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Research article

Metronidazole does not show direct antioxidant activity in *in vitro* global systems

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ABSTRACT

Metronidazole has been widely used topically and systemically for more than 50 years but data on its antioxidant properties are still incomplete, unclear and contradictory. Its antioxidant properties are primarily hypothesized based on *in vivo* results, therefore, studies have been performed to determine whether metronidazole has antioxidant activity *in vitro*. We used so-called global spectrophotometric and luminometric methods. Fe^{3+}/Fe^{2+}-reducing ability, hydrogen donor activity, hydroxyl radical scavenging property and lipid peroxidation inhibitory activity were investigated. Under the condition used, metronidazole has negligible iron-reducing ability and hydrogen donor activity. The hydroxyl radical scavenging capacity cannot be demonstrated. It acts as a pro-oxidant in the H_2O_2/˙OH-microperoxidase-luminol system, but it can inhibit the induced lipid peroxidation. According to our results, metronidazole has not shown antioxidant activity *in vitro* but can affect redox homeostasis by a ROS-independent mechanism due to its non-direct antioxidant properties.

1. Introduction

Metronidazole (MTZ, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) is a bactericidal drug that is administered systemically and topically to various protozoa (*Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis*) against a wide variety of gram-negative (Bacteroides and Fusobacterium spp.) and gram-positive anaerobic bacteria (*Peptostreptococcus* and *Clostridia* spp.). It is effective in *Helicobacter pylori* infections, Crohn's disease (CD) [1], prevention of postoperative wound infections [2], and external application e.g. in rosacea, acne skin [3, 4]. Since its introduction in 1959 [5], clinical use has been steadily increasing and is now one of the most commonly used drugs and it is included in the World Health Organisation's core list of medicines [6].

Depending on its use, the absorption, utilization and metabolism of metronidazole vary [7]. In humans, it decomposes under aerobic conditions to two major metabolites, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 1-acetic acid-2-methyl-5-nitroimidazole [8, 9].

Metronidazole is active against anaerobic organisms and converts to acetamide and N-(2-hydroxyethyl) oxamic acid [10]. Within the cell, its nitro group is reduced to hydroxylamine [11]. Several different enzymes participate in the reduction to form active metronidazole metabolites [12, 13, 14, 15] and several disorders can be observed in susceptible *Giardia*, e.g., protein disorder, DNA damage, and oxidative stress leading to cell death [16, 17]. Both metronidazole and its hydroxy metabolite are mutagenic to bacteria because they form base-pair substitutions [18, 19] and all effective against *Trichomoniasis* [20, 21].

To date, no reduced metabolites have been detected in human plasma and no DNA damage has been observed besides the application of metronidazole [22, 23]. Metronidazole is not considered carcinogenic, although some studies have found it may increase the risk of lung cancer [24, 25].

In vivo studies have suggested its antioxidant and free radical scavenging properties. Due to the putative antioxidant effect of MTZ, its effect is mediated by the inactivation of inflammatory mediators released by neutrophils (e.g., reactive oxygen species, IL-8) and reduction of oxidative stress [26, 27]. In the presence of neutrophils, it inhibits the formation of hydrogen peroxide and hydroxyl radicals in a dose-dependent manner but it has no effects without neutrophils [28]. The reduction of oxidative stress has been observed in periodontitis when used as an antibiotic [29]. Metronidazole has beneficial effects in inflammatory bowel diseases (IBD) by reducing damaged proteins in the colon [30].

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The use of metronidazole alone or in combination with ciprofloxacin in the treatment of Crohn's disease (CD) has a moderate beneficial effect [31]. Since recent data support that microbiota in the gut may participate in the pathogenesis of CD and colitis ulcerosa (CU), therefore, the application of metronidazole in perianal fistula can be favourable [31]. It significantly reduced the levels of malondialdehyde (MDA) in the blood of burn victims [32].

