CHEMIJA. 2018. Vol. 29. No. 4. P. 273–280

Enzymatic single-electron reduction and aerobic cytotoxicity of tirapazamine and its 1-oxide and nor-oxide metabolites

Jonas Šarlauskas[a],

Aušra Nemeikaitė-Čėnienė[b],

Audronė Marozienė[a],

Lina Misevičienė[a],

Mindaugas Lesanavičius[a],

Narimantas Čėnas[a]*

[a] *Institute of Biochemistry of Vilnius University, Saulėtekio St. 7, 10257 Vilnius, Lithuania*

[b] *State Research Institute Centre for Innovative Medicine, Santariškių St. 5, 08406 Vilnius, Lithuania*

Aerobic cytotoxicity of 3-amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine, TPZ), a bioreductively activated hypoxia-specific anticancer agent, is responsible for TPZ side effects in chemotherapy. In order to clarify its mechanisms, we examined the aerobic cytotoxicity of TPZ and its main metabolites, 3-amino-1,2,4-benzotriazine-1-oxide and 3-amino-1,2,4-benzotriazine in murine hepatoma MH22a cells, and their reduction by NADPH:cytochrome P-450 reductase (P-450R) and ferredoxin:$NADP^+$ reductase (FNR). Analogous studies of several quinones and nitroaromatic compounds with similar values of single-electron reduction midpoint potentials (E^1_7) were carried out. In single-electron reduction by P-450R and FNR, the reactivity of TPZ and its monoxide was similar to that of quinones and nitroaromatics, and increased with an increase in their E^1_7. The cytotoxicity of TPZ and its metabolites possessed a prooxidant character, because it was partly prevented by an antioxidant *N,N'*-diphenyl-*p*-phenylene diamine and desferrioxamine, and potentiated by 1,3-bis(2-chloroethyl)-1-nitrosourea. Importantly, the cytotoxicity of TPZ and, possibly, its 1-*N*-oxide, was much higher than that of quinones and nitroaromatics with similar values of E^1_7 and redox cycling activities. A possible additional factor in the aerobic cytotoxicity of TPZ is its reductive activation in oxygen-poor cell nuclei, leading to the formation of DNA-damaging species similar to those forming under hypoxia.

Keywords: tirapazamine, cytotoxicity, oxidative stress, reductive activation

Abbreviations: E^1_7, single-electron reduction midpoint potential; FNR, ferredoxin:$NADP^+$ reductase; k_{cat}, turnover number; k_{cat}/K_m, apparent bimolecular rate constant; NQO1, DT-diaphorase; P-450R, NADPH:cytochrome P-450 reductase; SOD, superoxide dismutase; TPZ, tirapazamine

INTRODUCTION

3-Amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine, TPZ) and its analogs are hypoxia-selective anticancer agents ([1], and references therein). TPZ (**1**) is enzymatically reduced in a single-electron way to a free radical (**2**), which forms DNA-damaging species under hypoxic conditions, namely, an oxidizing hydroxyl radical (OH·) ([2], and references therein), and/or a highly reactive

* Corresponding author. Email: narimantas.cenas@bchi.vu.lt

benzotriazinyl radical (**3**) that abstracts a hydrogen atom from DNA ([3, 4], and references therein) (Fig. 1). The nature of DNA-damaging species is still a matter of debate. In the cell, TPZ is converted into two main relatively nontoxic metabolites, its mono-1-oxide (**4**), possibly via free radical (**2**) intermediate [2], and its nor-oxide (**6**), presumably via 4-oxide (**5**) intermediate (Fig. 1) [5].

The single-electron reductive activation of TPZ under hypoxic and oxic conditions is carried out mainly by microsomal NADPH:cytochrome P-450 reductase (P-450R) ([6], and references therein), and/or by insufficiently characterized intranuclear NAD(P)H-oxidizing flavoenzymes [7]. DT-diaphorase (NQO1) also reduces TPZ into **4** and **6**, however, its role in TPZ cytotoxicity is minor [8, 9].

Because the aerobic cytotoxity of aromatic di-*N*-oxides is lower than under hypoxia, it attracted considerably less attention [10, 11]. In the simplest way, it is explained by the redox cycling of TPZ (Fig. 1), leading to the formation of superoxide ($O_2^{-}\cdot$) and subsequent oxidative stress [1]. This is considered as the side effect in tumour therapy ([11, 12], and references therein). However, some TPZ analogues possess anticancer activity at micromolar concentrations even under oxic conditions [13, 14]. Besides, redox cycling events play an important role in the antimicrobial and antiparasitic action of TPZ analogs ([15], and references therein). For these reasons, the aerobic cytotoxicity of TPZ deserves more thorough studies.

