Archives of the Balkan Medical Union
vol. 54, no. 3, pp. 445-454
September 2019

ORIGINAL PAPER

8-HYDROXY-DEOXYGUANOSINE AS AN INDEPENDENT PROGNOSTIC FACTOR IN PATIENTS WITH METASTATIC COLORECTAL CANCER

Anton BURLAKA[1], Tymur RUDIUK[2]✉, Anatoly BURLAKA[3], Olena KOLESNIK[1]

[1] National Cancer Institute, Ministry of Health of Ukraine, Kyiv, Ukraine
[2] Bogomolets National Medical University, Kyiv, Ukraine
[3] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

Received 12 June 2019, Accepted 02 August 2019
https://doi.org/10.31688/ABMU.2019.54.3.07

Abstract

Introduction. Surgical injury can stimulate tumor growth in metastatic colorectal cancer (mCRC), due to mitochondrial dysfunction.

The objective of the study was to analyze the clinical significance of the molecular marker of 8-hydroxydeoxyguanosine and the oncological influence of the liver parenchyma warm ischemia on the prognosis of mCRC.

Materials and methods. The study involved 89 CRC patients with metachronous metastatic liver disease treated in The National Cancer Institute of the Ministry of Health, Kyiv, Ukraine, from 2015 till 2018, and 19 conditionally healthy donors. 8-Hydroxy-deoxyguanosine (8-OHdG) level in the urine of patients with mCRC was determined. Urine sampling was done 24-hours before and on day 3 after surgery. Measurements of the marker of oxidative DNA damage were repeated three times. The state of the hepatocyte detoxification energy system, the levels of superoxide radicals (O_2-) in the tumour tissue were determined by electron

Résumé

8-oxo-2'-désoxyguanosine (8-oxo-dg) (ou 8-hydroxy-désoxyguanosine (8-OHdG) – facteur indépendant du pronostic du déroulement de la maladie chez des patients atteints de cancer colorectal métastatique

Introduction. Un traumatisme chirurgical peut stimuler la croissance de la tumeur pendant le cancer colorectal métastatique à la suite de la formation d'un dysfonctionnement mitochondrial.

But. Nous avons analysé l`importance clinique du marqueur moléculaire de 8- hydroxydésoxyguanosine (8-OHdG) et l`influence oncologique d`ischémie chaude de parenchyme hépatique.

Matériaux et méthodes. L'étude portait sur 89 patients atteints de cancer colorectal atteints de métastase hépatique métastatique traités à l'Institut National du Cancer du Ministère de la Santé, Kiev, Ukraine, de 2015 à 2018, et 19 donneurs sous condition. Le taux de 8-hydroxy-désoxyguanosine (8-OHDGu) dans l'urine des patients atteints de mCRC a été déterminé. La collecte d'urine a été effectuée un jour avant l'opération

✉ Address for correspondence: Tymur RUDIUK
Bogomolets National Medical University, Kyiv, Ukraine
Address: Taras Shevchenko Boulevard, 13, Kyiv, Ukraine
Email: tymur.rudiuk@gmail.com

paramagnetic resonance (EPR) and Spin Trapping Technology.

Results. The urinary 8-OHdG level of donors was within 0.24±0.063 nM/kg · day, whereas before the resection and on day 3 after the R_0-resection of liver in mCRC patients it was 3.42±0.18 nM/kg·day and 2.12±0.08 nM/kg·day ($p < 0.05$), respectively. On day 1 after the liver resection with a total duration of warm ischemia period < 30 min and > 30 min have had marker at level 2.108 ± 0.13 nM/kg·day and 2.9883 ± 0.159 nM/kg·day ($p < 0.0001$), respectively. The volume of metastatic tissue (V_{mts}) significantly and directly increased the level of urine 8-OhdG. The duration of surgical intervention (resection t) and duration of warm liver ischemia (t ischemia) significantly increased urine level of 8-OhdG.

Conclusions. R_0-resection of liver metastases in mCRC patients decreases urine 8-OHdG level after surgery. Warm liver ischemia during the Pringle maneuver (≥ 40 min), surgical intervention duration (≥ 300 min) and metastatic tissue volume (≥ 12 cm^3) in liver parenchyma in mCRC patients significantly increase urine 8-OHdG levels. The level of 8-OHdG correlates with the extent of organ resection, duration of ischemia, volume of post-resection liver tissue, volume of metastases and rate of superoxide radicals' generation.

Keywords: colorectal cancer, 8-hydroxy-deoxyguanosine, superoxide radicals, liver metastases, liver resection.

Abbreviations list

mCRC – metastatic colorectal cancer
DNA – Deoxyribonucleic acid
O2- – superoxide radicals
8-OHdG – 8-Hydroxydeoxyguanosine
EPR – electron paramagnetic resonance
V_{mts} – volume of metastatic tissue
resection t – time of resection
t ischemia – duration of warm liver ischemia
I/R – ischemia-reperfusion
PM – Pringle maneuver
CTX – chemotherapy
CT – computed tomography
CRC – colorectal cancer
ALF – acute liver failure
ISGLS – International Study Group of Liver Surgery
BMI – body mass index
ETC – electron transport chain
ATP – adenosine triphosphate

et trois jours après. Les mesures du marqueur des dommages oxydatifs à l'ADN ont été répétées trois fois. On déterminait l`état énergique et celui du système de détoxification des hépatocytes, les niveaux des ions superoxydes dans le tissu tumoral par la méthode de résonance paramagnétique électronique (RPE) et par les technologies des capteurs de spin (Spin Traps).

Résultats. Le niveau de 8-OHdGu dans l`urine des donneurs était dans les limites de 0,24±0,063 n×m/kg 24 heures, alors que avant et le troisième jour après la $R_0$ résection hépatique le niveau de 8-OHdGu dans l`urine était égal à 3,42±0,18 n×m/kg 24 heures et 2,12±0,08 n×m/kg 24 heures ($p<0,05$). Les patients atteints de cancer colorectal métastatique, le 1-er jour après la résection hépatique, avec la durée totale d`ischémie chaude < 30 minutes et >30 minutes avaient le niveau de 8-OHdGu de 2,108±0,13 n×m/kg 24 heures et 2,983±0,159 n×m/kg 24 heures ($p<0,0001$). Le volume de tissu métastatique ($V_{mts}$) augmentait véritablement le niveau de 8-OHdGu dans l`urine des patients opérés. La durée de l`opération ($t_{résection}$) et le temps de l`ischémie chaude ($t_{ischémie}$) véritablement augmentaient le niveau de 8-OHdGu des patients.

