CORRELATION OF THE FIRST REDUCTION POTENTIAL OF SELECTED RADIOSENSITIZERS DETERMINED BY CYCLIC VOLTAMMETRY WITH THEORETICAL CALCULATIONS

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Radiosensitizers are drugs that make cancer cells more sensitive to radiation therapy. The cytotoxic properties of such compounds are due to the fact that in the cell these compounds undergo one-electron reduction to generate radical anions. Therefore, their theoretical and/or experimental study is of high interest. To determine the correlation between reduction potential determined by cyclic voltammetry measurements and some physicochemical properties of selected radiosensitizers theoretical calculations of electron affinities based on the DFT method with B3LYP functional at the level of 6-311++G** basis set in vacuum were utilized. Very good correlation was found between electron affinities of radiosensitizers and their reduction potential and so called E_7^1 potential that account for the energy necessary to transfer the first electron to an electroactive group at pH 7 in aqueous medium to form a radical anion.

Keywords: Cyclic voltammetry; Radical ions; Radiochemistry; Radiosensitizers; Electron affinity; Cell.

Radiotherapy is one form of the cancer treatment that prevents malignant cells from growing and dividing. It is closely connected with free radical production. Radiosensitizers are drugs that make cancer cells more sensitive to radiation therapy. The cytotoxic properties of such compounds are due

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to the fact that in the cell these compounds undergo one-electron reduction to generate radical anions, which exhibit cytotoxicity towards cellular systems[1–4]. It is probable that these nitro radical anions interact with cellular DNA and DNA components causing DNA damage within the cell. It is also evident that nitro radical anions from heterocyclic drugs abstract electrons from the bases[5], forming the base cations, which subsequently undergo reactions to give different compounds, and as a result the base is degraded. An important property of some radiosensitizers is that they appear to radiosensitize hypoxic cells, but have no measurable effect in well-oxygenated cells, at least in vitro[6]. This is probably not because of some remarkable inherent property, but because of simple kinetic competition between oxygen and drugs (chemicals) for reaction with key DNA base radicals. This agent may also be useful as an imaging agent for identifying hypoxic, drug-resistant regions of primary tumors or metastases[1].

The reactivity of radical anions from most of the nitrogen-containing compounds has been studied mainly by the pulse radiolysis technique[7,8]. Recently electrochemical techniques have been used to study the behavior of the nitro radical anions[3,9–19].

It is known that there is a good correlation between physico-chemical properties of some nitro-compounds, e.g. radical anion production, with their ability act as hypoxic cell radiosensitizers[20]. Electron affinity (EA) of atoms and molecules, one of the fundamental properties, e.g. the EAs of the DNA bases are of interest owning to their significance to understanding of DNA radiation damage[21]. Drugs with high electron affinity (with low reduction potential) are generally highly toxic and mutagenic, being also more quickly metabolized. Furthermore, there are a lot of other studies[22,23] dealing with the relationship between reduction potentials and pharmacological activity showing that this parameter is of interest from electrochemical and pharmacological points of view.

In this study we tried to correlate the first reduction potential (anion radical production potential) of several radiosensitizers with electron affinity, HOMOs of the radiosensitizer anions, LUMOs of the neutral radiosensitizers and so called E_7^1 potential that account for the energy necessary to transfer the first electron to an electroactive group at pH 7 in aqueous medium to form a radical anion. Therefore, in the case of nitro compounds, the E_7^1 values represent the ability to form the nitro radical anion. All these correlations could be useful if effects of different radiosensitizers in the human body are being compared.