In this work, we examined the aerobic cytotoxicity and enzymatic redox properties of TPZ and its metabolites **4** and **6**. We found that the cytotoxicity of TPZ is considerably higher than that of model quinones and nitroaromatic compounds with similar values of single-electron reduction midpoint potentials (E^1_7) and redox cycling activities.

MATERIALS AND METHODS

TPZ, 3-amino-1,2,4-benzotriazine-1-oxide and 3-amino-1,2,4-benzotriazine were synthesized as described in [10, 16, and 17], respectively. The compound purity was characterized by IR and NMR spectrometry, the melting point, and elemental analysis. NADPH, cytochrome *c*, superoxide dismutase, and other reagents were obtained from Sigma, and used as received. Recombinant rat NADPH:cytochrome P-450 reductase (P-450R) was prepared as described in [18], and was a generous gift of Dr. Alexey Yantsevich (Institute of Bioorganic Chemistry, Minsk, Belarus). The enzyme concentration was determined spectrophotometrically according to ε_{456} = 21.4 mM^{-1} cm^{-1}.

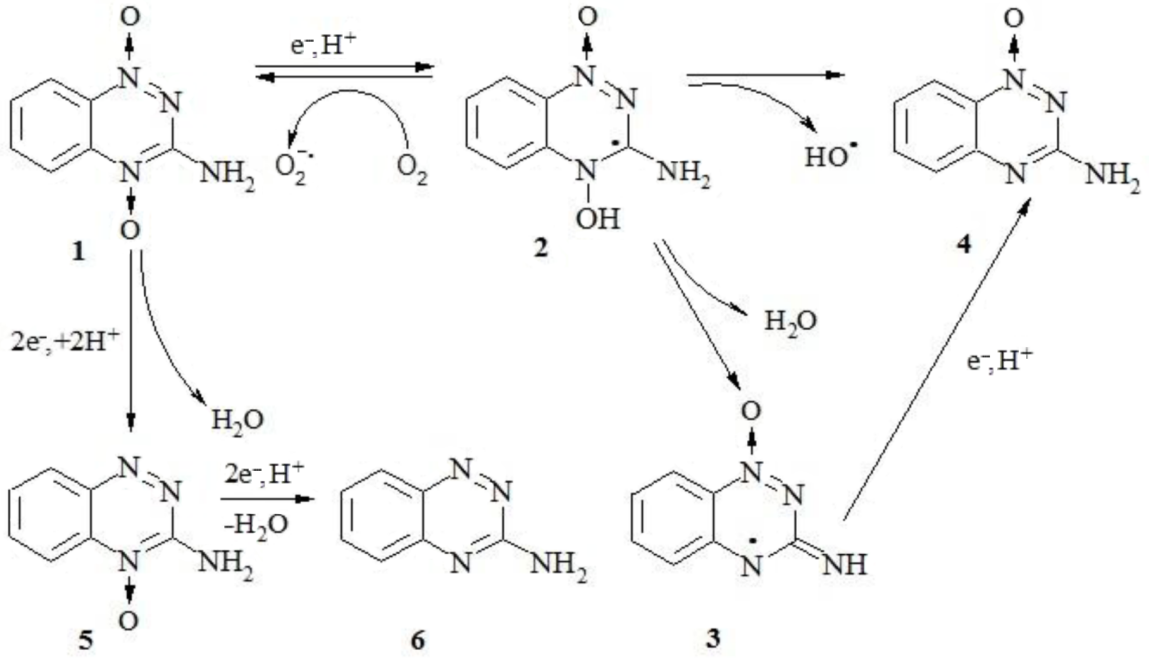

Fig. 1. A scheme of the reduction of tirapazamine in the cell

Recombinant *Plasmodium falciparum* ferredoxin:NADP$^+$ reductase (FNR) was prepared as described in [19], and was a generous gift of Dr. Alessandro Aliverti (Universita degli Studi di Milano, Italy). The enzyme concentration was determined according to ε_{461} = 10.1 mM^{-1} cm^{-1}.