Conclusions. R_0 résection de métastases hépatiques provoque la baisse du niveau de 8-OhdGu dans l`urine des malades après l`opération. La durée de l`ischémie durant la manoeuvre de Pringle (≥40 minutes), le temps de l`opération ((≥300 minutes), le volume de tissu métastatique ((≥12cm^3) dans le parenchyme de foie des patients atteints de cancer colorectal métastatique augmentent véritablement les niveaux de 8-OhdGu dans l`urine des malades. Le niveau de 8-OhdGu est en corrélation avec le volume de la résection de l`organe, la durée de l` ischémie, le volume de tissus hépatique après la résection, le volume des métastases, la vitesse de génération des ions superoxydes.

Mots-clés: le cancer colorectal, 8-hydroxy-désoxyguanosine, les ions superoxydes, les métastases dans le foie, la résection hépatique.

Introduction

In 30% of patients with colorectal cancer with metastatic lesion of liver (mCRC), during the first year after the liver resection, repeated metastatic lesions are diagnosed, and the recurrent metastatic lesion occurs since the time of first resection in 50–60% of patients[1]. Local and systemic control of the disease course in metastatic colorectal cancer (mCRC) patients is a key point of modern oncology and individualized prediction of the disease course in mCRC patients is the subject of active research. Recently, prognosis of such patients depended predominantly on clinical, radiological, pathological, and molecular criteria, but this approach did not demonstrate sufficient clinical efficacy[2].

Ischemia-reperfusion (I/R) effect in liver is usually recorded in case of large as well as advanced liver resections using Pringle maneuver (PM) and/or total blood supply blockage in organ[3]. It's proven that liver oxygenation is significantly reduced during the organ mobilization/retraction and its preparation for the latter transection of parenchyma, which leads to additional hepatocytes damage and death[4]. Liver with signs of fibrosis, steatosis due to toxic effect of chemotherapy (CTX) is much more susceptible to damage caused by I/R[5]. It was shown that damage by PM and I/R during the liver resection leads to hepatocytes dysfunction, increases the anti-inflammatory cytokines levels and enhances metastatic metalloproteinases activity in animal experiments[6]. That may stimulate the progression of micro metastases of mCRC carcinoma further[6]. Surgical trauma creates favourable conditions for the growth of uncontrolled concentrations of superoxide radicals (O2-) which in turn creates a new redox state in surgery-treated organ and organism in general, which is a key factor in local relapse and distant metastases after the tumour resection performed[7].

Despite the fact that this is considered to be the main factor in the pathogenesis of an increasing list of clinical conditions, the role of oxidative-induced DNA damage in ischemia in the operated liver remains undetermined. In particular, in the study by Yamashita et al, an independent predictor of oncological prognosis for patients with mCRC the degree of ischemia that arose as a result of a blood supply violation in liver parenchyma during the first 30 days after the resection (liver stroke) was assumed[3]. The authors observed a direct-proportional dependence between non-recurrent survival rate and stroke severity in resection area according to CT data. This approach and scale of ischemia described by these authors as an evidence proving that level of liver ischemia after the surgery can be a significant predictive factor of mCRC patients survival rate since the time of surgical intervention. These findings were confirmed by our results, as well.

It is known that O2- causes DNA oxidation leading to formation of a number of oxidative bases and nucleotides in DNA. Such lesions become a cause of mutations and carcinogenesis initiation in healthy people or in malignant neoplasms progression, including those with CRC. 8-oxo-2'-deoxyguanosine (8-oxodGu) is the most frequently reported form of oxidative bases in DNA or in a nuclear pool. It has dangerous effects because of capacity to form pairs with adenine and/or cytosine in DNA molecule.

8-oxoguanine (8-oxoG) is able to accumulate in both nuclear and mitochondrial DNA. That is why the latter considered a highly informative marker of malignant neoplasms formation.

The aim of our study is to evaluate the clinical significance of 8-oxodGu marker, to assess the oncological effects of warm ischemia of liver parenchyma on disease prognosis in patients with mCRC and to determine the correlation relationship between the latter and the marker under study.

Table 1. Characteristics of participating patients.

Characteristics	*Value*
Age	61.4 ±2.3
Body mass index	26.5 ± 5.3
Sex (male/female)	36/33
Metastatic liver injury (synchronous/metachronous)	14/55
Primary tumour location (rectum/colon)	35/54
Metastatic injury of other sites at the moment of liver resection (lungs/abdomen cavity)	4/5
Number of resected metastases	3.41 ± 1.3 (4 – 7)
Volume of metastatic tissue in liver, cm^3	9.2 ± 7.3 (1.0 – 43.2)
Type of liver resection (major/minor)	55/34
Neoadjuvant CTX (yes/no)	27/62
Patient condition (ASA scale):	
$G_{1,2}$ tumors	56
$G_{3,4}$ tumors	33
Warm ischemia duration in time of the PM, min	19.7 ± 20.5
Liver parenchyma transection duration, min	228.9 ± 118.2
Pringle maneuver applied (yes/no)	61/28

Material and methods

The study involved 89 CRC patients with metachronous metastatic liver disease ($pT_{1-4}N_{0-2}M_{0-1}$ colon cancer and $pT_{1-3}N_{0-2}M_{0-1}$ rectal cancer) treated in National Cancer Institute of the Ministry of Health, Kyiv, Ukraine, from 2015 till 2018, and 19 conditionally healthy donors. 46 men and 43 women were included in the study. The average age of patients was 61.4±2.3 years. 38 patients were diagnosed with rectal cancer, while 51 — with colon cancer. Based on the degree of tumour differentiation, the patients were distributed as follows: G_{1-2} tumors found in 33 patients, G_{3-4} tumors — in 56 patients .