EXPERIMENTAL

Electrochemical measurements were performed using AUTOLAB instrument PG STAT 30 equipped with FRA2 module (ECO Chemie, The Netherlands). An electrochemical data from cyclic voltammetry, phase-sensitive AC polarography, and DC polarography were analyzed using AUTOLAB software. A three-electrode electrochemical cell was used. The reference electrode (RE), Ag|AgCl|1 M LiCl, was separated from the test solution by a salt bridge. The working electrode (WE) was a valve-operated static mercury electrode (SMDE2; Laboratorní Přístroje, Praha) with an area of 5.15×10^{-3} cm^2. The counter electrode (CE) was cylindrical platinum wire with area ca. 100 times higher than area of WE. Radiosensitizers (etanidazole (ETN), tirapazamine (TIR; Sigma Aldrich), metronidazole (MET; Acros Organics, France), megazol (MEG; Bios Chemicals, France), nimorazole (NIM; Carbon Scientific Co., Ltd. UK), tinidazole (TIN), ornidazole (ORN; LKT Laboratories, Inc., USA)) were used without further purification. Density functional theory (DFT) calculations of EAs, HOMOs and LUMOs of the selected radiosensitizers were calculated using Spartan'08 program (Wavefunction, Inc., Irvine, CA, USA)[24] with B3LYP (Becke, three-parameter, Lee–Yang–Parr) exchange-correlation functional at the level of 6-311++G** basis set in vacuum (6-311 is a split-valence triple-zeta basis; it adds one GTO to 6-31G (Pople's split-valence double-zeta basis set; the core orbital is a CGTO made of 6 Gaussians, and the valence is described by two orbitals – one CGTO made of 3 Gaussians, and one single Gaussian); two asterisks, **, indicate that polarization functions are also added to hydrogen, two plus signs, ++, indicate that diffuse functions are also added to hydrogen). Addition of polarization functions is an important when considering accurate representations of bonding between atoms, because the very presence of the bonded atom makes the energetic environment of the electrons spherically asymmetric. Similarly, addition of diffuse functions is useful when considering anions, what is important for our systems. This approach was successfully utilized in calculating of EAs of several chemical compounds[25–27].

RESULTS AND DISCUSSION

In general, the electrochemical behavior of nitroaromatic compounds has been studied at different electrodes. These experiments have been usually focused on study of nitro radical anion formed during reduction step in various, mostly, aprotic and mixed solvents. This study is aimed on the redox properties of the selected drugs in aprotic media. Radiosensitizers utilized in this study are depicted in Fig. 1.

As stated above, the main in vivo effect of radiosensitizers is the radical anion production. In Fig. 2, an example of cyclic voltammetry behavior of the selected radiosensitizer metronidazole radical anion is shown.

As can be seen from Fig. 2, the formation of radical anion of metronidazole in aprotic medium is a reversible one electron process controlled by the diffusion of the reactive species (Fig. 3). Slight enhancement of the difference between cathodic and anodic peak potential with increasing scan rate is probably caused by adsorption of the compounds at the electrode surface.