The kinetic measurements were carried out spectrophotometrically using a Cary 60 UV–VIS spectrophotometer (Agilent Technologies) in the 0.1 M K-phosphate buffer (pH 7.0) containing 1 mM EDTA at 25°C. Enzymatic NADPH oxidation rates were determined according to $\Delta\varepsilon_{340}$ = 6.2 mM^{-1} cm^{-1} after the substraction of intrinsic NADPH oxidase activities of enzymes, 0.05 s^{-1} (P-450R) and 0.07 s^{-1} (FNR). The values of the turnover rate, k_{cat}, reflecting the maximal number of moles NADPH oxidized or oxidant reduced per mole of the enzyme active center per second, and k_{cat}/K_m, the apparent bimolecular rate constant, correspond to the inverse intercepts and slopes in Lineweaver–Burk coordinates, $[E]/v$ vs 1/[oxidant]. These rate constants were obtained by fitting the experimental data to the Michaelis–Menten equation using the Sigmaplot software. The rates of reduction of cytochrome *c* and ferricyanide were monitored according to $\Delta\varepsilon_{550}$ = 20 nM^{-1} cm^{-1} and $\Delta\varepsilon_{420}$ = 1.0 mM^{-1} cm^{-1}, respectively. At the saturating concentration of substrates, 100 μM NADPH and 50 μM cytochrome *c* or 1.0 mM ferricyanide, the k_{cat} of P-450R was equal to 39 s^{-1} (cytochrome *c*), and the k_{cat} of FNR was equal to 50 s^{-1} (ferricyanide). The k_{cat} of NQO1 was monitored according to the rate of menadione-mediated reduction of cytochrome *c*.

Murine hepatoma MH22a cells were grown and maintained at 37°C in the DMEM medium, supplemented with 10% fetal bovine serum and antibiotics, as described in [20]. In the cytotoxicity experiments, 3.0 × 10^4/ml cells were seeded on 18×18 mm glass slides in 5-ml flasks either in the presence or in the absence of compounds, and were grown for 24 h. Then, the slides were rinsed 3–4 times with phosphate buffered saline and stained with Trypan blue. The cells adherrent to the slides were counted under a light microscope. Typically, they did not accumulate Trypan blue and their viability was 98.5–99.3%. Stock solutions of compounds were prepared in dimethyl sulfoxide. Its concentration in cultivation media did not exceed 0.2%, and did not affect the cell viability. The experiments were conducted in triplicate.

The statistical analysis was performed using Statistica (version 4.3, StatSoft, 1993) and octanol/water partition coefficients (log P_{calc}) were calculated using a Log P calculator (version 2015, ACD Labs).

RESULTS

Frequently, the aerobic cytotoxicity of quinones and nitroaromatic compounds increases with an increase in their single-electron reduction midpoint potential at pH 7.0 (E^1_7) with the relationship $\Delta\log cL_{50}/\Delta E^1_7 \sim -10$ V^{-1}, where cL_{50} is the concentration of the compound for 50% cell survival ([21, 22], and references therein). It points to the oxidative stress being the main factor of cytotoxicity, because, as a rule, the rates of single-electron reduction of quinones or nitroaromatics by flavoenzymes initiating redox cycling increase with E^1_7 of an oxidant [23–25]. To examine the relationship of TPZ cytotoxicity with its redox cycling activity, we determined the steady-state reduction rate constants of TPZ and its metabolites by NADPH:cytochrome P-450 reductase (P450R) which is supposed to be one of the major determinants of TPZ cytotoxicity [6], and by a model single-electron transferring enzyme, ferredoxin:NADP$^+$ reductase (FNR) [24]. In parallel, the reduction rate constants of a number of quinones and nitrobenzenes with low E^1_7 values, –0.260 V – –0.485 V, were determined (Table 1).

In the FNR-catalyzed reactions, the rates of reduction of added cytochrome *c* (50 μM) were equal to 180–190% of the NADPH oxidation rate in the presence of TPZ, its 1-oxide and nor-oxide. The reaction was inhibited by 100 U/ml superoxide dismutase by 40–50%. It shows that cytochrome *c* is reduced by free radicals of these compounds undertaking the redox equilibrium with a $O_2/O_2^{-\cdot}$ couple. The data of Fig. 2 show that the reactivity (log k_{cat}/K_m) of TPZ and its 1-oxide in P-450R-catalyzed reduction is in the range of reactivities of quinones and nitroaromatic compounds with similar values of E^1_7. In general, the log k_{cat}/K_m of compounds increases with an increase in their E^1_7, although a linear regression between these two parameters is scattered (r^2 = 0.7985). A similar although more scattered relationship is characteristic of FNR reactions (data not shown). Interestingly, 3-amino-1,2,4-benzotriazine reacted with P-450R and FNR with rates similar to TPZ and its 1-*N*-oxide (Table 1). The E^1_7 value of

Table 1. **Single-electron reduction midpoint potentials (E^1_7) of compounds and their enzymatic reduction apparent bimolecular rate constants (k_{cat}/K_m)**

