The relationship of the redox-homeostasis and the redox-associated systems in gastro-intestinal diseases

Doctoral theses

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1. Introduction

The importance of redox homeostasis in physiological processes is becoming more and more certified in various disorders. Oxidative stress can be observed in cardiovascular and tumorous diseases (which have the highest mortality in western societies), as well as in terminal oxidation or during the processes of the immune system. Researches on the redox and redox-associated systems are important in cancer cases, especially during chemo- or radiotherapy, and in the prevention of metastases.

In spite of these, the evaluation of oxidative stress in the clinical practice is not common. Main problems: currently there are currently no internationally approved standards for the measurements, and it is disputed that the data from one tissue can characterize well another tissue.

However, as more and more studies reveal further details of antioxidants and changes in the redox homeostasis, it raises the question: Is it really negligible to examine the redox status in the clinical practice? In recent decades, our research team has made countless observations on free radicals and antioxidants that have been executed on major instruments (electron spin resonance, impulse radiolysis) as well as by simple methods. These experiences highlight the importance of the continuous and diverse research on the redox homeostasis, which can be further applied in the clinical practice and help to avoid diseases, improve diagnoses and therapy. Furthermore, the research on redox-associated systems - such as the levels of various elements or the determination of the bound formaldehyde (HCHO) levels - also seems valuable. These data make the information obtained from the measured redox parameters more accurate.

2. Aims

The aim of our research was to study the relationship between redox homeostasis and transmethylation, as the literature points to a close relationship between free radical reactions, nutrition factors and methylation level in the body.

During our preliminary studies about the nutritional factors, the main goal was to evaluate the differences between the redox systems and the transmethylation processes. Accordingly, the redox parameters and bound HCHO levels were measured in some dietary components which are rich in bioactive substances [wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.), beetroot (*Beta vulgaris* L. var. rubra), cabbage (*Brassica oleracea* L.), and livers of poultry (*Gallus gallus domesticus* L.) and rabbit (*Oryctolagus cuniculus* var. domestica)]. At the same time, the redox parameters and associated substances were measured in processed foods, *i. e.* in watery extracts of blueberry (*Vaccinium corymbosum* L.), blackberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.), and hard candies, that contains blackberry,

pineapple (*Ananas comosus* L.), black currant (*Ribes nigrum* L.) and sour cherry (*Cerasus vulgaris* Mill.) extracts, as well as in commercial 100 % orange juice and hand-squeezed orange juice (*Citus sinensis* L.).

Studies have been carried out to determine the effects of some certain nutrition factors which can affect redox homeostasis and the related systems. Rat experiments were conducted to model obesity and alcoholism, which are well known risks of cancer. We also wondered if liposomal glycyrrhizine treatment is suitable for the prevention of alcohol related disorders.

For the human studies we developed a high performance liquid chromatographic (HPLC) method that can be used in routine clinical laboratories to determine the bound HCHO level in erythrocytes. The differences in redox and transmethylation parameters between controls and cancerous patients were analyzed. During our work, we noticed that patients treated with cancer often develop a dementia-like "chemobrain". In previous studies, element analyzes were performed in some cancerous patients during the study the redox homeostasis, hence a retrospective study could have been conducted. The aim was to determine whether the element contents of the erythrocytes were changed during chemotherapy.

3. Experiments

3.1. Preparation of plant derived samples

Extracts were made by pouring water on the lyophilized berry samples and incubating them for one day on room temperature. The concentration was adjusted to 1 g lyophilized berry sample in 10 ml water. After filtration on Whatman filter paper the aqueous extracts were diluted and measured.

In our experiments with orange samples, commercially available "Salustiana", "Navel" and "Lane late" orange juices (*Citrus sinensis* L.) and commercially available 100 % orange juice (Cappy 100 %, Happy Day, Rauch 100 %, Spar Orange 100 %, Topjoy 100%) were used.

Watery solutions from hard candies that contained blackberry (*Vaccinium myrtillum* L.), pineapple (*Ananas comosus* L.), black currant (*Ribes nigrum* L.) and sour cherry (*Cerasus vulgaris* Mill.) extracts were 10 mg/100ml.