However, several *in vivo* studies have shown that metronidazole induces oxidative stress, at the same time other studies have shown that it does not cause oxidative damage in neurons and has no effect on reactive oxygen species (ROS) production, suggesting that metronidazole induces the neuronal cell death by a ROS-independent mechanism [33]. Metronidazole is a potent anti-inflammatory agent *in vivo*, but its mechanism of action has been mainly hypothesized in the literature [34]. There are only references to its antioxidant properties *in vitro* in the literature, and there is no evidence to date. Therefore, this work aimed to investigate the *in vitro* antioxidant, free radical scavenging and lipid peroxidation inhibitory properties of metronidazole by global methods. The experimental design is shown in Figure 1.

2. Materials and methods

2.1. Materials

Metronidazole of European Pharmacopoeia quality was provided by Aarti Drugs Limited (Mumbai, India). Ferrous sulphate heptahydrate, acetic acid, hydrochloric acid, sodium acetate and perchloric acid of analytical grade was purchased from Reanal (Budapest, Hungary). 2,4,6-tripyridyl-S-triazine (TPTZ), ascorbic acid, 1,1-diphenyl-2-picrylhydrazil (DPPH), hydrogen peroxide, luminol, microperoxidase and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich Ltd. We purchased malondialdehyde (MDA) in the form of diethyl acetate from Merck Ltd.

2.2. Measurement of ferric reducing ability

Fe^{3+}/Fe^{2+}-reducibility was determined spectrophotometrically at 593 nm by the modified FRAP (ferric reducing ability of plasma or plant) method of Benzie and Strain [35]. A calibration series was prepared from an aqueous solution of $FeSO_4*7H_2O$ for calibration. Composition of FRAP reagent: 25 ml acetate buffer (pH 3.6; 3.1 g sodium acetate*$3H_2O$ and 16 ml acetic acid in 1000 ml buffer solution), 2.5 ml TPTZ solution (0.1559 g 4,6-tripyridyl-S -triazine (TPTZ) and 0.2 ml HCl in 50 ml water) and 2.5 ml $FeCl_3*6H_2O$ solution (0.2689 g $FeCl_3*6H_2O$ in 100 ml distilled water). The reaction mixture consists of 3 ml FRAP reagent and 10 µl sample solution [36].

2.3. Measurement of hydrogen donor activity

Hydrogen donor activity was determined according to the method of Blois modified by Hatano et al. [37, 38]. The DPPH compound binds hydrogen in the presence of hydrogen donor molecules, and thus its absorbance reduces. 4 ml sample solution was added to 1 ml DPPH solution (9 mg DPPH dissolved in 100 ml methanol). After thorough mixing, the reaction mixture was allowed to stand at room temperature for 30 min. The absorbance was read at 517 nm against methanol blanks. The degree of reduction (% inhibition) indicates hydrogen donor activity.

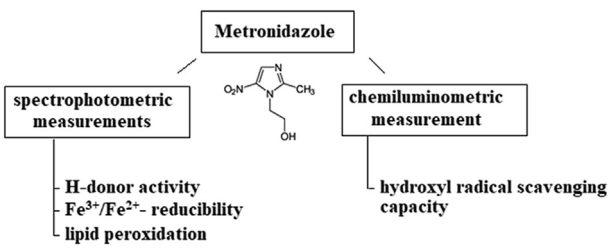

Figure 1. Protocol of global measurements of metronidazole antioxidant property.

2.4. Determination of hydroxyl radical scavenging capacity

Hydroxyl radical scavenging capacity was determined in $H_2O_2/^{\cdot}OH$-microperoxidase-luminol system by the method of Blázovics et al. [39]. The resulting radicals excite the luminol present in the reaction mixture. Upon the excited molecule's return to the ground state, inactive aminophthalic acid is formed, while monochromatic light ($\lambda = 425$ nm) is emitted. The intensity of the light emitted is proportional to the amount of free radicals present in the system. In the presence of radical scavengers, the intensity of the measurable light decreases significantly depending on the molecule's radical scavenging capacity. The intensity of chemiluminescent light is expressed as RLU (relative light unit), which is the percentage of the total light emitted during the study period relative to the background intensity. The composition of the reaction mixture was as follows: 300 µl H_2O_2 (10^{-4} M), 300 µl microperoxidase (3×10^{-7} M), 50 µl luminol (7×10^{-7} M), 50 µl sample or bidistilled water. The total volume was 850 µl. A stock solution of 1 g/100 ml was prepared from metronidazole.