No.	Compounds	E^1_7 (V)[a]	k_{cat}/K_m (M^{-1} s^{-1})	
			P-450R	FNR
1.	3-Amino-1,2,4-benzotriazine-1,4-dioxide	–0.455	$1.1 \pm 0.1 \times 10^4$	$4.4 \pm 0.3 \times 10^3$
2.	3-Amino-1,2,4-benzotriazine-1-oxide	–0.568	$2.4 \pm 0.2 \times 10^3$	$4.2 \pm 0.2 \times 10^3$
3.	3-Amino-1,2,4-benzotriazine	–	$6.0 \pm 0.4 \times 10^3$	$1.4 \pm 0.2 \times 10^4$
4.	Tetramethyl-1,4-benzoquinone	–0.260	$1.0 \pm 0.1 \times 10^6$	$8.6 \pm 0.7 \times 10^4$
5.	1,8-Dihydroxy-9,10-anthraquinone	–0.325	$8.5 \pm 1.0 \times 10^5$	$3.2 \pm 0.4 \times 10^5$
6.	2-Hydroxy-1,4-naphthoquinone	–0.41	$1.3 \pm 0.2 \times 10^4$	$1.0 \pm 0.1 \times 10^4$
7.	1,3-Dinitrobenzene	–0.345	$1.3 \pm 0.1 \times 10^5$	$2.7 \pm 0.3 \times 10^3$
8.	4-Nitroacetophenone	–0.355	$4.0 \pm 0.4 \times 10^4$	$3.3 \pm 0.4 \times 10^3$
9.	4-Nitrobenzyl alcohol	–0.475	680 ± 40	390 ± 55
10.	Nitrobenzene	–0.485	670 ± 80	55 ± 10

[a] From [3, 26].

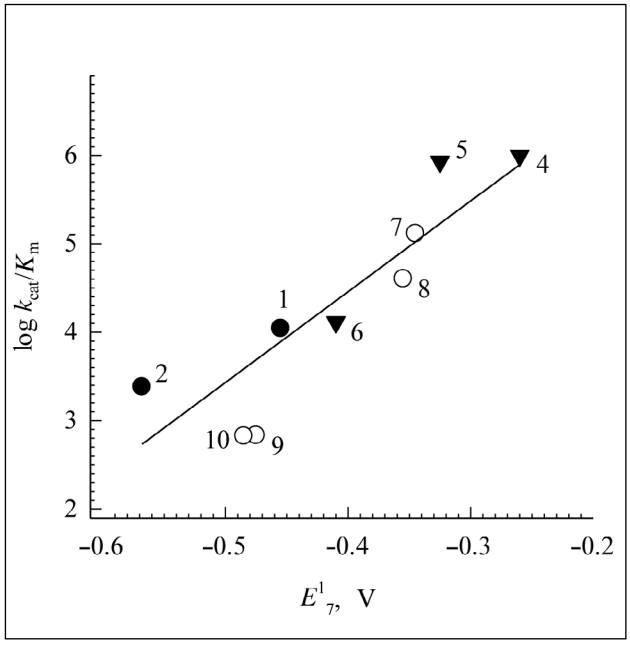

Fig. 2. Dependence of the reactivity of P-450R on the E^1_7 value of electron acceptors: TPZ (1), its 1-oxide (2), tetramethyl-1,4-benzoquinone (4), 1,8-dihydroxy-9,10-anthraquinone (5), 2-hydroxy-1,4-naphthoquinone (6), 1,3-dinitrobenzene (7), 4-nitroacetophenone (8), 4-nitrobenzyl alcohol (9), and nitrobenzene (10)

this compound is unavailable, however, it is electrochemically reversibly reduced to 1,4-dihydro derivative at –0.61 V vs Ag/AgCl (pH 7.4), which is close to the reduction potential of TPZ and its 1-*N*-oxide [27].

The cytotoxicity studies were performed using the murine hepatoma MH22a line, which is characterized by the activity of P-450R and NQO1 of 6.2 ± 1.2 nmol × mg^{-1} × min^{-1} and 79.5 ± 7.5 nmol × mg^{-1} × min^{-1}, respectively [20]. In this cell line, log cL_{50} of quinones exhibit a negative linear dependence on their E^1_7 values in the range of –0.15 V – –0.41 V [20]. We found that the log cL_{50} of the examined nitroaromatic compounds also followed this rule, however, the cytotoxicity of TPZ and its 1-*N*-oxide metabolite was higher than expected (Table 2, Fig. 3A).