3.2. Animal experiments

Animal experiments were performed according to the 40/2013. (II.14.) (amendment to Act XXVIII of 1998). Approval number for rat experiments: 770/004/04; XIV-I-001/229-4/2012; for broiler experiment: 22.1/613/001/2010; for rabbit experiment: 22.1/5/003/2010. The euthanasia of animals was conducted by Government Decree 40/2013 (II. 14.) on animal testing [Annex 4 ("Methods of killing animals")].

3.2.1. Experiments on animal-origin food

The broiler chickens (N = 6) (Babadi Baromfikeltető Kft., Ócsa, Hungary) receiving commercial "finishing" poultry feed were terminated on day 42 after CO_2 stunning. The exsanguinated livers were washed with isotonic NaCl solution, homogenized with Potter-Elvehjem equipment and stored at -20 ° C until measurements. Standardization was performed on the basis of the protein content, measured according to Lowry et al. (1951), and diluted to 10 mg/ml with isotonic NaCl. Standard was bovine serum albumin.

We examined 6 rabbits (Lab-Nyúl Kft., Gödöllő, Hungary) consuming commercially available rabbit food. The 4 month old animals were terminated with T61 injection (2 ml/rabbit i. p., Intervet International B.V. Boxmeer, The Netherlands). Livers were exsanguinated and treated as it has been described in the previous paragraph.

3.2.2. Rat experiments

With our rat experiments, we tried to model systems that reflect social patterns and provide relevant information on problems affecting Western societies.

For experiments on fat-rich diet we used male Wistar rats (200-250 g) (Biofarm Prompt Ltd., Gödöllő, Hungary). Control group (N = 5) consumed only standard rat chow. The strongly fat-rich diet (N = 5) was composed of 2 % cholesterol, 20 % sunflower oil (commercially available), 0,5 % cholic acid mixed to the control diet. The moderately fat-rich diet (N = 5) composed of 1 % cholesterol, 11 % sunflower oil, 0,3 % cholic acid mixed to the control diet for the third group. On day 10, termination was executed by exsanguination through the abdominal vein in deep narcosis [75 mg/kg ketamine (Calypsol 50 mg/ml solution), 7,5 mg/kg xylazine (Rompun 20 mg/ml solution)]. Livers were treated as it has been described in the previous part.

Nineteen male Harlan-Wistar rats were used (175-200 g at the beginning of the experiment). Three groups were randomly formed. Two groups (7 animals / group) were treated with continuous alcohol consumption (EC), one of them was *i. v.* treated by liposomal glycyrrhizin (ECLGT). There was a non-alcoholic control group, which contained 5 rats. After a week of acclimation period, because of a new environment, an alcohol-adaptation period was applied: ethanol was added to the animals' *ad libitum* offered drinking water, starting from 1%, and increasing in every 4 days to 2-4-6-8-10-12-14 %. The 14 % ethanol-containing water was offered for the animals for 8 weeks, and for an additional 4 weeks 20 % ethanol in drinking water was applied, *ad libitum*. Conventional dry pelleted rodent food was added *ad libitum* too. At the end of treatment period the rats were deeply anesthetized by isoflurane (4% in O_2), the chest was opened. Left lateral lobes of the livers were treated as it has been described in the previous part.

3.3. Human studies

The studies have been in accordance with ethical rights. Permissions from Medical Research Council, Scientific and Research Committee [Egyészségügyi Tudományos Tanács, Tudományos és Kutatásetikai Bizottság (ETT TUKEB)]: TUKEB 167/1997, TUKEB 15/2004, IKEB 3944/2004, TUKEB 133/2015.

Human blood samples were collected into citrate containing tubes, and were centrifuged at 2800 rpm, at 4 °C for 10 minutes. Plasma was collected to a new tube, buffy coat were removed and remnant erythrocytes were additionally diluted with isotonic NaCl solution. Plasma samples were centrifuged again and collected to another tube. Erythrocyte samples were washed in isotonic NaCl solution twice and standardized to hemoglobine concentration, measured with CHR hemoglobin D reagent, with standard methods. Erythrocyte and plasma samples were stored at -20 °C until determination.

3.4. Analysis of the samples

Ascorbic acid concentration was determined according to Pharmacopoeia Hungarica VII. (National Institute of Pharmacy, 1986). The total polyphenol content was measured according to Pharmacopoeia Hungarica VIII. (National Institute of Pharmacy, 2003). A gallic acid unit (GAU) is 1 mg gallic acid in 1 ml liquid chromatography grade water.