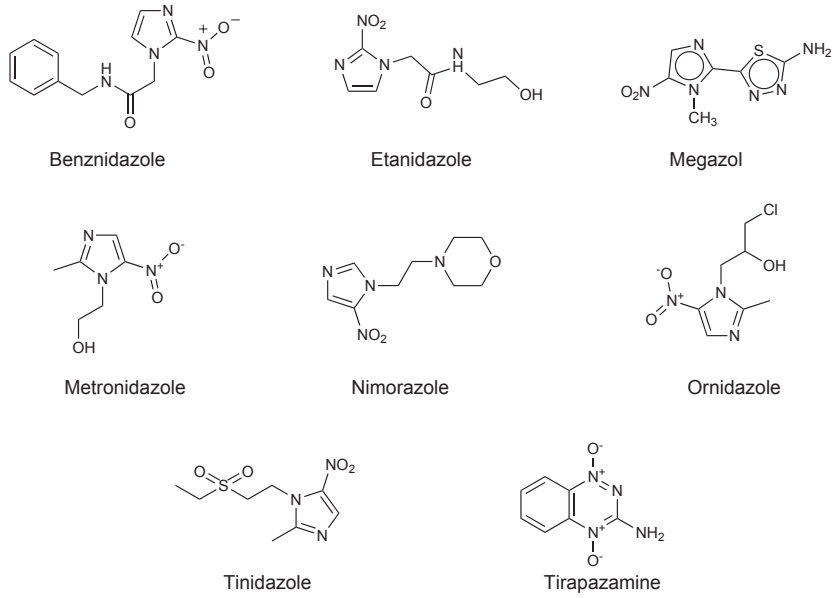

FIG. 1
Chemical structure of the radiosensitizers utilized in this study

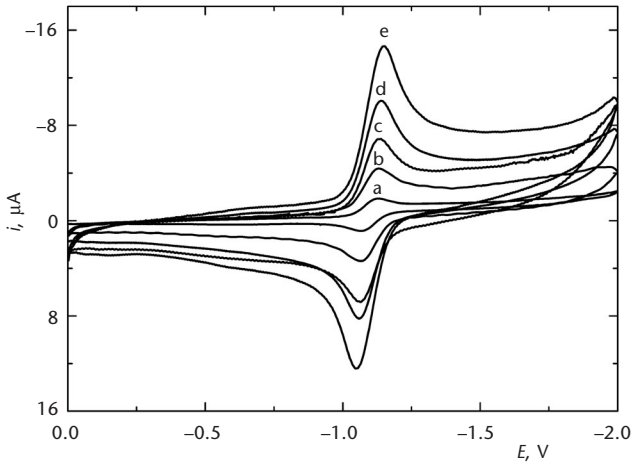

FIG. 2
Cyclic voltammograms of metronidazole in dimethylformamide at various scan rates: 10 (a), 50 (b), 125 (c), 250 (d), 500 mV/s (e). 0.1 M $TBAPF_6$ as a supporting electrolyte, *c*(metronidazole) = 4.2 mM

Behavior of the other radiosensitizers radical anion in aprotic media is very similar to that of metronidazole. Despite the structural differences among some of radiosensitizers utilized are not very high, significant differences in the reduction potentials of anion radical of respective radiosensitizers are observed[1–3,5,10,12]. Moreover, it is well known that slight structural modifications may confer the big chemical and/or biological activities in vitro and/or in vivo.

As stated higher, values of potential characterize the energy necessary to transfer the first electron to an electroactive group at pH 7 in aqueous medium to form a radical anion, i.e. in the case of nitro radiosensitizers the E_7^1 values represent the ability to form the nitro radical anion of respective radiosensitizers. Moreover, the E_7^1 values of the $RNO_2/R\text{–}NO_2^{\bullet-}$ couple may be used to assess not only the thermodynamic feasibility of one-electron reduction of RNO_2 by any possible "nitroreductase" but also the probability of electron donation from the $R\text{–}NO_2^{\bullet-}$ to oxygen in the well-known "futile reduction" present in aerobic conditions[3]

$$R\text{–}NO_2^{\bullet-} + O_2 \xrightarrow{K_{O2}} RNO_2 + O_2^{\bullet-} . \qquad (1)$$

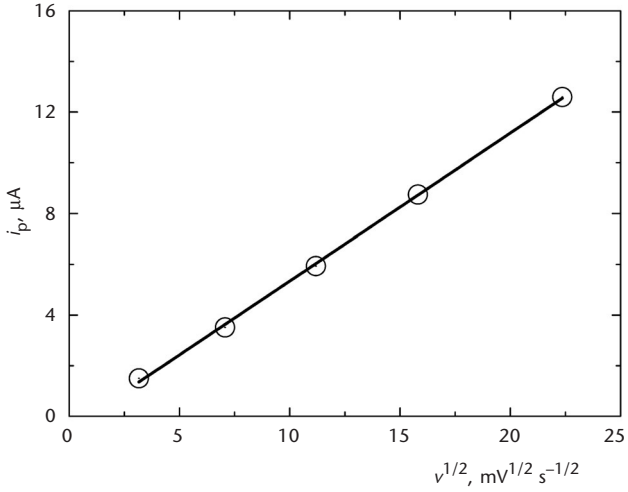

FIG. 3
Dependence of the cathodic peak current of metronidazole at various scan rates on the square root of the scan rate. All experimental conditions are the same as in Fig. 2