Alternatively, for the data analysis involving 3-amino-1,2,4-benzotriazine with an unavailable value of E^1_7, we applied the previously used approach for description of the prooxidant cytotoxicity of nitroaromatics and quinones, namely the geometrical mean of their reactivity in P-450R and FNR-catalyzed reactions (0.5 log k_{cat}/K_m (P-450R) + 0.5 log k_{cat}/K_m (FNR)) [28, 29] (Table 2). In this case, a significant deviation from a linear regression is evident only for TPZ (Fig. 3B). An enhanced cytotoxicity of TPZ and, possibly, its 1-oxide, may not be attributed to their higher lipophilicity, because their calculated log *P* values are even lower than those of other examined compounds (Table 2).

The data of Fig. 4 show that the cytotoxicity of TPZ and its nor-oxide derivative was decreased in the presence of antioxidant *N,N'*-diphenyl-*p*-phenylene diamine (DPPD) and Fe-ion chelator desferroxamine, and enhanced by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), the latter inactivating glutathione reductase and depleting reduced glutathione [30]. Analogous effects were observed studying the cytotoxicity of 1-oxide of TPZ and other compounds (data not shown).

Table 2. **Compound concentrations for the 50% survival of MH22a cells (cL_{50}), the geometrical means of their k_{cat}/K_m in the reactions with P-450R and FNR (0.5 log k_{cat}/K_m (P-450R) + 0.5 log k_{cat}/K_m (FNR)), and their calculated octanol/water partition coefficients (log P_{calc})**

No.	Compounds	cL_{50}, μM	0.5 log k_{cat}/K_m (P-450R) + 0.5 log k_{cat}/K_m (FNR)	Log P_{calc}
1.	3-Amino-1,2,4-benzotriazine-1,4-dioxide	33 ± 5.0	3.84	–0.31 ± 0.20
2.	3-Amino-1,2,4-benzotriazine-1-oxide	≥600	3.50	–2.05 ± 0.64
3.	3-Amino-1,2,4-benzotriazine	≥1200	3.94	0.08 ± 0.53
4.	Tetramethyl-1,4-benzoquinone	59 ± 5.0a	5.47	2.63 ± 0.37
5.	1,8-Dihydroxy-9,10-anthraquinone	120 ± 15a	5.72	4.57 ± 0.77
6.	2-Hydroxy-1,4-naphthoquinone	500 ± 80a 540 ± 70	4.05	1.55 ± 0.75
7.	1,3-Dinitrobenzene	130 ± 17	4.27	1.84 ± 0.23
8.	4-Nitroacetophenone	239 ± 31	4.05	1.42 ± 0.24
9.	4-Nitrobenzyl alcohol	2800 ± 500	2.71	0.76 ± 0.23
10.	Nitrobenzene	1800 ± 200	2.28	1.95 ± 0.20

[a] From [20].