The H-donor ability was determined by Hatano et al. (1988) with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical. The free sulfhydryl-group concentration was measured by the method of Ellman and Lysko with 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) reagent (1967). The reducing power was measured by Oyaizu (1986). The inhibition of ascorbic acid-induced lipid peroxidation was measured by Horváth et al. (1993) with thiobarbituric acid containing reagent. Free radical scavenger capacity was measured by Blázovics et al. (1999) with a luminometric method.

Transmethylation capacity at plant and animal samples were measured with dimedone by overpressure layer chromatographic (OPLC) method. At human samples an HPLC method has been developed and validated.

For element determinations in human erythrocyte samples an inductively coupled plasma optical emission spectrometric (ICP-OES) method has been used.

Routine laboratory parameters were measured with Advia 120 or ABX Micros 60 hematological analyzer. Serum parameters were determined with kits from Roche. HbA1c was measured with Variant II HPLC system. Carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), and alpha-fetoprotein (AFP) tumor markers were measered with LIA-mAT immunoluminometric methods.

The reduced NADH dinatrium and the reduced NADPH tetra(cyclohexylammonium) salts were solved in liquid chromatography grade water and standard solutions were made. The H-donor ability was determined by Hatano et al. (1988) with DPPH radical.

For the histological examination liver sections were fixed in neutral buffered 4% formaldehyde solution, and embedded in paraffin. Hematoxylin-eosin stain was executed on 5 μm thick slices of the samples. During light-microscopic evaluation, the presence of dysfunctions was scored and statistically analyzed.

3.5. Statistical analysis

Statistical analysis was made with Microsoft Office Excel 2003 and 2013 (Microsoft Corp., Redmond, USA) and Statistica 11 and 12 (StatSoft Inc., Tulsa, USA). Significance level was 5 % except for the retrospective study, where it was 1 % because of the numerous mathematical analyses.

4. Results

4.1. Partial phytochemical analysis of plant samples

Uncontrolled intake of food-derived antioxidants may become harmful because of the "rebound" effect. Therefore, it is important to know the differences between the original sources and the processed products. Accordingly, we have measured some plants and products that are also common in our country as well.

We made squeezed juices from three orange varieties ("Salustiana", "Navel", "Lane Late") and compared to five kinds of 100 % orange juices (Cappy 100 %, Happy Day, Rauch 100%, Spar Orange 100%, Topjoy 100%). Significant difference has been only detected between the ascorbic acid contents. However, it was also demonstrated that a glass of (250 ml) from any kinds of orange juices are good sources of ascorbic acid, as it is follows from the recommended daily intake of vitamin C, proposed by OGYÉI.

Data show, that our berry extracts, especially blackberry extracts notably inhibit the lipid peroxidation, underpinned by reducing power, total polyphenol and ascorbic acid contents.

After dissolving the hard candies that contained blackberry, pineapple, black currant and sour cherry extracts, polyphenol content, H-donor activity and free radical scavenging capacity were measured. The antioxidant parameters appeared to be significant.

Among the plant-derived samples we have determined the transmethylation capacity of several plants (wheat, beans, beetroot, cabbage). We found significant differences between the samples. These differences might be affected for example by differences in agrotechnical conditions and varieties.

4.2. Analysis of the methyl-pool in animal-origin food

Continuing our measurements in plant-based samples, we investigated the H-donor activity, as a redox parameter and transmethylation capacity in animal-origin samples. We found that the more commonly used poultry liver has a higher methyl-pool and H-donor activity than the rabbit liver.

4.3. Rat experiments: Disturbances in the transmethylation processes during fat-rich diet and alcohol consumption

In our rat experiment, we wanted to examine, how the methyl-pool changes in the fatty liver due to the energy-rich (fat-rich) diet, which can be noticed in the western diet. It has been observed that the redox parameter (H-donor activity) was only slightly changed ($p = 0{,}166$). In the moderately fat-rich diet, a mild increase was observed compared to the control values, while in the strongly fat-rich diet a slight, statistically non-significant decrease was observed. The slight increase could be attributed to the role of NADH and NADPH cofactors. To prove the supposed effect of NADH and NADPH, their salts were also tested in the same system. The two reducing coefficient showed dose-dependent H-donor ability, because the regression was significant and the coefficient of determination was high ($r^2(\text{NADH}) = 0{,}988$; $p(\text{NADH}) < 0{,}001$; $r^2(\text{NADPH}) = 0{,}999$; $p(\text{NADPH}) < 0{,}001$). The bound HCHO levels in fatty liver were significantly decreased ($p_{HCHO} = 0{,}002$). Statistically significant differences have been found between the three groups ($p_{\text{control/moderately fat-rich}} = 0{,}004$; $p_{\text{moderately fat-rich / strongly fat-rich}} = 0{,}029$).