In each clinical case, a multidisciplinary approach was used, where surgeons, oncologists, chemotherapists and radiologists took part. In all cases, the diagnosis of metastatic liver damage was confirmed using cytology/histology techniques and fine-focal biopsy of the pathological liver sites. Routine computed tomography (CT) scan with intravenous contrast of thoracic, abdominal and pelvic cavity was applied; in complicated cases (suspicion of canceromatosis or bilobate lesions), the examination was supplemented by magnetic resonance imaging with intravenous contrast. Positron-emission tomography was used only in case of metastatic involvement of other organs/sites.

The surgical technique included the implementation of radical resection with the maximum possible preservation of parenchyma and ensuring adequate retreat from the tumour edge (4–10 mm). Each operation was accompanied by intraoperative ultrasound examination in order to mark sites in relation to major hepatic veins and Glissonian pedicles and also to detect small metastases. Major liver resections referred to removal of 3 or more liver segments (class Couinaud). The technique of ischemia assumed the use of classic and selective PM (15 min — ischemia, and 5 min — reperfusion)[8]. The parenchyma transection was performed using the „crash-clamp" method. Resected parenchyma haemostasis was performed by prolene 4.0, 5.0 flashing, bipolar forceps, clipping of LT200, LT300.

The patients received adjuvant CTX according to international standards, FOLFOX-6/FOLFIRI/XELOX (4–6 courses). Patients treated with CTX and with signs of disease progression did not undergo liver resection. The liver functional capacity was assessed using Child – Turcotte – Pugh score and MELD score. The manifestations of CTX toxicity were documented according to STCAE 5.0 criteria. The degree of acute liver failure (ALF) in the postoperative period was determined by the International Study Group of Liver Surgery (ISGLS) classification.

Urine sampling was done 24-hours before and on day 3 after the surgery. Then, 20 mL of 24-hour volume test urine filtered through a solid-phase extraction column. Measurement of 8-OHdG level in eluate was carried out using spectrophotometric method[9]. The energy system and hepatocyte detoxification system state, O2- generation speed in tumour tissue, hepatocyte mitochondria and tissue-associated neutrophils was determined using method of electron paramagnetic resonance (EPR) and technology of spin traps (SpinTraps) at room temperature. Measurements were carried out on a computerized EPR spectrometer RE-1307. EPR spectra were recorded at liquid nitrogen temperature (–196 °C) in a paramagnetically pure quartz dewar on a computerized spectrometer PE-1307 with resonator H_{011}. Following parameters were used: power of microwave source level 40 mW, modulation frequency 100 kHz, amplitude 10 Gaussian, the receiver's constant time $\tau = 0.3$ s. As a standard of intensity a specially oriented sample of a single crystal Al_2O_3 with specific concentration of Cr^{3+} ions were used. Method of double integration evaluated the concentration of molecules by comparing the signals intensity in EPR spectra to standard intensity. The error of spectrum integration method and spread of spectrum reproduction of one sample was within 3%.

Generation by mitochondria in surgical tissue samples of tumors was determined by electron paramagnetic resonance (EPR) method with computerized spectrometer RE-1307 using a spintrap TEMPONE-H (2,2,6,6, –tetrametyl-4 oxypiperidine) (Sigma, USA) at the room temperature in a special paramagnetic pure quartz cuvette[10].

Statistical Methods. Statistical analysis of the results was performed using IBM SPSS Statistics (Version 20.0, Armonk, NY, USA). T-test was used to evaluate the difference of urine 8-OHdG between donors and mCRC patients. Concentration on urine 8-OHdG at different disease stages was compared using One-way ANOVA test. Logistic regression model designed to determine the relation between the urine 8-OHdG concentrations in patients with CRC and other factors i.e. body mass index, presence of ischemic liver tissue, presence of distant metastases, volume of metastatic tissue in liver, stage of process, age, sex, degree of tumour differentiation. Statistical tests were two-sided and statistical significance was assumed when $p < 0,05$. Patient's survival was analysed by the Kaplan–Meier estimating method. Results are presented as M±m.