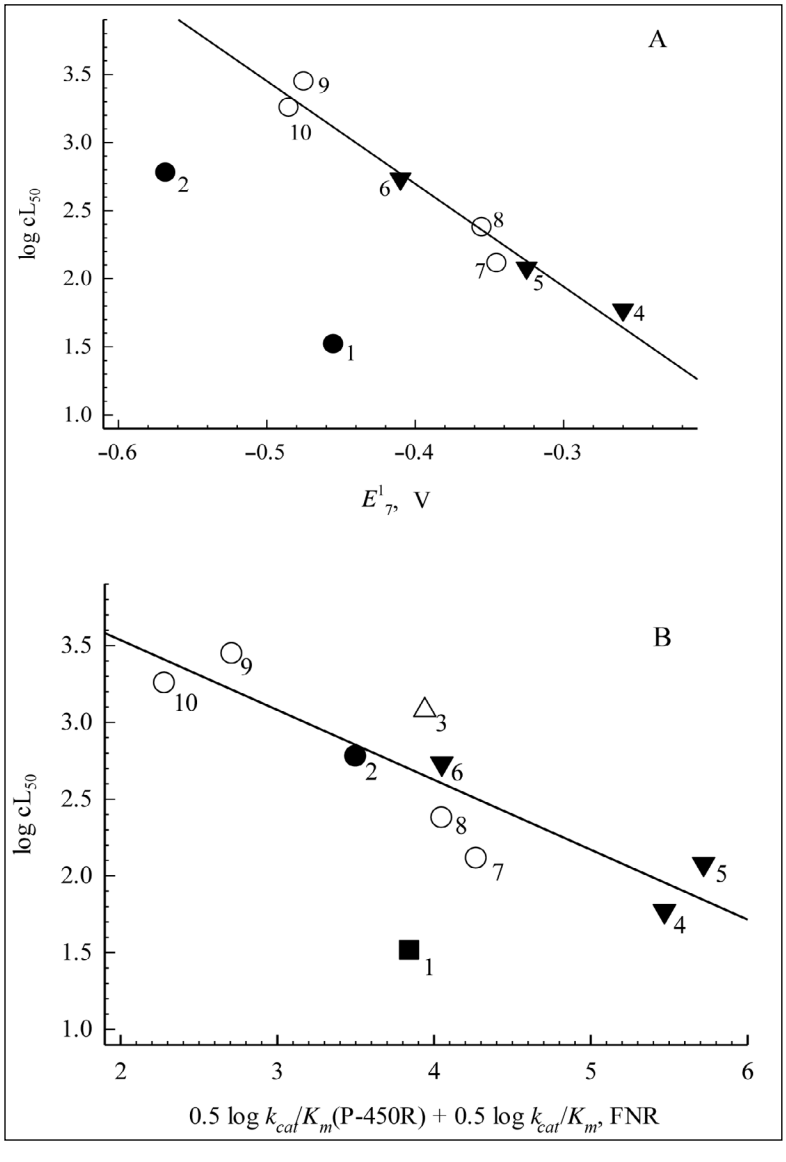

Fig. 3. The dependence of cytotoxicity of the examined compounds in MH22a cells on their single-electron reduction midpoint potential (E^1_7) (A), and on the geometrical mean of their reactivity in P-450R and FNR-catalyzed reactions (0.5 log k_{cat}/K_m (P-450R) + 0.5 log k_{cat}/K_m (FNR)) (B). The numbers of compounds are taken from Tables 1 and 2. The first order regression lines were drawn through the values of cL_{50} of compounds 4–10 (A) and 2–10 (B)

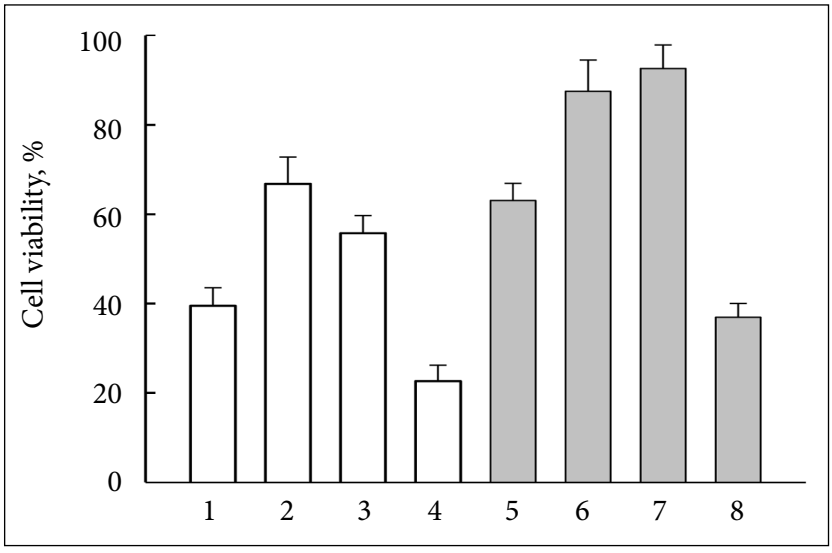

Fig. 4. Viability of MH22a cells in the presence of 50 μM TPZ (1–4) and 1000 μM 3-amino-1,2,4-benzotriazine (5–8). Additions: compound (1, 5), compound + 3.0 μM DPPD (2, 6), compound + 300 μM desferrioxamine (3, 7), compound + 20 μM BCNU (4, 8), $n = 3$, $p < 0.02$ for 1 against 2–4, and 5 against 6–8

An inhibitor of NQO1, dicumarol (20 μM), protected against the TPZ cytotoxicity, however, its effects were statistically insignificant.

DISCUSSION

Our data show for the first time that the reactivity of TPZ and its 1-oxide towards single-electron transferring flavoenzymes P-450R and FNR is comparable to that of quinones and nitroaromatics, and varies according to their E^1_7 (Table 1, Fig. 2). This provides a background for the quantitative interpretation of TPZ cytotoxicity.