2.5. Measurement of lipid peroxidation inhibition

To determine the inhibition degree of lipid peroxidation, the method of Placer et al. was used with some modifications [40, 41]. In the acidic medium and heat-induced reaction MDA from the oxidative degradation of polyunsaturated fatty acids and thiobarbituric acid (TBA) form trimethine, which is a yellowish-red complex, which has an absorption maximum at 532–535 nm [42]. In choosing the temperature, we took into account that according to differential scanning calorimetric (DSC) and thermogravimetric (TG) studies, metronidazole melts at 160 °C and begins to decompose at 200 °C. Therefore, different amounts (0.25 ml, 0.5 ml and 1 ml) of a 1% metronidazole solution were heated in an oven at 150 °C for 10 min with 10 ml sunflower oil. Composition of TBA reagent: 20 ml perchloric acid of 70%, 120 ml deionized Millipore water, 0.75% supersaturated thiobarbituric acid solution. After centrifugation of the solution, 30 ml trichloroacetic acid (20%) was added to the supernatant TBA solution (10 ml). For the measurement, 0.5 ml sample was placed in 4.5 ml TBA reagent, covered and set in a 100 °C water bath for 30 min. After cooling, the clear solution was measured against the blank. Calibration was performed with malondialdehyde diethyl acetate. Lipid peroxidation inhibition is given by the percentage of mM MDA by the effect of metronidazole divided by mM MDA formed from sunflower oil.

2.6. Statistical calculations

Measurements were made in each case from three parallel samples, from which mean and standard deviation were calculated. One way ANOVA was performed to evaluate the significant differences using Statistica 12 (StatSoft Inc., Tulsa, USA). The level of significance was set at $p < 0.05$ in all cases.

3. Results

The Fe^{3+}-reducibility of organic compounds can be determined *in vitro* by the FRAP method. For comparison and evaluation of the result, ascorbic acid was used as a standard since, at certain concentrations and conditions, it is an antioxidant with very good reducing ability [43]. Based on the results, ascorbic acid has good reducing power (37.59 µM at 105 µg/ml), while metronidazole did not reduce Fe^{3+} to Fe^{2+} in the concentration range of 100–500 µg MTZ/ml. By increasing the concentration of the active substance to 9 910 µg/ml, metronidazole showed a

mild Fe^{3+}-reducing ability (2.42 μM) in one hundred times the highest ascorbic acid concentration studied.

The results of hydrogen donor activity indicate that metronidazole has no significant hydrogen donor activity. DPPH stable free radical takes up H in the presence of H-donor molecules. But it seems metronidazole does not have an H-donor property. At the lowest concentration (10 μg MTZ/ml), the percentage inhibition is 4.99%, and the H-donor activity does not increase proportionally with the increasing concentrations. There was a small significant increase ($p < 0.05$) at 500 μg/ml (5.87%) and after this concentration, a significant decrease ($p < 0.05$) at 2000 μg/ml (3.99%) and 10000 mg/ml (3.76%) compared to the per cent inhibition at the lowest concentration (Figure 2). Despite the significant differences compared to the inhibitions value at the lowest concentration, the measured 4–6% inhibition is negligible from in hydrogen donor activity point of view since the significant hydrogen donor activity would be close to 100%. In our case, however, DPPH free radical appears to be stabilized in the presence of metronidazole and at higher concentrations.

In the presence of a radical scavenger, in the H_2O_2/·OH-microperoxidase-luminol system, the intensity of the measurable light would be significantly reduced depending on the molecule's radical scavenging activity. However, metronidazole slightly increased the intensity of the emitted light. This shows that the metronidazole molecule does not have H_2O_2/·OH radical scavenging capacity, but excites the radicals slightly. We measured light intensities higher in the presence of metronidazole than the background intensity, which gave higher values than 100% in RLU (Figure 3), but significant differences ($p < 0.05$) were found only in the lowest (10 μg MTZ/ml with 106.7%) and the higher concentrations (>500 μg metronidazole/ml) because of the standard deviations. Above a concentration of 500 μg MTZ/ml, the amount of induced free radicals increased continuously, the relative light unit reaches 105.5% at 500 μg/ml, 111.9% at 1000 μg/ml, 113.5% at 2000 μg/ml, and 130.5% at 10000 μg/ml. Based on these measurements, metronidazole has a mild prooxidant property that increases above 500 μg/ml.