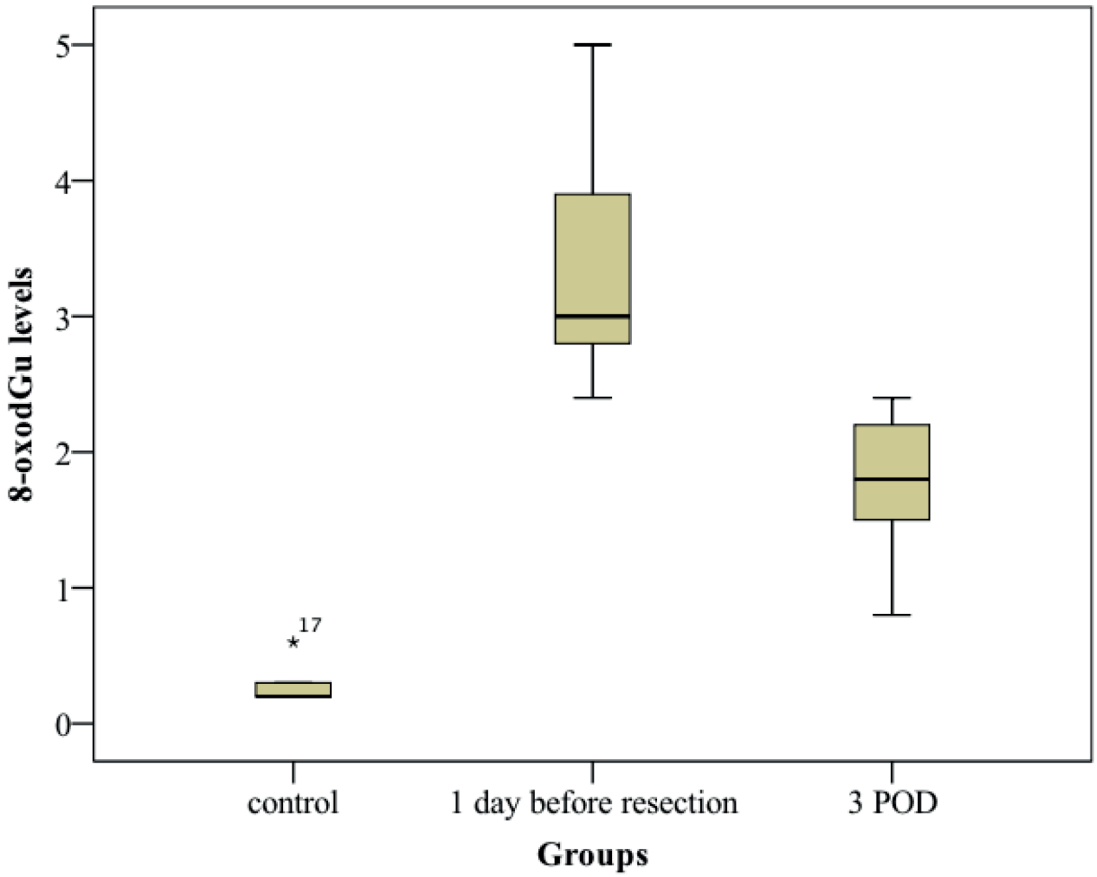

Fig. 1. Urine 8-OHdG levels (nM/kg · day) in patients' groups: normal — conditionally healthy donors (n = 17); „day 1 before resection (n = 30)/day 3 after the liver resection" (n = 30) in mCRC patients.

Results and discussion

The main reason for the development of mCRC in the intestinal mucosa is considered to be the mutation process in the genes controlling the cell cycle (proliferation, differentiation, adhesion and apoptosis)[7,11]. Oxidative damage of nuclear and mitochondrial DNA, hypermethylation of gene promoters are major events in all stages of carcinogenesis. The prolonged exposure of intestinal mucosa to O2- initiates chronic inflammation and dysplasia. Disorders caused by O2- include oxidative-induced mutations in genome, its functional instability and consequently cell proliferation dysregulation[12-14]. The integral quantitative analysis of urine 8-OHdG provides an opportunity to non-invasive evaluation of the degree of DNA oxidation and redox condition of tissue after surgery.

We have analysed the urine 8-OHdG levels in mCRC patients before and after removal of liver metachronous metastases (Fig. 1). The obtained data and their analysis showed that metastatic tissue removal leads to normalization of oxidative DNA damage. Thus, the average level of the marker in donors' urine is 0.244±0.063 nM/kg · day, whereas on day 3 after the R_0-resection of liver due to its metastatic lesion, it was 3.42±0.18 and 2.12±0.08 nM/kg·day ($p < 0.05$).

Fig. 2 shows the results of urine 8-OHdG levels in healthy donors and mCRC patients depending on the warm liver ischemia duration during parenchyma transection. Thus, the mean value of urine 8-OHdG in healthy donors is 0.244±0.063 nM/kg · day. In patients with mCRC on 1 day after the liver resection due to its metastatic lesion with a total duration of warm ischemia < 30 min and > 30 min was 2.108±0.13 nM/kg·day and 2.9883±0.159 nM/kg·day ($p < 0.0001$), respectively. Obviously, the urine level of 8-OHdG is significantly increased in patients with mCRC and depends on warm ischemia duration caused by PM (Fig. 2).

We have studied a number of factors that could negatively affect the level of oxidative DNA damage and subsequently the oncologic prognosis in surgically operated patients (Table 2). A statistically significant effect was registered only with volume of metastatic tissue in liver (p=0.037), duration of warm