The modulation of cytotoxicity of TPZ and its metabolites by antioxidants and prooxidant BCNU (Fig. 4) is in line with the suggested oxidative stress-type aerobic cytotoxicity of TPZ [1]. The lower cytotoxicity of TPZ metabolites as compared with the parent compound (Table 2) is in line with the previous findings ([11], and references therein). However, the data of Fig. 3A, B show that the cytotoxicity of TPZ and, possibly, its 1-oxide is much higher than may be expected from their E^1_7 value and redox cycling ability. Concerning the possible additional factors of TPZ cytotoxicity, let us first address the role of NQO1, which, according to the earlier studies, was modest or even absent [8, 9]. In our case the protecting effects of the inhibitor of NQO1, 20 μM dicumarol were also statistically insignificant. Thus, the action of NQO1 may be not the factor determining an enhanced cytotoxicity of TPZ over the 'model' redox cycling compounds. An attractive alternative possibility is that a high aerobic cytotoxicity of TPZ stems from the specific redox chemistry of aromatic *N*-oxides, namely, the formation of oxidizing and DNA-damaging species from their radicals (Fig. 1), which is not characteristic of quinones and nitroaromatics. Although these events take place under hypoxia, they may manifest themselves in relatively poorly oxygenated cell compartments, e.g. nucleus [31], but with a lower efficiency. This assumption is in line with the data of [7], which point to the leading role of the intranuclear reductive activation of TPZ in its hypoxic cytotoxicity. Our studies on further differences between the mechanisms of the aerobic cytotoxicity of aromatic di-*N*-oxides, quinones and nitroaromatic compounds including the apoptosis induction are currently underway.

CONCLUSIONS

Our data show that the aerobic cytotoxicity of TPZ and, possibly, its 1-oxide in MH22a hepatoma cells is much higher than may be expected from their E^1_7 value and redox cyling ability. It possibly stems from the specific redox chemistry of aromatic *N*-oxides, namely, the formation of oxidizing and DNA-damaging species from their radicals, which is not characteristic of quinones and nitroaromatics. Although these events take place under hypoxia, they may manifest themselves in relatively poorly oxygenated cell compartments, e.g. nucleus, but with a lower efficiency.

ACKNOWLEDGEMENTS

This work was supported by the European Social Fund (Measure No. 09.33-LMT-K-712, Grant

No. DOTSUT-34/09.3.3.-LMT-K712-01-0058/LSS-600000-58).

Received 13 September 2018
Accepted 18 September 2018

References

1. P. Wardman, M. F. Dennis, S. A. Everett, K. B. Patel, M. R. L. Stratford, M. Tracy, *Biochem. Soc. Symp.*, **61**, 171 (1995).
2. X. Shen, A. Rajapakse, F. Gallazzi, et al., *Chem. Res. Toxicol.*, **22**, 111 (2013).
3. S. S. Shinde, A. Maroz, M. P. Hay, A. V. Patterson, V. A. Denny, R. F. Anderson, *J. Am. Chem. Soc.*, **132**, 2591 (2010).
4. R. F. Anderson, P. Yadav, S. S. Shinde, C. R. Hong, S. M. Pullen, J. Reynisson, *Chem. Res. Toxicol.*, **25**, 1310 (2016).
5. T. Fuchs, G. Chowdhury, C. L. Barnes, K. S. Gates, *J. Org. Chem.*, **66,** 107 (2001).
6. F. W. Hunter, R. J. Young, Z. Shalev, et al., *Cancer Res.*, **75**, 4211 (2015).
7. Y. M. Delahoussaye, J. W. Evans, J. M. Brown, *Biochem. Pharmacol.*, **62**, 1201 (2001).
8. A. Cahill, T. C. Jenkins, I. N. H. White, *Biochem. Pharmacol.*, **45**, 321 (1993).
9. S. Y. Sharp, L. R. Kelland, M. R. Valenti, L. A. Brunton, S. Hobbs, P. Workman, *Mol. Pharmacol.*, **58**, 1146 (2000).
10. M. P. Hay, S. A. Gamage, M. S. Kovacs, et al., *J. Med. Chem.*, **46**, 169 (2003).
11. K. O. Hicks, B. G. Siim, J. K. Jaiswal, et al., *Clin. Cancer Res.*, **16**, 4946 (2010).
12. Y. Gu, T. T.-A. Chang, J. Wang, et al., *Front. Pharmacol.*, **8**, 531 (2017).
13. B. Zarranz, A. Jaso, I. Aldana, A. Monge, *Bioorg. Med. Chem.*, **12**, 3711 (2004).
14. G. Chowdhury, U. Sarkar, S. Pullen, et al., *Chem. Res. Toxicol.*, **12**, 197 (2012).
15. G. Cheng, W. Sa, C. Cao, et al., *Front. Pharmacol.*, **7**, 64 (2016). DOI: 10.3389/fphar.2016.00064.
16. P. Pazdera, M. Potacek, *Chem. Papers*, **42**, 527 (1988).
17. J. Gauthier, J. S. Duceppe, *J. Heterocycl. Chem.*, **21**, 1081 (1984).
18. T. A. Pechurskaja, I. N. Harnastai, I. P. Grabovec, A. A. Gilep, S. A. Usanov, *Biochem. Biophys. Res. Commun.*, **353**, 598 (2007).
19. E. Balconi, A. Pennati, D. Crobu, et al., *FEBS J.*, **276**, 4249 (2009).
20. A. Nemeikaitė-Čėnienė, R. Jarašienė, H. Nivinskas, J. Šarlauskas, N. Čėnas, *Chemija*, **26**, 46 (2015).
21. P. J. O'Brien, W. C. Wong, J. Silva, S. Khan, *Xenobiotica*, **20**, 945 (1990).
22. A. Nemeikaitė-Čėnienė, J. Šarlauskas, Ž. Anusevičius, H. Nivinskas, N. Čėnas, *Arch. Biochem. Biophys.*, **416**, 110 (2003).
23. N. Čėnas, Ž. Anusevičius, D. Bironaitė, G.I. Bachmanova, A.I. Archakov. K. Öllinger, *Arch. Biochem. Biophys.*, **315**, 400 (1994).
24. Ž. Anusevičius, M. Martinez-Julvez, C. G. Genzor, H. Nivinskas, C. Gomez-Moreno, N. Čėnas, *Biochim. Biophys. Acta*, **1320**, 247 (1997).
25. Ž. Anusevičius, H. Nivinskas, J. Šarlauskas, M.-A. Sari, J.-L. Boucher, N. Čėnas, *Acta Biochim. Pol.*, **60**, 217 (2013).
26. P. Wardman, *J. Phys. Chem. Ref. Data*, **18**, 1637 (1989).
27. J. H. Tocher, N. S. Virk, D. I. Edwards, *Biochem. Pharmacol.*, **39**, 781 (1990).
28. N. Čėnas, A. Nemeikaitė-Čėnienė, E. Sergedienė, H. Nivinskas, Ž. Anusevičius, J. Šarlauskas, *Biochim. Biophys. Acta*, **1528**, 31 (2001).
29. A. Nemeikaitė-Čėnienė, E. Sergedienė, H. Nivinskas, N. Čėnas, *Z. Naturforsch.*, **57c**, 822 (2002).
30. K. Öllinger, A. Brunmark, *J. Biol. Chem.*, **266**, 21496 (1991).
31. H. Kurokawa, H. Ito, M. Inoue, et al., *Sci. Rep.*, **5**, 10657 (2015).