The inhibition of lipid peroxidation was studied in the presence of sunflower oil. The metronidazole solution was able to reduce the concentration of malondialdehyde in the sunflower oil exposed to 150 °C for 10 min. Thus it was able to reduce the lipid peroxidation by 7.3%, 17.8% and 25.2% depending on the increasing concentration. The results obtained are values expressed as a percentage of the amount of MDA formed during the decomposition of sunflower oil, 92.7% at 250 μg MTZ/ml, 92.25 at 500 μg/ml and 74.8% at 1000 μg/ml (Figure 4). The standard deviations were below one per cent in all cases, so the inhibition values of metronidazole compared to the sunflower oil were significant ($p < 0.05$).

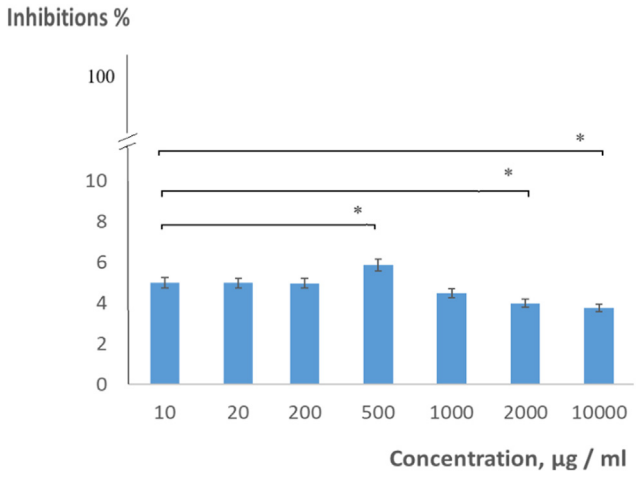

Figure 2. Hydrogen donor activity of metronidazole as a function of concentration. *Significant difference ($p < 0.05$) compared to the inhibition % at the lowest concentration.

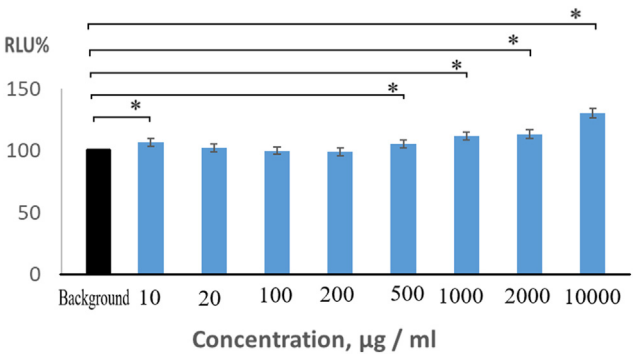

Figure 3. Concentration-dependent hydroxyl radical generation of metronidazole in H_2O_2/□OH microperoxidase-luminol system (RLU: relative light unit). *Significant difference ($p < 0.05$) compared to the background intensity.

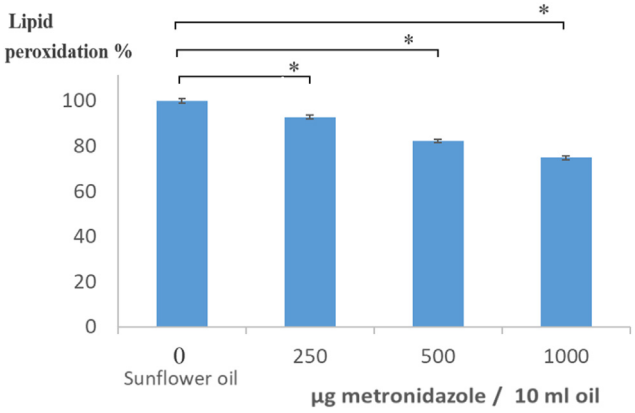

Figure 4. Lipid peroxidation inhibition of metronidazole. *Significant difference ($p < 0.05$) compared to the lipid peroxidation value of sunflower oil.