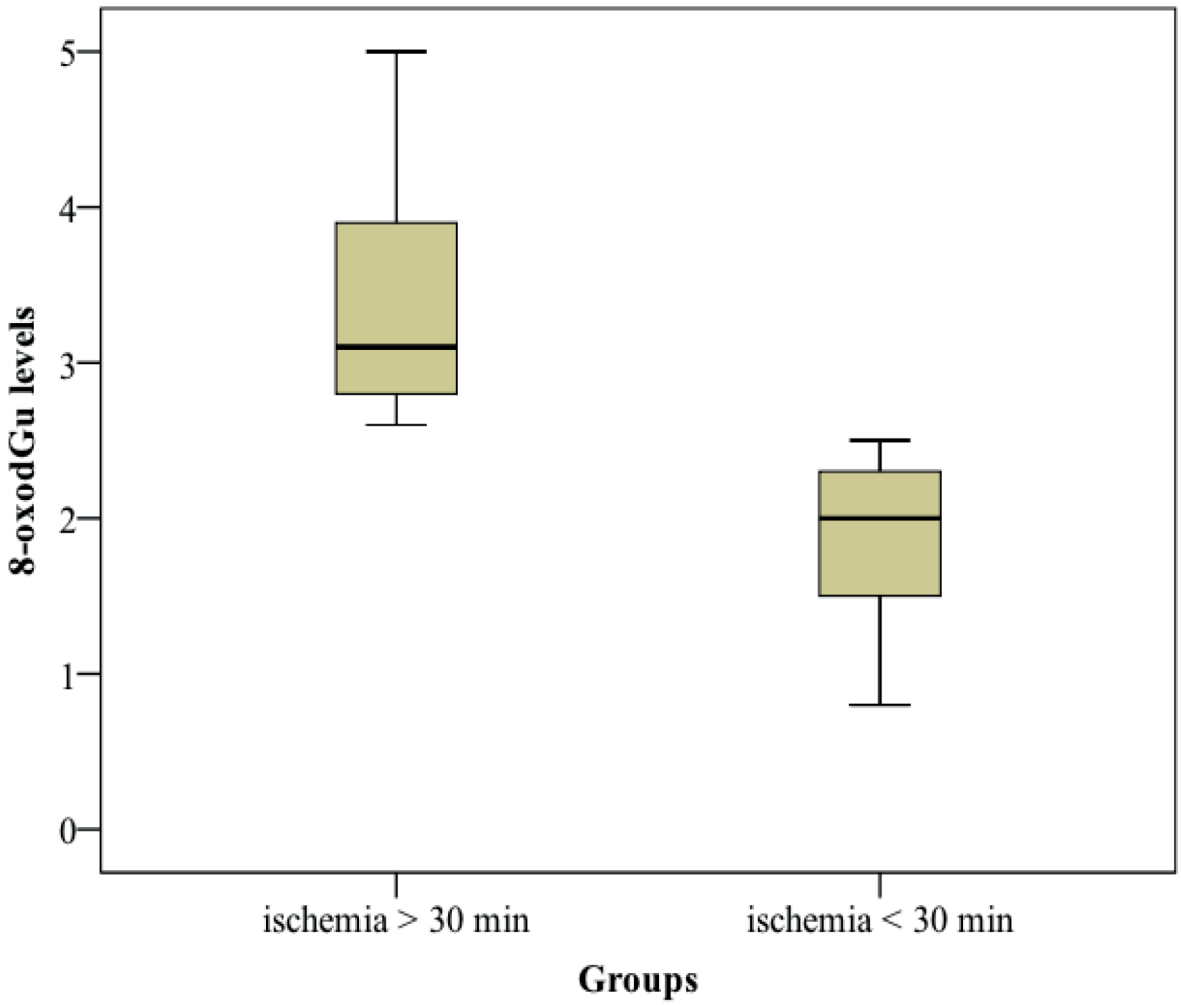

Fig. 2. The effect of warm ischemia duration on the 8-OHdG level in the urine of the studied patients: ischemia < 30 min in mCRC patients (on day 3 after R_0-resection of liver) whom PM applied with a total duration of warm ischemia < 30 min; ischemia > 30 min in mCRC patients (on day 3 after R_0-resection of liver) whom PM applied with a total duration of warm ischemia > 30 min.

ischemia during the liver parenchyma transection (p = 0.001) and duration of surgery (p = 0.006).

Statistical analysis of studied factor dependence on oxidative DNA damage (8-oxodGu) level showed that duration of liver parenchyma transection, warm ischemia duration using the Pringle maneuver and volume of metastatic tissue in liver are independent factors that negatively affect the level of studied marker (Fig. 3). In particular, the volume of metastatic tissue (V_{mts}) significantly and proportionally increases the level of urine 8-oxodGu in surgery-treated patients (R^2 = 0.54; 95% CI 0.037–0.091 at p < 0.000) (Fig. 3a). Surgery time ($t_{resection}$) and duration of warm ischemia during the surgical manipulations ($t_{ischemia}$) significantly increased the level of urine 8-oxodGu in surgery-treated patients (R^2 = 0.54; 95% CI 0.001–0.004 at p < 0.001).

Linear regression model equation

We have created a linear regression equation for 8-oxodGu marker levels = $1.253 + 0.483 \bullet V_{mts} + 0.342 \bullet t_{resection} + 0.352 \bullet t_{ischemia}$

Using EPR method under conditions of low temperature stabilization (T77 K) the study of ischemic liver tissue samples and liver tissue samples dissected at a distance of 5 cm from the tumour node (postoperative material) was done. In EPR spectra of healthy liver tissue following spectra were detected: EPR signals g=1.94, which characterize the state of mitochondrial electron transport chain (ETC) (iron-sulphur proteins of Complex I, NADN-dehydrogenase 1a); EPR signal g=2.00 – level of flavo-, ubisemiquinone, a main electron transfer in ETC; EPR signal g=2.03 – level of NOFeS-protein complexes; EPR signal g=2.25 and g=2.42 – activity level of cytochrome P-450 (CYP) in redox cycle of hepatocyte detoxification system;

Table 2. Results of the linear logistic regression analysis of the studied factors in mCRC patients.

Model	Non-standard coefficients		Standard coefficients	t	Significance	95.0% CI lower limit
	B	Standard error	Beta			
Constant	1.235	0.172		7.167	0.000	0.891
V_{mts}	0.064	0.013	0.486	4.738	0.000	0.037
$t_{resection}$	0.003	0.001	0.345	3.369	0.001	0.001
Age	0.023	-0.034	0.071	0.042	0.930	1.076
T	0.443	0.300	0.150	0.089	0.659	1.517
N	0.638	0.414	0.111	0.066	0.461	2.170
G	-0.071	-0.373	-0.323	-0.201	0.834	1.199
PM	0.786	0.453	0.308	0.190	0.721	1.387
BMI	0.016	-0.020	-0.121	-0.072	0.900	1.111
$t_{ischemia}$	0.017	0.005	0.352	3.277	0.002	0.006

BMI – body mass index; Pringle maneuver (PM) – yes/no; G – degree of tumour differentiation; N – status of regional lymph nodes; T – status of primary tumour; $t_{ischemia}$ – duration of warm ischemia during the transection of liver parenchyma (min); $t_{resection}$ –duration of the surgery on the liver, min; V_{mts} – volume of metastatic tissue based on CT-volumetry data, cm^3.