Jonas Šarlauskas, Aušra Nemeikaitė-Čėnienė, Audronė Marozienė, Lina Misevičienė, Mindaugas Lesanavičius, Narimantas Čėnas

TIRAPAZAMINO IR JO 1-OKSIDO BEI NOR-OKSIDO METABOLITŲ VIENELEKTRONINĖ FERMENTINĖ REDUKCIJA IR JŲ AEROBINIS CITOTOKSIŠKUMAS

S a n t r a u k a

Bioredukciškai aktyvuojamo hipoksijai selektyvaus priešnavikinio agento 3-amino-1,2,4-benzotriazino-1,4-dioksido (tirapazamino, TPZ) aerobinis citotoksiškumas sukelia pašalinius efektus chemoterapijoje. Siekdami išaiškinti jų mechanizmus, ištyrėme aerobinį TPZ ir jo pagrindinių metabolitų 3-amino-1,2,4-benzotriazino-1-oksido ir 3-amino-1,2,4-benzotriazino citotoksiškumą pelės hepatomos MH22a ląstelėse ir jų redukciją NADPH: cytochromo P-450 reduktaze (P-450R) ir ferredoksin:$NADP^+$ reduktaze (FNR). Analogiškai buvo ištirti keli chinonai ir nitroaromatiniai junginiai, turintys panašius vienelektroninės redukcijos potencialus (E^1_7). Vienelektroninės redukcijos P-450R ir FNR atveju TPZ ir jo monoksido reakcingumas buvo artimas chinonų ir nitroaromatinių junginių reakcingumui ir didėjo didėjant jų E^1_7. TPZ ir jo metabolitų citotoksiškumas buvo prooksidantinis, nes jis buvo slopinamas antioksidantų *N,N'*-difenil-*p*-fenilendiamino ir desferioksamino bei stiprinamas 1,3-bis(2-chloretil)-1-nitrozokarbamido. Svarbu tai, kad TPZ ir, galimai, jo 1-oksido citotoksiškumas buvo daug didesnis nei panašaus E^1_7 ar ciklinių redokso virsmų aktyvumo chinonų arba nitroaromatinių junginių. Galimas papildomas TPZ aerobinio citotoksiškumo veiksnys yra jo redukcinė aktyvacija ląstelės branduolyje, kur dėl žemesnės deguonies koncentracijos susidaro DNR pažeidžiantys produktai, analogiški susidarantiesiems hipoksijos sąlygomis.