CHEMIJA. 2015. Vol. 26. No. 1. P. 46–50
© Lietuvos mokslų akademija, 2015

# Cytotoxicity of anticancer aziridinyl-benzoquinones in murine hepatome MH22a cells: the properties of RH1-resistant subline

**Aušra Nemeikaitė-Čėnienė**[1],

**Rasa Jarašienė**[2],

**Henrikas Nivinskas**[2],

**Jonas Šarlauskas**[2],

**Narimantas Čėnas**[2]*

[1] *State Research Institute Centre for Innovative Medicine, Molėtų Rd. 29, LT-08409 Vilnius, Lithuania*

[2] *Institute of Biochemistry of Vilnius University, Mokslininkų St. 12, LT-08662 Vilnius, Lithuania*

2,5-Diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1) is a potential anticancer agent, which underwent both preclinical and phase-I clinical trials. However, the data of the previous studies did not sufficiently distinguish the relative impact of its main mechanisms of cytotoxicity, the bioreductive activation by DT-diaphorase (NQO1), and redox cycling under the action of single-electron transferring enzymes. For the discrimination between the roles of these mechanisms, we examined the cytotoxicity of RH1 and other quinones in MH22a murine hepatoma cells and their RH1-resistant subline. In MH22a cells, the cytotoxicity of aziridinyl-unsubstituted quinones increased with an increase in their single-electron reduction potential ($E^1_7$). Taken together with the protection by the antioxidants, it points to a dominating oxidative stress-type cytotoxicity mechanism. The cytotoxicity of RH1 and other aziridinyl-substituted benzoquinones was higher than expected from their $E^1_7$ values. Taken together with the protection by dicumarol, it points to an additional involvement of NQO1 in the cytotoxicity. The derived subline of MH22a with 16.6-fold resistance to RH1 was not cross-resistant to duroquinone and daunorubicin. In this subline, the activity of NQ01 was decreased by 24 times, whereas the activities of NAD(P)H:cytochrome *c* reductases, catalase, superoxide dismutase, and glutathione reductase decreased much less significantly. It points to the dominating role of NQO1 in the cytotoxicity of RH1.

**Key words:** aziridinylbenzoquinones, cytotoxicity, NAD(P)H:quinone oxidoreductase, oxidative stress

**Abbreviations:** BCNU – 1,3-bis(2-chloromethyl)-1-nitrosourea; BZQ – 2,5-bis(2'-hydroxyethylamino)-3,6-diaziridinyl-1,4-benzoquinone; $cL_{50}$ – the concentration of compound for 50% cell survival; DPPD – *N,N'*-diphenyl-*p*-phenylene diamine; DZQ – 2,5-diaziridinyl-1,4-benzoquinone; $E^1_7$ – redox potential of quinone/semiquinone couple at pH 7.0; MeDZQ – 2,5-dimethyl-3,6-diaziridinyl-1,4-benzoquinone; NQO1 – NAD(P)H:quinone oxidoreductase; P-450R – NADPH:cytochrome P-450 reductase; RH1 – 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone.

## INTRODUCTION

Aziridinyl-substituted 1,4-benzoquinones (Fig. 1) comprise an important group of antitumour agents ([1], and references therein). In general, their cytotoxicity / antitumour activity stems from the following: i) the net two-electron reduction by flavoenzyme NAD(P)H: quinone oxidoreductase (NQO1, EC 1.6.99.2) into the coresponding aziridinylhydroquinones, which alkylate DNA more rapidly than the parent quinones [1]. This may increase their toxicity in certain cancer cell lines with the increased levels of NQO1 [2]; ii) the aziridinyl-substituted benzoquinones with strong electron-donating substituents, e. g. 2,5-bis(2'-hydroxyethylamino)-3,6-diaziridinyl-1,4-benzoquinone (BZQ, Fig. 1) may alkylate DNA directly, without the bioreductive activation [3]; and iii) both aziridinyl-substituted and -unsubstituted quinones exert the 'oxidative stress-type' cytotoxicity due to their reduction by NADPH: cytochrome P-450 reductase (P-450R, EC 1.6.2.4) or by other single-electron transferring enzymes into their free radicals. The free radicals further undergo the redox cycling with the formation of reactive oxygen species [4–7]. This mode of cytotoxicity, the oxidative stress, is neutralized by the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase.