Therefore, radiosensitizers should have electron affinities smaller than oxygen. On the other side, too negative value of the electron affinity may results to a loss of their selectivity[28]. Similarly, the electron affinity of nitro compounds (radiosensitizers) is associated to the ability to form radical anion and, therefore, it is associated to the biological activity of the radiosensitizers. Some authors identify one of the two terms of electron affinity, so called adiabatic electron affinity with E_7^1 potential[28]. The E_7^1 values were firstly obtained experimentally by electron pulse radiolysis but according to the study published by Breccia et al.[29], it is possible to obtain an excellent correlation between the cathodic peak potentials E_{pc} of the first reduction peak of the several nitrocompounds measured in aprotic media with the E_7^1 values obtained by pulse radiolysis in water. Therefore, cyclic voltammetry (and other electrochemical techniques) is a good alternative to the classic pulse radiolysis method to obtain reliable values of the E_7^1 parameter for nitro radical anions[29]. According to the linear dependence of the first reduction peak E_{pc} of several nitrocompounds on E_7^1 potential in water obtained by pulse radiolysis[29], the value of E_7^1 potential for all radiosensitizers utilized in this study can be determined. Thus obtained values of E_7^1 together with measured values of the peak potential E_{pc} of radiosensitizers can be correlated with theoretically calculated values of electron affinities, HOMO (anion) and LUMO of the neutral form of the same compounds.

As mentioned above, the biological activity of radiosensitizers is associated to their respective electron affinities and E_7^1 potentials. Moreover, relationship between sensitizing efficiency and electron affinity is supported by pulse radiolysis experiments of the first electron transfer on some radiosensitizers[30]. The electron affinity is defined as the energy of neutral molecule minus that of the anion radical

$$\mathrm{EA} = E^0_{\text{Radiosensitizer}} - E^{\bullet -}_{\text{Radiosensitizer}} . \qquad (2)$$

In the present work we study the relations between theoretical electron affinities of selected radiosensitizers and their "first electron" reduction potentials which is important for sensitizing efficiency characterization. To determine EA of the selected radiosensitizers the DFT calculations with B3LYP functional at the level of 6-311++G** basis set in vacuum were used. In the Fig. 4, the correlation between theoretically calculated electron affinities of selected radiosensitizers and their respective peak potentials E_{pc} determined from cyclic voltammetry measurements is plotted.

As one can easily see, very good correlation between theoretically calculated electron affinities by DFT/B3LYP/6-311++G** method and peak potentials measured by cyclic voltammetry can be found.

Similarly, good correlation between theoretical electron affinities of selected radiosensitizers and so called E_7^1 potential which is associated with the ability of the radiosensitizers to accept electron and form anion radical is visible in Fig. 5.

From both Fig. 4 and Fig. 5 it is clear that reduction of the radiosensitizers used in this study is more favorable in aqueous solution than in aprotic media. This is in good agreement with our previous studies[1–3] and with study of Ramalho et al.[28]. These observations are in the line with the proposition of the probable in vivo mechanism of the reduction of these drugs: it is suggested that reduction takes place inside the cell at the cytoplasm or at the cytoplasm/cell membrane interface rather than in the cell membrane[32,33]. Molecules having higher electron affinity could be more reactive under reductive conditions. However, the reactivity of the radiosensitizers depends not only on their electron affinity but also on the environmental media. The better is electron donating ability of the surrounding media the better efficiency can be expected in the case of the presented radiosensitizer.

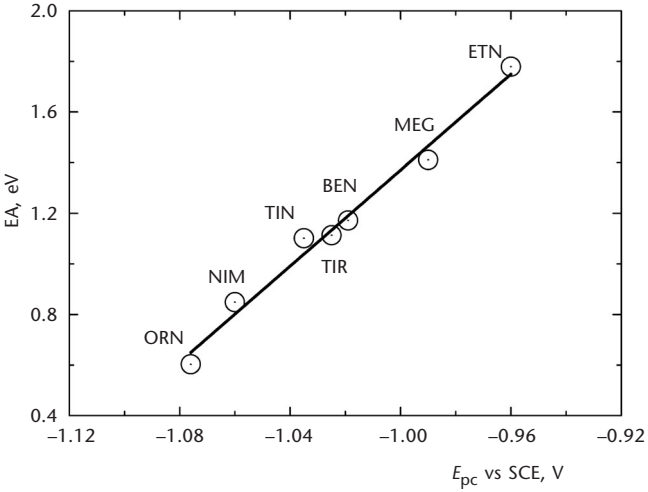

FIG. 4
Correlation between cathodic peak potential of radiosensitizers determined by cyclic voltammetry measurements and their theoretical electron affinities. E_{pc} for benznidazole taken from ref.[31]