In our next study, alcohol consumption and liposomal glycyrrhizin treatment were investigated. The effect of the glycyrrhizin treatment in the presence of liver injury were not significant ($p=1{,}00$). The H-donor ability in the liver was enhanced in the EC group, which effect could be in agreement with the high H-donor ability of the reducing equivalents, as it has been proven in the previous paragraph but the difference was not significant between the groups ($p=0{,}415$). Alcohol consumption lowered the free sulfhydryl-group ($p = 0{,}355$) and the bound HCHO concentration ($p = 0{,}002$; $p_{\text{EC/control}} = 0{,}001$) in the rat livers. Glycyrrhizin treatment showed mild but beneficial effects on the concentration of the free sulfhydryl-group and the transmethylating ability. It should be also noticed, that the significant difference between the control group and the ECLGT group in the bound formaldehyde content ($p_{\text{ECLGT/control}} = 0{,}073$) has diminished.

4.4. Determination of bound HCHO-levels in erythrocyte samples

The OPLC method was a fast and accurate determination method, but HPLC technique is more easily available. The chromatographic analysis was performed using a JASCO (Tokyo, Japan) HPLC system, equipped with a PU-980 pump, an LG-980-02 solvent mixer, a PU-975

diode-array UV detector and an ERC-3113 degasser. HPLC column was a Kinetex C18 column (250*4,6 mm; 5 μm particle size) from Phenomenex (Torrance, CA, USA). Mobile phase A was 0,2% (v/v) acetic acid in water and mobile phase B was methanol. The mobile phase consisted of A/B 20/80 (v/v) at a flow rate of 0,7 mL/min. Measurements were carried out at 25 °C. Injection volume was 20 μl. Chromatograms were registered at λ = 260 nm. The 0,4 ml erythrocyte sample (5,0 g/100ml hemoglobin), or the HCHO samples (0,583; 1,167; 2,042; 2,917; 4,025 mg/100ml HCHO) were diluted with 1 ml methanol and mixed with 0,1 ml 0,07% dimedone solution. After six days at ambient temperature (24 °C), samples were centrifuged (2800 rpm, 4 °C for 10 min), and supernatant was filtered with 0,2 μm pore size Phenex RC membrane filters (Phenomenex Inc.; Torrance, CA, USA). The method was validated in accordance with the ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guideline Q2(R1) and the guideline of the FDA (Food and Drug Administration). Results show that our method was appropriate to determine bound formaldehyde in erythrocyte samples. The relationship between detector response and formaldemethone concentrations were linear between 0,583-4,025 mg/100ml HCHO, but this range may be extendable if it is necessary. Therefore standard curve was constructed with linear regression. Determination coefficient (r^2) was higher than 0,99. Data from the intraday and interday precision and accuracy showed that the method is reproducible and accurate. Stability has been proved in five hours. Specificity has been proved with methanol and with adequately prepared blood sample without dimedone reagent. The latter study showed that our method can be an easy and inexpensive way to determine erythrocyte bound HCHO concentration, with the avoidance of airborne formaldehyde.

4.5. Human studies

In our human studies, we wanted to study the relationship between the redox homeostasis, the transmethylation and the element levels in cancerous patients.

In the first study, 25 Caucasian individuals and patients from the Semmelweis University Oncology Center were compared. Colectomysed patients (N = 12, mean age ± standard deviation = 59,8 ± 11,2) were operated within 8 months prior to the study. Age-matched control group was 13 (mean age ± standard deviation = 50,8 ± 11,7). There were just a few significant differences in the routine laboratory parameters between patients and controls. The mean corpuscular volume, percentage of lymphocytes, percentage of monocytes were statistically different ($p < 0,05$) between the two groups. In addition, the number of lymphocytes was lower in tumorous patients, while the number of granulocytes showed higher values. Significantly higher platelet counts and tendentiously lower mean platelet volume were observed in tumorous patients.