4. Discussion

According to our results, metronidazole has only a weak ferric reducing ability and hydrogen donor activity, but it shows prooxidant behaviour in the H_2O_2/·OH-microperoxidase-luminol system. Similar *in vitro* studies have not been reported in the literature. Nevertheless, Trecy and Webster obtained hydroxyl radical scavenging ability when neutrophils were present in the system and without neutrophils, the compound showed no radical scavenging activity [44]. *In vivo* experiment without neutrophils, neither dose-dependence nor antioxidant effects were observed [28]. Andrioli et al. studied the toxicity of metronidazole on the roots of *Allium cepa* for 30 h. They found a significant increase in the antioxidant defence parameters attributed to metronidazole-induced oxidative stress [45]. Metronidazole applied against *Trichomoniasis* hydrogen peroxide, superoxide and nitrogen-free radicals were activated [20, 21]. These are consistent with our test results that metronidazole has prooxidant property. However, metabolites of metronidazole are also prooxidant *in vivo*, as metronidazole enters microorganisms to form toxic intermediates, free radicals (e.g. MTZ-$NO_2^{\cdot}$, MTZ- $NO_2^{\cdot}H$, MTZ- NO, $ONOO^{-\cdot}$, MTZ-NHOH), which cause DNA damage and trigger oxidative stress [12, 16, 22, 28, 29, 32, 33]. To the formation of active metronidazole metabolites, NADH oxidase, alcohol dehydrogenase, thioredoxin peroxidase, nitroreductase 1, nitroreductase 2, ferredoxin, nicotinamide adenine dinucleotide phosphate (NADPH) oxidoreductase are required [12, 13, 14, 15]. After activation, the formed MTZ-NO can react with superoxide to form more radical peroxynitrite (ONOO−), converted to nitrate [15]. In wastewater treatment, an H_2O_2-photocatalytic process is used to degrade metronidazole in the presence of zinc. Hydroxyl radicals formed in the aqueous medium decompose the organic molecules [46,

47, 48]. The process conditions are similar to those for measuring hydroxyl radical scavenging ability, except that iron microperoxidase is the catalyst in our method. Therefore, under these conditions used, metronidazole does not scavenge hydroxyl radicals but oxidizes itself to produce free radicals and then decomposes. During high-energy photocatalysis, metronidazole is degraded by NO_2 release, while *in vivo* at low energy exposure it dissociates by NOOH release to form several intermediates [49]. These processes also support the prooxidant behaviour of metronidazole.

The result of inhibition of lipid peroxidation is consistent with the results obtained by Narayanan et al. [50] in a simple skin lipid model in which MDA concentrations were reduced by 25, 36, and 49% in the presence of 10, 100, and 500 μg metronidazole/ml, respectively. They concluded from previous experiments by themselves and others that the antioxidant effect of metronidazole can be mediated through two mechanisms: by reducing free radical production through neutrophil activity and by reducing free radical concentration due to its free radical scavenging property [21, 50]. Our studies did not confirm the free radical scavenging activity *in vitro*. However, we found moderate inhibition of lipid peroxidation. Therefore, questions arise as to why the results are so contradictory, why it inhibits lipid peroxidation, and why it does not have radical scavenging property. Rodrigez and Caseli recently reported that metronidazole interacts with phospholipids and depending on the chemical nature of lipid, the interaction is different [51], furthermore, metronidazole also interacts with the surface of DNA [17]. According to these, many side reactions are conceivable, in which metronidazole interacts with certain molecules to inhibit further lipid peroxidation.

5. Conclusion

The general antioxidant properties of metronidazole were not confirmed because even at high concentrations, it had only minimal iron-reducing ability and hydrogen-donor activity. Its hydroxyl radical scavenging capacity in the H_2O_2/˙OH-microperoxidase-luminol system cannot be demonstrated either, as the compound behaved as a prooxidant. However, it was able to inhibit the induced lipid peroxidation based on *in vitro* studies.

