of resected ML, median (min–max)	5 (1–19)	4 (1–17)	5 (3–19)	0.03
Bilobar ML in liver	77 (53.1)	41 (53.3)	36 (46.7)	0.34
Volume of metastatic tissue in liver (cm ³)	15.2 ± 7.3	13.6 ± 5.5	14.1 ± 8.2	0.16
Median ± st. error (min–max)	(1.0–43.2)	(3.6–43.2)	(3.9–34.2)	
Without ischemia/Classic PM/Modified PM	24/98/23	24/52/15	0/46/8	
Duration of ischemia mean ± st. error	40 ± 24.1	20 ± 15.1	55 ± 12.2	< 0.001
Perioperative hemotransfusion	24 (16.5)	13 (14.3)	11 (20.4)	0.51
Clear resection margin:				
R0	138 (95.2)	89 (97.8)	40 (74.1)	0.33
R1	2 (1.4)	0	2 (3.7)	0.12
R1vasc	5 (3.4)	2 (2.2)	3 (5.5)	0.2
Acute hepatic failure:				
A	24 (16.5)	6 (6.6)	18 (33.3)	< 0.001
B	11 (7.5)	4 (4.4)	7 (12.9)	0.16
C	2 (1.4)	0	2 (3.7)	0.09
Severe complications (≥ III grade)	14 (9.6)	4 (4.4)	10 (18.5)	0.015
Postoperative lethality	1 (0.7)	1 (1.1)	0	0.32

of microcirculatory barrier, induction of hypoxia and angiogenesis [40, 41]. According to some data, the surgical resection of MLs may be connected with the recurrent tumor growth. The surgical procedures in liver may trigger the spreading of colon cancer cells to the blood flow [42–44]. Such cells as well as the residual micrometastases that were not found intraoperatively may represent the source of the recurrence. In addition, the partial hepatectomy may be accompanied with the increased levels of SR, NO, MMP-2, -9, cytokines, growth factors and adhesion molecules facilitating metastasizing and tumor growth [17, 21, 45–48].

To sum up, the ischemic injury of liver in course of the surgical resection of metastases represents the response of cells to interrupted blood flow followed by the restoration of blood supply and oxygenation. The key factor in the genesis of reperfusion damage is non-regulated increase in the levels of SR and their metabolites — reactive oxygen species as well as increasing MMP activity. We have found out that I/R results in high activity of xanthine oxidase and monoamine oxidase that generate SR through metabolism of hypoxanthine and oxidizing deamination of biogenic amines, respectively. Therefore, in the I/R setting, mitochondria, xanthine oxidase and monoamine oxidase of hepatocytes, NOX of neutrophils and macrophages and their damaged or deficient NOS represent the major sources of SR. All the listed SR sources may be considered as challenging targets for minimization of both recurrence and oxidizing-induced dysfunctions in mCRC patients.

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