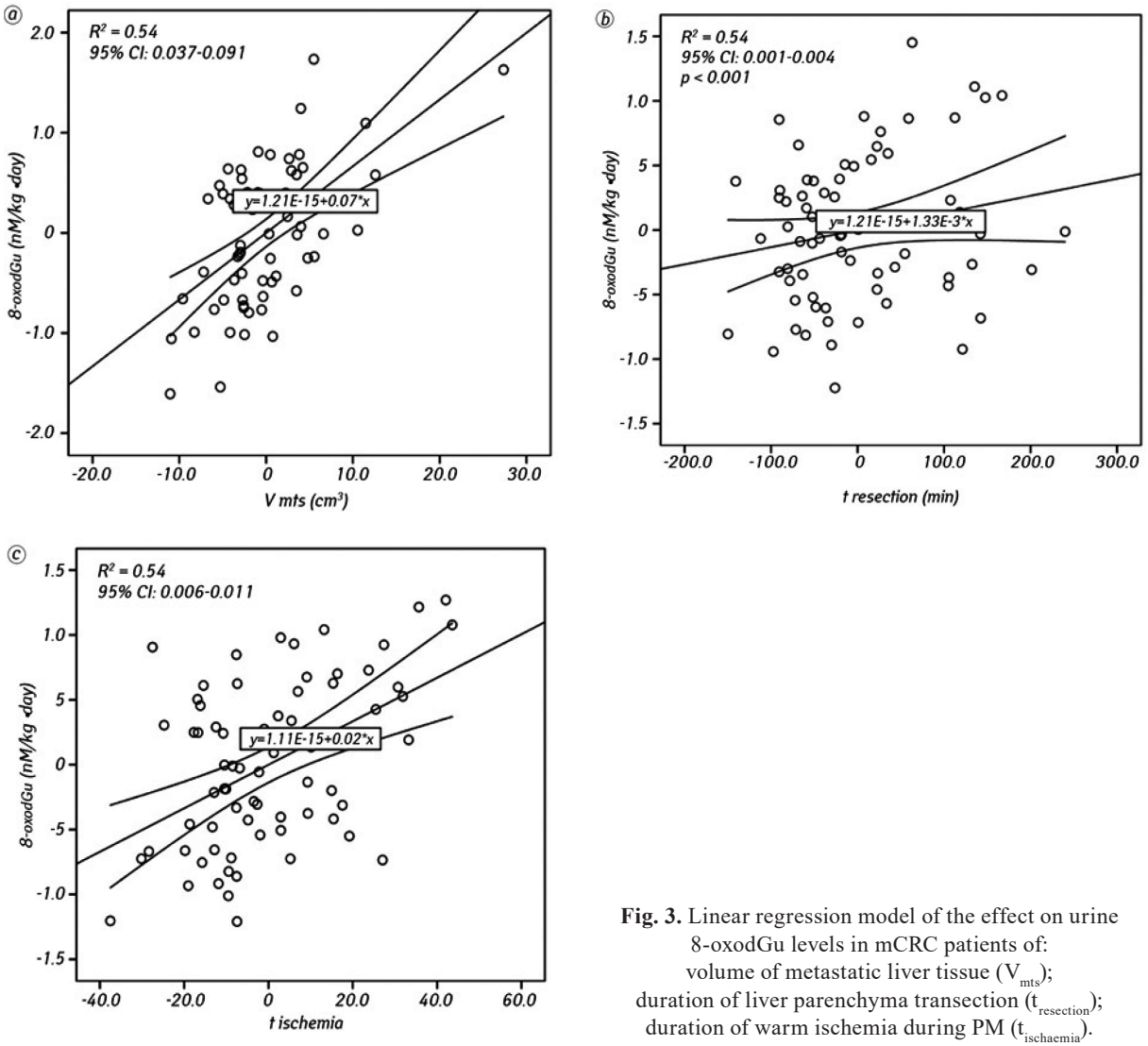

Fig. 3. Linear regression model of the effect on urine 8-oxodGu levels in mCRC patients of: volume of metastatic liver tissue (V_{mts}); duration of liver parenchyma transection ($t_{resection}$); duration of warm ischemia during PM ($t_{ischaemia}$).

ERPR signal g=2.65 – free iron level; EPR signal with g=4.25 – level of lactoferrin, transferrin functioning in oxidative metabolism in mitochondria (Fig. 4). Qualitative and quantitative changes in energy and detoxification systems of ischemic liver tissue mitochondria were detected. In conditionally healthy liver tissue at a distance of 5 cm from the metastasis, the value of cytochrome P-450 activity was detected at level 0.44±0.08 a.u. while in healthy tissue – 1.48±0.13 a.u. In ischemic tissue activity of cytochrome P-450 was detected at level 0.11±0.07 a.u., which is an evidence of significant decrease of detoxification system effectiveness (Fig. 4). In mitochondria of these cells reduced activity of Complex 1 (NADH-ubiquinone oxidoreductase) to value 0.31±0.06 a.u. was detected, while in control group 1.51±0.10 a.u. and 0.59±0.09 a.u. in conditionally healthy liver tissue. Decreased activity of the electron transport complexes in ETC occurs due to NO complexes with FeS proteins (g=2.03) formation, which levels are increased; and this refers to formation of triplet structure in EPR, which is typical for mitochondria of malignant tumors (Fig. 4, ESR spectrum 2). In liver tissue at a distance of 5 cm from the metastatic node, complexes NO-FeS-proteins in ETC detected at level 0.25±0.07 a.u. These changes in hepatocytes ETC functioning in ischemic liver tissue cause an increase in mitochondrial O2- generation rate up to 0.81±0.09 nM/g tissue · min. Mitochondria of conditionally healthy liver tissue generate O2- at level 0.73±0.07 nM/g tissue · min. Ischemic liver cells generate O2- at level 1.42±0.15 nM/g tissue · min, conditionally healthy tissue cells produce O2- at level 0.96±0.12 nM/g tissue · min (Fig. 4). These results show the contribution of other producers of oxygen radicals, in particular NOX neutrophils, which infiltrate affected tissues. In liver tissue, under ischemia, a decrease of molybdenum-containing enzymes activity – xanthine and aldehyde oxidase (g=1.97) was found, which leads to toxic products of purines and aldehydes destruction accumulation.

Detected changes in ischemic tissue mitochondria functioning cause a violation of energy