We can say that depending on the use of metronidazole, in addition to its absorption, utilization and metabolism, its mechanism of action is also different. The compounds formed during metabolic degradation of MTZ may cause ambivalent behaviour *in vivo*. It can be stated that metronidazole does not reduce free radical processes as a direct antioxidant and has no radical scavenging nature *in vitro*. Rather, it reduces free radical reactions by killing the bacteria, reducing the inflammatory processes caused due to the bactericidal properties, and stopping the consequent "respiratory burst" defence mechanism in the body. Thus metronidazole can affect redox homeostasis by a ROS-independent mechanism due to its non-direct antioxidant properties.

Declarations

Author contribution statement

Klára Szentmihályi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Krisztina Süle; Anna Egresi: Zoltán May: Performed the experiments.

Anna Blázovics: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] C.D. Freeman, N.E. Klutman, K.C. Lamp, Metronidazole. A therapeutic review and update, Drugs 4 (1997) 679–708.
[2] D.A. Ramasubbu, V. Smith, F. Hayden, P. Cronin, Systemic antibiotics for treating malignant wounds, Cochrane Database Syst. Rev. 24 (2017) CD011609.
[3] I.K. Aronson, J.A. Rumsfield, D.P. West, J. Alexander, J.H. Fischer, F.P. Palouceket, Evaluation of topical metronidazole gel in acne rosacea, Drug Intell. Clin. Pharm. 21 (1987) 346–351.
[4] C. Hoffmann, N. Fock, G. Franke, M. Zsciesche, W. Siegmund, Comparative bioavailability of metronidazole formulations (Vagimid) after oral and vaginal administration, Int. J. Clin. Pharmacol. Ther. 33 (1995) 232–239.
[5] C. Cosar, L. Julou, Activitiè de1'(hydroxy-2-ethyl)-1-methyl-2-nitro-5-imidazole (8.823R.P.) vis-a-vis des infections expérimentales à *Trichomonas vaginalis*, Ann. Inst. Pasteur. 96 (1959) 238–241.
[6] World Health Organization, Essential drugs, WHO Drug Inf. 13 (1999) 249–262.
[7] A.H. Lau, N.P. Lam, S.C. Piscitelli, L. Wikes, L.H. Danziger, Clinical pharmakokinetics of metronidazole and other nitroimidazole anti-infectives, Clin. Pharmacokinet. 23 (1992) 328–364.
[8] I. Amon, K. Amon, H. Hüller, Pharmacokinetics and therapeutic efficacy of metronidazole at different dosages, Int. J. Clin. Pharmacol. 16 (1978) 384–386.
[9] S. Loft, S.V. Otton, M.S. Lennard, G.T. Tucker, H.E. Poulsen, Characterization of metronidazole metabolism by human liver microsomes, Biochem. Pharmacol. 41 (1991) 1127–1134.
[10] R.L. Koch, B.B. Beaulieu, E.J. Chrystal, P. Goldman, A metronidazole metabolite in human urine and its risks, Science 211 (1981) 398–400.
[11] M. Müller, Mode of action of metronidazole on anaerobic bacteria and protozoa, Surgery 93 (1983) 165–171.