The improvements in the observed values of the redox homeostasis and the non-significant increase in transmethylation capacity raised additional questions. To resolve anomalies due to unexpected results, a short, recorded interview were executed about the dietary habits of colectomized people. In the studied group, 58 % of patients changed their diet at least 1 month prior to the first chemotherapy treatment, and another 83 % changed their diet at the first day of chemotherapy, when the samples were collected. They usually started to consume a more fruits and vegetables, and the consumption of beetroot once or twice a week had become characteristic. Both these changes can improve redox homeostasis and transmethylation capacity. All in all the role of diet and healthy food in redox homeostasis and transmethylation have been underpinned.

Improved or stagnant values in the redox homeostasis and in the transmethylation capacity raise the question of how the metallic and non-metallic element levels can be changed in colectomysed patients, because they are in a close relation to the redox homeostasis. Our retrospective study was made with the data of 49 Caucasian patients. Colectomysed cancerous patients (N = 27; mean age ± standard deviation = 63,9 ± 8,0), operated within the last 3 years, were involved into the study. Patients of the control group (N = 22; mean age ± standard deviation = 46,8 ± 13,8) were outpatients. Exclusion criteria for outpatients were colorectal malignancy and inflammatory bowel diseases. Additionally 10 healthy volunteers (mean age ± standard deviation = 55,3 ± 14,9) from both genders were included in the study to compare the element contents in erythrocytes of both colectomysed and outpatient groups, because only scarce data was found as reference intervals for the measured elements. The levels of elements were tested in washed erythrocytes, considering that the lifespan of erythrocytes is greater than 3 months, so it can characterize a longer period, than the relatively variable blood plasma (or serum). Cu, Fe, Zn are the most typical metals of the redox system. The highest mean values of these three elements in erythrocytes were recorded in the colectomysed patients. Furthermore mean Al concentration was the highest in colectomysed patients and a marked difference was detected between colectomysed patients and healthy controls ($p < 0,01$). The measured routine laboratory parameters showed no significant differences, except for corpuscular haemoglobin concentration, red cell distribution width and alkaline phosphatase ($p < 0,01$). There were no significant differences between tumour markers of colectomysed patients and outpatients. Most data were in the normal range in general. All in all, marked changes in the levels of metal elements could already be observed while the routine laboratory parameters were just slightly altered. Furthermore, in the background of these alterations could have positive or negative events.

It can be also supposed, that element contents were elevated in the background of our prospective human study like in this retrospective study that can be the consequent of a "healthier" diet, rich in trace elements and antioxidants. Based on these observations, studies are ongoing to clarify the effect of nutritional factors.

7. Theses

1. A close relationship can be demonstrable between redox homeostasis, transmethylation ability and element homeostasis.
2. Significant differences can be found in the antioxidant parameters and transmethylation ability of physiologically relevant plant derived and animal-origin nutritional factors.
3. In a rat experiment it could be demonstrated that the levels of methyl pool was significantly reduced after a long term alcohol consumption.
4. In a rat experiment the antioxidant parameter (H donating ability) was just slightly changed in mild fatty liver as well as in severe fatty liver, while the methyl pool was significantly and stepwise reduced after the two kind of fat-rich diet.
5. In a retrospective study, it could be demonstrated that the metal ion homeostasis of colectomysed, chemotherapy treated patients differ significantly from healthy controls.
6. The significantly higher levels of erythrocyte aluminum content in colectomysed, chemotherapy treated patients can play a role in oxidative stress related disorders, for example in the redox-associated neurological disorder: “chemobrain”.
7. We have developed an HPLC method to study the transmethylation processes in the human erythrocytes, which allows us to follow the status of the disease, the changes in their nutritional status, and the consumption of dietary supplements.