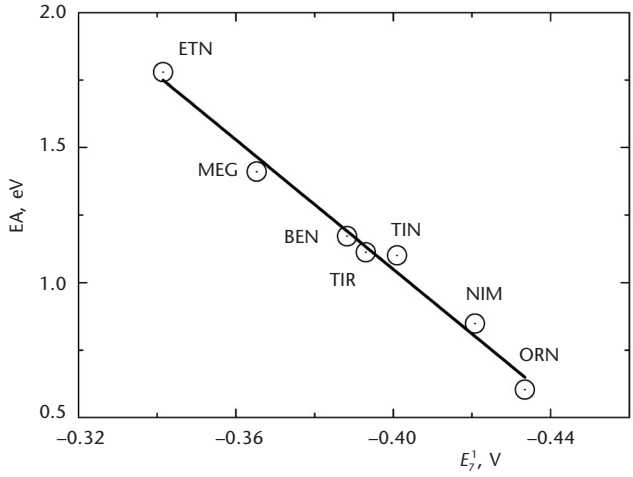

FIG. 5
Correlation between E_7^1 potentials of the selected radiosensitizers calculated according to ref.[29] with their theoretical electron affinities calculated utilizing DFT/B3LYP/6311++G** method

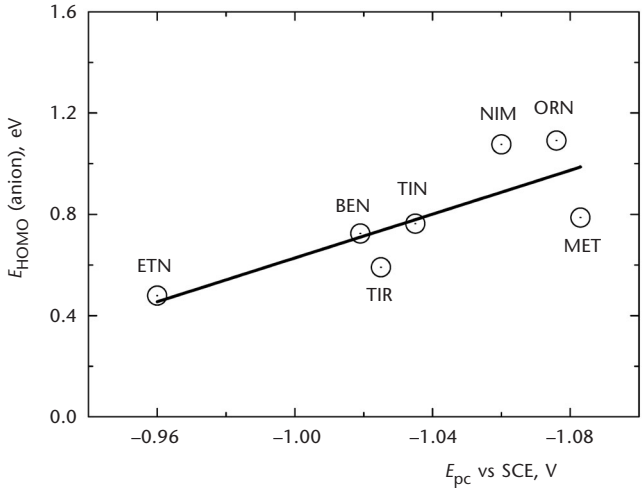

FIG. 6
Correlation between cathodic peak potential of radiosensitizers determined form cyclic voltammetry measurements and the energy of the HOMO of respective anions. E_{pc} for benznidazole taken from ref.[31]

Another approach how to estimate the ability of the radiosensitizers to accept single electron and form anion radical may be calculation of the energy of the highest occupied molecular orbital (HOMO) of the anion of the respective compound or calculate the energy of the lowest unoccupied molecular orbital (LUMO) of the neutral species[30]. The correlation between HOMOs of radiosensitizer anions and their cathodic peak potentials is depicted in Fig. 6.

In Fig. 6, the linear dependency of HOMO energies of anion radicals of radiosensitizers on cathodic peak potentials of respective drugs can be observed. It is also clear that the linear curve in Fig. 6 has the same tendency than that in Fig. 4. However, the spread of points in Fig. 6 (correlation coefficient $R^2 = 0.5602$) is not as good as in Fig. 4 ($R^2 = 0.9817$). Very similar observation ($R^2 = 0.6735$) can be made in the case of LUMO energies of basic radiosensitizers (graph not shown). It is possible to summarize that calculation of electron affinities represents more precise method than the calculation of HOMO energies of anion radical species of radiosensitizers or LUMO energies of neutral species. Similar conclusion was made also by Ramalho et al.[28]. Correlation of HOMO-LUMO energy gap of radiosensitizers with reduction peak potential or E_7^1 potential was even worse than previous two correlations (HOMO (anion) – E_{pc}/E_7^1, LUMO – E_{pc}/E_7^1).

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