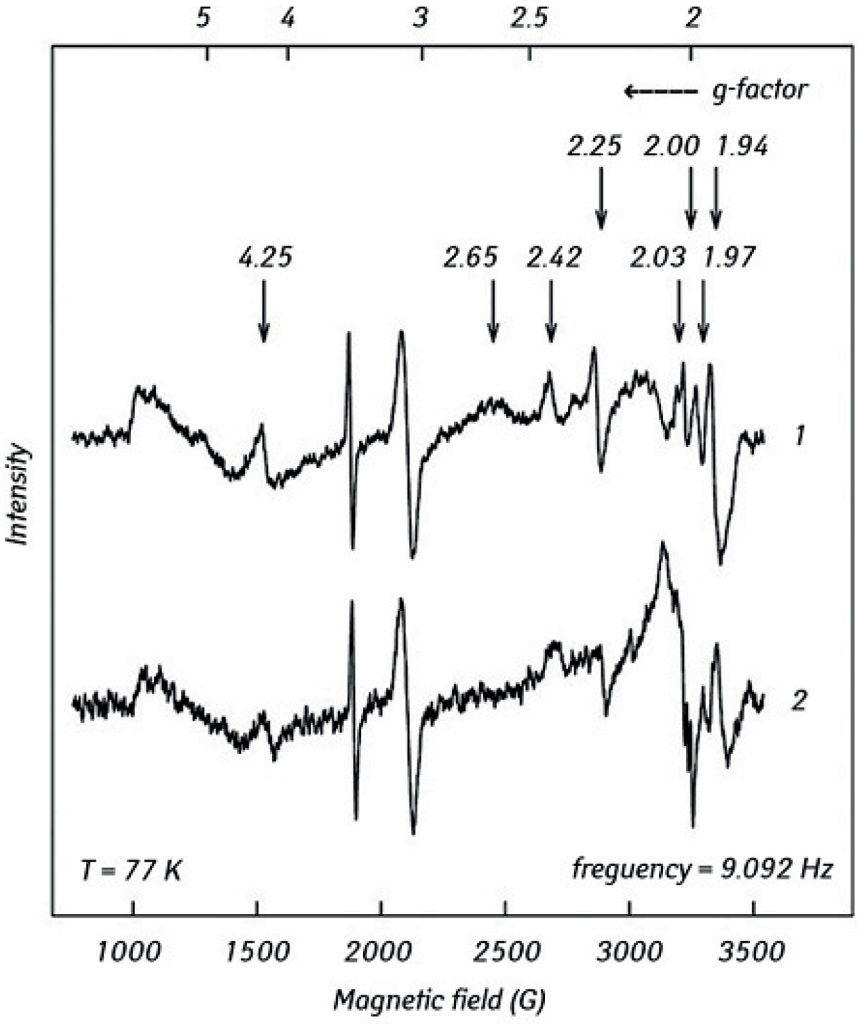

Fig. 4. ESR spectra of tissue: 1 — conditionally healthy liver; 2 — ischemic liver

metabolism (decrease ATP levels), enhanced anaerobic glucose metabolism, lactate accumulation. An ATP synthesis reduction leads to acute cellular swelling (edema) — one of the earliest manifestations of ischemic injury, decreased intracellular levels of Na^+ and increased intracellular Ca^{2+} concentration, which facilitates the activation of proapoptotic pathways[15]. Restoration the oxygen tissues supply as a result of reperfusion causes unregulated O2- generation in hepatocyte mitochondria and NOX of leukocytes and platelets, inducing the opening of mitochondrial pores and cell apoptosis[16-18].

Liver ischemia is a risk factor in case of its transplantation and resection[19,20], and the growth of O2- levels in hepatocyte and tissue mitochondria is a specific feature not only of ischemic tissue, but also of many diseases (neurodegenerative, cardiovascular, chronic inflammation)[21-23], those associated with enhanced O2- generation[20]. However, O2- is known to be a key factor in cell apoptosis induction, in particular in hepatocytes, which in turn plays an important role in ALF development after resection[24,25]. In addition, persistent oxidative stress contributes to pathological progression of liver fibrosis and increases the risk of surgery-treated organ functional failure.

Our results and also the results of other authors determine that human mCRC tumors, experimental tumor strains, tumor cell lines have significantly higher levels of 8-OHdG than normal tissues or normal cell lines[10,26-27]. The key point is that the growth and development of malignant neoplasms is accompanied with steadily increase of O2-. generation level produced as a result of mitochondrial ETC dysfunction[28-31]. Oxidative modification of DNA can lead to cytotoxic effects that are fundamental in the pathogenesis of many diseases[32,33], including neurodegenerative, cardiovascular, chronic inflammatory diseases and cancer[34-37]. 8-OHdG formation leads to transcription violations due to switch G:C to T:A. Moreover, formation of 8-OHdG may cause decreased microsatellites formation and accelerate the telomere reduction[10]. The obtained results indicate a significant increase of urine 8-OHdG levels in mCRC patients, which can be an informative biomarker for evaluation of metastatic organ damage in mCRC patients.

Conclusions

Thermal ischemia of liver during the PM (≥ 40 min), long-term surgery (≥ 300 min), and metastatic tissue volume (≥ 12 cm^3) in liver parenchyma in patients with mCRC cause damage of mitochondrial ETC (formation of NO-FeS-proteins), resulting in an increase of O2-generation rate in mitochondria and tissue in these patients. Unregulated levels of O2- oxidize guanine in DNA with 8-OHdG formation. R_0-resection of metastases in liver in mCRC patients leads to decrease of urine 8-OHdG already at day 3 after the surgery. The level of urine 8-OHdG in mCRC patients correlates with the volume of resection, duration of ischemia, volume of post-ischemic liver tissue, volume of tumour tissue in liver and the rate of O2- generation in tumour tissue and degree of tumour differentiation. Urine 8-OHdG levels are significantly higher in patients with long-term metastases as compared to those in patients who did not have metastases. Levels of urine 8-OHdG, level of intraoperative and postoperative ischemia of liver parenchyma are new factors of oncological prognosis in patients with rectal forms of mCRC that affect the liver.

Compliance with ethic requirements:

„The authors declare no conflict of interest regarding this article".

„The authors declare that all the procedures and experiments of this study comply with the ethical standards of the Declaration of Helsinki 1975, revised in 2008, as well as national legislation".

„There was no funding for this study".

References

1. Strandberg Holka P, Eriksson S, Eberhard J, et al. Significance of poor performance status after resection of colorectal liver metastases. *World J Surg Oncol* 2018;16: 3.
2. Tsai HL, Chen YT, Yeh YS, et al. Apical lymph nodes in the distant metastases and prognosis of patients with stage III colorectal cancer with adequate lymph node retrieval following FOLFOX adjuvant chemotherapy. *Pathol Oncol Res* 2019;25(3):905-913.
3. Yamashita S, Venkatesan AM, Mizuno T, et al. Remnant liver ischemia as a prognostic factor for cancer-specific survival after resection of colorectal liver metastases. *JAMA Surg* 2017;152: e172986.
4. Govaert KM, Emmink BL, Nijkamp MW, et al. Hypoxia after liver surgery imposes an aggressive cancer stem cell phenotype on residual tumour cells. *Ann Surg* 2014;259:750–9.
5. Miyashita T, Nakanuma S, Ahmed AK, et al. Ischemia reperfusion-facilitated sinusoidal endothelial cell injury in liver transplantation and the resulting impact of extravasated platelet aggregation. *Eur Surg* 2016;48: 92–8.
6. Tohme S, Kameneva MV, Yazdani HO, et al. Drag reducing polymers decrease hepatic injury and metastases after liver ischemia-reperfusion. *Oncotarget* 2017;8: 59854–66.
7. Ortega AL, Mena S, Estrela JM. Oxidative and nitrosative stress in the metastatic microenvironment. *Cancers (Basel)* 2010;2: 274–304.
8. Chouillard EK, Gumbs AA, Cherqui D. Vascular clamping in liver surgery: physiology, indications and techniques. *Ann Surg Innov Res* 2010; 4: 2.
9. Burlaka AP, Sidorik EP. Radical forms of oxygen and nitrogen oxide in the tumour process. Kyiv: Naukova Dumka, 2006. 227 p. (in Ukrainian).