[12] C.S. Saghaug, C. Klotz, J.P. Kallio, H.R. Brattbakk, T. Stokowy, T. Aebischer, I. Kursula, N. Langeland, K. Hanevik, Genetic variation in metronidazole metabolism and oxidative stress pathways in clinical Giardia lamblia assemblage A and B isolates, Infect. Drug Resist. 12 (2019) 1221–1235.
[13] S. Löfmark, C. Elund, C.E. Nord, Metronidazole is still the drug of choice for treatment of anaerobic infections, Clin. Infect. Dis. 50 (Suppl.1) (2010) S16–S23.
[14] J. Samuelson, Why metronidazole is active against both bacteria and parasites, Antimicrob. Agents Chemother. 43 (1999) 1533–1551.
[15] P. Upcroft, J.A. Upcroft, Drug targets and mechanisms of resistance in the anaerobic protozoa, Clin. Microbiol. Rev. 14 (2001) 150–164.
[16] D. Leitsch, S. Schlosser, A. Burgess, M. Duchéne, Nitroimidazole drugs vary in their mode of action in the human parasite Giardia lamblia, Int. J. Parasitol.: Drugs Drug Resist. 2 (2012) 166–170.
[17] M. Uzlikova, E. Nohynkova, The effect of metronidazole on the cell cycle and DNA in metronidazole-susceptible and -resistant Giardia cell lines, Mol. Biochem. Parasitol. 198 (2014) 75–81.
[18] M. De Meo, P. Vanelle, E. Bernadini, M. Laget, J. Maldonado, O. Jentzer, M.P. Crozet, G. Duménil, Evaluation of the mutagenic and genotoxic activities of 48 nitroimidazoles and related imidazole derivatives by the Ames test and the SOS chromotest, Environ. Mol. Mutagen. 19 (1992) 167–181.
[19] D. Raj, E. Ghosh, A.K. Mukherjee, O. Nozaki, S. Ganguly, Differential gene expression in Giardia lamblia under oxidative stress: significance in eukaryotic evolution, Gene 535 (2014) 131–139.
[20] M.O. Chacon, T.H.A. Fonseca, S.B.V. Oliveira, M.A. Alacoque, L.L. Franco, C.A. Tagliati, G.D. Cassali, G.P. Campos-Mota, R.J. Alves, L.S.A. Capettini, M.A. Gomes, Chlorinated metronidazole as a promising alternative for treating trichomoniasis, Parasitol. Res. 117 (2018) 1333–1340.
[21] R.L. Dunne, L.A. Dunne, P. Upcroft, P.J. O'Donoghue, J.A. Upcroft, Drug resistance in the sexually transmitted protozoan Trichomonas vaginalis, Cell Res. 13 (2003) 239–249.
[22] D.I. Edwards, Reduction of nitroimidazoles in vitro and DNA damage, Biochem. Pharmacol. 35 (1986) 53–58.
[23] J.E. Stambaugh, L.G. Feo, R.W. Manthei, The isolation and identification of the urinary oxidative metabolites of metronidazole in man, J. Pharmacol. Exp. Ther. 161 (1968) 373–381.
[24] D.A. Danielson, M.T. Hannan, H. Jick, Metronidazole and cancer, J. Am. Med. Assoc. 247 (1982) 2498–2499.
[25] G.D. Friedman, H.K. Ury, Initial screening for carcinogenicity of commonly used drugs, J. Natl. Cancer Inst. 65 (1980) 723–733.
[26] Y. Miyachi, S. Imamura, Y. Niwa, Antioxidant action of metronidazole: a possible mechanism of action in rosacea, Br. J. Dermatol. 114 (1986) 231–234.