8. List of own publications

8.1. Publications related to the dissertation

1. Blázovics A, Kursinszki L, Papp N, Kleiner D, Szőke É, Hegyi G, Szilvás Á. (2016) Is professional prescribing of a commercially derived dietary supplement in colectomysed patients necessary? Eur J Integr Med, 3: 219-226. **[IF (2015).: 0,769]**
2. Czigány Z, Turóczi Zs, Kleiner D, Lotz G, Homeyer A, Harsányi L, Szijártó A. (2015) Neural elements behind the hepatoprotection of remote perconditioning. J Surg Res, 193: 642-651. **[IF (2015).: 2,198]**
3. Ditrói K, **Kleiner D**, Böszörményi A, Szentmihályi K, Fébel H. (2013) The alimentary impact of the hemp seed. Acta Aliment, 42: 410-416. **[IF (2013).: 0,427]**
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5. **Kleiner D**. Alkoholos májkárosodás és transzmetilezés. In: Blázovics A. Rőth E. Mézes M. (szerk.), Oxidatív stressz és betegségek. Szent István Egyetemi Kiadó Nonprofit Kft. Gödöllő. 2015: 141-147.
6. **Kleiner D**, Bersényi A, Fébel H, Hegedűs V, Mátis E, Sárdi E. (2013) Transzmetilezési folyamatok és a redox homeosztázis. Orv Hetil, 154: 1180-1187.
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8. **Kleiner D**, Hegyi G. Urbanics R, Dézsi L, Robotka H, Fehér E, Sárdi É, Szebeni J, Blázovics A. (2016a) Hepatoprotective liposomal glycyrrhizin in alcoholic liver injury. Eur J Integr Med, 8: 23-28. **[IF (2015).: 0,769]**
9. **Kleiner D**, Kurucz D, Bersényi A, Szentmihályi K, Skesters A, Liga Z, Blázovics A. (2016) Berries, the antioxidant sources of the boreal cold and arid regions. Acta Aliment, 45: 317–322. **[IF (2015).: 0,333]**
10. **Kleiner D**, Mátis E, Süle K, Molnár J (2015a) A szelén élettani szerepe és jelentősége. Gyógyszerészet. 58: 148-153.
11. **Kleiner D**, Sárdi É, Ficsor E, Balázs A, Lemberkovics É, Blázovics A. A redox-homeosztázis és a transzmetilezés kapcsolata táplálkozás-élettani szempontból. Aktualitások a táplálkozástudományi kutatásokban, workshop. Megjelent: Aktualitások a táplálkozástudományi kutatásokban című workshop össszefoglalói. Budapest, 2014: 6. (ISBN 978-963-88108-7-8)
12. **Kleiner D**, Süle K, Windisch V, Szabó G, Blázovics A. Citrus sinensis levének redox-paraméterei frissen facsart és gyárilag feldolgozott mintáiban. Aktualitások a

táplálkozástudományi kutatásokban, Ph.D. konferencia. Megjelent: Aktualitások a táplálkozástudományi kutatásokban című V. PhD Konferencia összefoglalói. Budapest, 2015b: 26. (ISBN 978-963-88108-8-5)

13. **Kleiner D**, Szilvás Á, Szentmihályi K, Süle K, Blázovics A. Fémelem-akkumuláció kemoterápia indukált kognitív funkcióromlás esetén. A Magyar Szabadgyök-Kutató Társaság VIII. Kongresszusa. Budapest, 2015c.
14. **Kleiner D**, Szilvás Á, Szentmihályi K, Süle K, Blázovics A. (2016) Changes of erythrocyte element status of colectomysed cancerous patients: Retrospective study. J Trace Elem Med Biol, 33: 8-13. **[IF (2015).: 2,55]**
15. Skesters A, **Kleiner D**, Blázovics A, May Z, Kurucz D, Silova A, Szentmihályi K. (2014) Mineral element content and antioxidant capacity of some Latvian berries. Eur Chem Bull, 3: 98-101.
16. Tóth G, Sándor GL, **Kleiner D**, Szentmáry N, Kiss H, Blázovics A, Nagy ZZs. (2016) Szabadgyök-felszabadulás vizsgálata femtoszekundumos lézerrel asszisztált capsulotomiát követően. Orv Hetil, 157: 1880–1883. **[IF (2015).: 0,291]**

8.2. Publications not related to the dissertation

1. Kleiner D, Mátis E, Ditrói K. (2012) A kender (Cannabis sativa L.) magyar orvoslásban betöltött szerepe kábítószerré nyilvánításáig. Farmakognóziai Hírek, 7: 2-4.