10. Burlaka AP, Ganusevich II, Golotiuk VV, et al. Superoxide- and NO-dependent mechanisms of antitumor and antimetastatic effect of L-arginine hydrochloride and coenzyme Q10. *Exp Oncol* 2016; 38: 31–5.
11. Burlaka AP, Sidorik EP. Redox-dependent signal molecules in mechanisms of tumour process. Kyiv: Naukova Dumka, 2014. 255 p. (in Ukrainian).
12. Saha SK, Lee SB, Won J, et al. Correlation between oxidative stress, nutrition, and cancer initiation. *Int J Mol Sci* 2017;18:1544-57.
13. Ribeiro ML, Priolli DG, Miranda DD, et al. Analysis of oxidative DNA damage in patients with colorectal cancer. *Colorectal Cancer* 2008;7:267–72.
14. Szymczak I, Sliwińska A, Drzewoski J. DNA damage and efficacy of DNA repair in patients with type 2 diabetes and coexisting colorectal cancer. *Polskie archiwum medycynyw nętrznej*. 2014; 124: 7 – 8.
15. Ginghina O, Negrei C, Hudita A, et al. In vitro impact of some natural compounds on HT-29 colorectal adenocarcinoma cells. *Farmacia* 2017;65(6):947-953.
16. Hu H, Nan J, Sun Y, et al. Electron leak from NDUFA13 within mitochondrial complex I attenuates ischemia-reperfusion injury via dimerized STAT3. *Proc Natl Acad Sci USA* 2017; 114: 11908–13.
17. Sun K, Liu ZS, Sun Q. Role of mitochondria in cell apoptosis during hepatic ischemia-reperfusion injury and protective effect of ischemic postconditioning. *World J Gastroenterol* 2004; 10: 1934–8.
18. Vardanian AJ, Busuttil RW, Kupiec-Weglinski JW. Molecular mediators of liver ischemia and reperfusion injury: a brief review. *Mol Med* 2008; 14: 337–45.
19. Bystrom P, Foley N, Toledo-Pereyra L, Quesnelle K. Ischemic preconditioning modulates ROS to confer protection in liver ischemia and reperfusion. *EXCLI J* 2017; 16: 483–96.
20. Draghici T, Negreanu L, Bratu OG, et al. Liver abnormalities in patients with heart failure. *Arch Balk Med Union*. 2018;53(1):76-81.
21. Diaconu C, Dumitru N, Fruntelata A, Lacau S, Bartos D. Apical hypertrophic cardiomyopathy: the ace-of-spades as the disease card. *Acta Cardiologica Sinica* 2015;31(1):1:83-86.
22. Manea M, Marcu D, Pantea Stoian A, et al. Heart failure with preserved ejection fraction and atrial fibrillation: a review. *Rev Chim (Bucharest).* 2018;69(11):4180-4184.
23. Diaconu C, Balaceanu A, Morosan E. Sepsis biomarkers: past, present and future. *Farmacia* 2015;63(6):811-815.
24. Reiniers MJ, van Golen RF, van Gulik TM, et al. Reactive oxygen and nitrogen species in steatotic hepatocytes: a molecular perspective on the pathophysiology of ischemia-reperfusion injury in the fatty liver. *Antioxid Redox Signal* 2014;21:1119-1142.
25. Burlaka AP, Vovk AV, Ganusevich II, et al. Superoxide-and NO-dependent mechanisms of formation of metastatic microenvironment distant sites of metastasis in patients with colorectal cancer. *Oncology* 2017;19: 64–70 (in Ukrainian).
26. Scott TL, Rangaswamy S, Wicker CA, Izumi T. Repair of oxidative DNA damage and cancer: recent progress in DNA base excision repair. *Antioxid Redox Signal* 2014; 20:708–26.
27. Tudek B, Speina E. Oxidatively damaged DNA and its repair in colon carcinogenesis. *Mutat Res* 2012; 736: 82–92.
28. Burlaka AP, Sidorik EP, Ganusevich II, et al. Effects of radical oxygen species and NO: formation of intracellular hypoxia and activation of matrix metalloproteinases in tumour tissues. *Exp Oncol* 2006; 28: 49–53.
29. Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. *J Gastroenterol Hepatol* 2011; 26: 173–9.
30. Peiris-Pagès M, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Metastasis and oxidative stress: are antioxidants a metabolic driver of progression? *Cell Metab* 2015; 22: 956–8.
31. Diaconu CC, Arsene D, Paraschiv B, Balaceanu A, Bartos D. Hyponatremic encephalopathy as the initial sign of neuroendocrine small cell carcinoma – case report. *Acta Endocrinologica.* 2013;IX(4): 637-642.
32. Pallag A, Rosca E, Tit DM, Mutiu G, Bungau SG, Pop OL. Monitoring the effects of treatment in colon cancer cells using immunohistochemical and histoenzymatic techniques. *Romanian Journal of Morphology and Embriology* 2015;56(3):1103-1109.
33. Yeung AWK, Tzvetkov N, El-Tawil OS, Bungau SG, Abdel-Daim MM, Atanasov AG. Antioxidants: scientific literature landscape analysis. *Oxidative Medicine and Cellular Longevity* 2019;2019:1-11, ID 8278454.
34. Stagos D, Goutzourelas N, Ntontou AM, et al. Assessment of eccentric exercise-induced oxidative stress using oxidation-reduction potential markers. *Oxidative Medicine and Cellular Longevity* 2015; Article ID 204615
35. Suehiro K, Tanaka K, Matsuura T, et al. Preoperative hydroperoxide concentrations are associated with a risk of postoperative complications after cardiac surgery. *Anaesth Intensive Care* 2014; 42: 487–94.
36. Schwarz C, Fitschek F, Bar-Or D, et al. Inflammatory response and oxidative stress during liver resection. *PLoS One* 2017; 12: e0185685.
37. Sliwinska A, Kwiatkowski D, Czarny P, et al. The levels of 7,8-dihydrodeoxyguanosine (8-oxoG) and 8-oxoguanine DNA glycosylase 1 (OGG1) – potential diagnostic biomarkers of Alzheimer's disease. *J Neurol Sci* 2016; 368: 155–9.