[27] Y. Miyachi, Potential antioxidant mechanism of action for metronidazole: implications for rosacea management, Adv. Ther. 18 (2001) 237–243.
[28] H. Akamatsu, M. Oguchi, S. Nishijima, M. Takahashi, T. Ushijima, Y. Niwa, The inhibition of free radical generation by human neutrophils through the synergistic effects of metronidazole with palmitoleic acid: a possible mechanism of action of metronidazole in rosacea and acne, Arch. Dermatol. Res. 282 (1990) 449–454.
[29] S. Boia, S.T. Stratul, M. Boariu, S. Ursiniu, S.L. Gotia, E.R. Boia, C. Borz, Evaluation of antioxidant capacity and clinical assessment of patients with chronic periodontitis treated with nonsurgical periodontal therapy and adjunctive systemic antibiotherapy, Rom, J. Morphol. Embryol. 59 (2018) 1107–1113.
[30] M.A. Pélissier, P. Marteau, P. Pochart, Antioxidant effects of metronidazole in colonic tissue, Dig. Dis. Sci. 52 (2007) 40–44.
[31] O. Nitzan, M. Elias, A. Peretz, W. Saliba, Role of antibiotics for treatment of inflammatory bowel disease, World J. Gastroenterol. 22 (2016) 1078–1087.
[32] C.M. Rao, A. Ghosh, C. Raghothama, K.L. Bairy, Does metronidazole reduce lipid peroxidation in burn injuries to promote healin? Burns 28 (2002) 427–429.
[33] Y. Xiao, T. Xiong, X. Meng, D. Yu, Z. Xiao, L. Song, Different influences on mitochondrial function, oxidative stress and cytotoxicity of antibiotics on primary human neuron and cell lines, J. Biochem. Mol. Toxicol. 33 (2019), e22277.
[34] S.A. Dingsdag, N. Hunter, Metronidazole: an update on metabolism, structure-cytotoxicity and resistance mechanisms, J. Antimicrob. Chemother. 73 (2018) 265–279.
[35] I.E.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: direct measure of the total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Method Enzymol. 299 (1999) 15–27.
[36] C. Lado, M. Then, I. Varga, É. Szőke, K. Szentmihályi, Antioxidant property of volatile oils determined by the ferric reducing ability, Z. Naturforsch. 59c (2004) 354–358.
[37] M.S. Blois, Antioxidant determination by the use of a stable free radical, Nature 4617 (1958) 1198–1200.
[38] T. Hatano, H. Kagawa, T. Yasuhara, T. Okuda, Two new flavonoids and other constituents in licore root: their relative astringency and radical scavenging effects, Chem. Pharm. Bull. 36 (1988) 2090–2097.
[39] A. Blázovics, É. Sárdi, Methodological repertoire development to study the effect of dietary supplementation in cancer therapy, Microchem. J. 136 (2018) 121–127.
[40] L. Ma, G. Liu, X. Liu, Amounts of malondialdehyde do not accurately represent the real oxidative level of all vegetable oils: a kinetic study of malondialdehyde formation, Int. J. Food Sci. Technol. 54 (2019) 412–423.
[41] Z.A. Placer, L.L. Cushman, B.C. Johnson, Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems, Anal. Biochem. 16 (1966) 359–364.
[42] A. Zeb, F. Ullah, A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods, J. Anal. Methods Chem. (2016). Article ID 9412767.
[43] D. Njus, P.M. Kelley, Y.J. Tu, H.B. Schlegel, Ascorbic acid: the chemistry underlying its antioxidant properties, Free Rad. Biol. Med. 159 (2020) 37–43.
[44] J.W. Tracy, L.T. Webster Jr., Drugs used in the chemotherapy of protozoal infections: trypanosomiasis, leishmaniasis, amebiasis, giardiasis, trichomoniasis, and other protozoal infections, in: J.G. Hardman, L.E. Limbird, P.B. Molinoff, A. Gilman (Eds.), Goodman and Gilman's the Pharmacological Basis of Therapeutics, ninth ed., McGraw-Hill, New York, 1996, pp. 987–1008.
[45] N. Andrioli, S.E. Sabatini, M.D. Mudry, M.C.R. de Molina, Oxidative damage and antioxidant response of *Allium cepa* meristematic and elongation cells exposed to metronidazole, Environ. Toxicol. Chem. 31 (2012) 968–972.
[46] Z. Dongshuo, C. Haon, G. Kaiyin, Preparation and visible-light photocatalytic degradation on metronidazole of Zn2SiO4-ZnO-biochar composites, J. Inorg. Mater. 35 (2020) 923–930.
[47] S. Yang, P. Wang, X. Yang, G. Wei, W. Zhang, L. Shan, A novel advanced oxidation process to degrade organic pollutants in wastewater: microwave-activated persulfate oxidation, J. Environ. Sci. 21 (2009) 1175–1180.
[48] N. Ta, J. Hong, T. Liu, C. Sun, Degradation of atrazine by microwave-assisted electrodeless discharge mercury lamp in aqueous solution, J. Hazard Mater. 138 (2006) 187–194.
[49] S. Ahmadi, C. Osagie, S. Rahdar, N.A. Khan, S. Ahmed, H. Hajini, Efficacy of persulfate-based advanced oxidation process (US/PS/Fe_3O_4) for ciprofoxacin removal from aqueous solutions, App. Water Sci. 10 (2020) 187.
[50] S. Narayanan, A. Hünerbein, M. Getie, A. Jäckel, R.H.H. Neubert, Scavenging properties of metronidazole on free oxygen radicals in a skin lipid model system, J. Pharm. Pharmacol. 59 (2007) 1125–1130.
[51] E. Itälä, J. Niskanen, L. Pihlava, E. Kukk, Fragmentation patterns of radiosensitizers metronidazole and nimorazole upon valence ionization, J. Phys. Chem. A 124 (2020) 5555–5562.