* Corresponding author. E-mail: narimantas.cenas@bchi.vu.lt

Recently, 2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1) (Fig. 1) emerged as a well-promising potential antitumour agent, demonstrating its activity at submicromolar concentrations [1, 4], and the encouraging results in preclinical and phase-I clinical trials [8–10]. Although it was initially suggested that the anticancer activity of RH1 stems mainly from its two-electron reduction by NQO1 [4, 11], the further studies challenged this suggestion [5, 6, 12], pointing to a possible significant role of its oxidative stress-type cytotoxicity, resulting from the single-electron reduction by P-450R and other single-electron transferring enzymes.

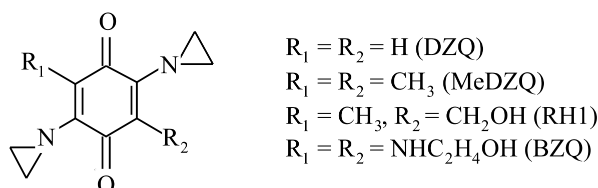


**Fig. 1.** The formulae of diaziridinyl-substituted benzoquinones studied in this work

In order to clarify this problem, we attempted to distinguish between the roles of NQO1-catalyzed reactions and single-electron reduction-induced redox cycling in the cytotoxicity of RH1 in MH22a murine hepatoma cells. For this purpose, we examined the relationships between the cytotoxicity of the series of aziridinyl-substituted and unsubstituted quinones, and their single-electron reduction potential ($E^1_7$, redox potential of quinine / semiquinone couple). Further, we for the first time derived RH1-resistant MH22a subline, which was not cross-resistant to other prooxidant compounds, and possessed a significantly decreased level of NQO1. Taken together, our data point to the dominating role of NQO1-catalyzed bioreductive activation in the cytotoxicity of RH1.

## EXPERIMENTAL

Xanthine oxidase, xanthine, $H_2O_2$, NADH, NADPH, cytochrome *c,* dicumarol, desferrioxamine, *N,N'*-diphenyl-*p*-phenylene diamine (DPPD), 1,3-bis(2-chloromethyl)-1-nitrosourea (BCNU), model quinones, and oxidized glutathione (GSSG) were obtained from Sigma-Aldrich and were used as received. The synthesis of aziridinyl-substituted quinones (Fig. 1) was performed according to the established methods [13–16]. All synthesized compounds were characterized by a melting point and $^1$H-NMR, UV, and IR spectroscopy. Daunorubicin was obtained from Minmedprom (Russia).

The culture of murine hepatoma MH22a cells [17] was grown and maintained at 37 °C in the DMEM medium, supplemented with 10% fetal bovine serum and antibiotics in 5–10 ml flasks until reaching the confluence. For the cytotoxicity tests, the cells were trypsinized, twice washed with phosphate buffered saline (PBS), and reseeded ($3.0 \times 10^4$/ml) on glass slides in 6 well cell culture plates in the absence or presence of compounds, and were grown for 24 h. Further, the slides were rinsed 2–3 times with PBS and stained with Trypan blue. The cells on the slide were calculated under a light microscope. Cell viability was expressed as the percentage of viable (Trypan blue excluding) cells, remaining adherent after the compound treatment with respect to their amount after 24 h growth in the absence of a compound. The adherent MH22a cells showed 98–99% viability, while the detached ones were found to be 98–99% nonviable. The tested quinone compounds were dissolved in DMSO, making 0.2% concentration of DMSO in the medium. This concentration of DMSO did not affect the cell viability.

The RH1-resistant subline of MH22a (MH22a-R) was obtained as follows: MH22a cells were grown till 80% confluence in the absence of RH1, then 0.15 μM RH1 was introduced. Following this, the cells were grown in the presence of 0.15 μM RH1 for 5 months, involving the change of the growth medium each 7th day, and the reseeding of the cells reaching the confluence. In total, 16 cell passages were performed during 5 months, which resulted in a resistant subline. The attempts of a subsequent increase in the RH1 concentration up to 0.30 μM after a cultivation period of 2 months lead to the complete suppression of cell proliferation, monitored for 50 days.

For the enzymatic analysis, MH22a cells and their resistant subline were grown until confluence, detached by trypsinization, twice washed with PBS, and sonicated on ice in four cycles of 20 s. The homogenate was centrifuged at 14 000 × *g* for 45 min and the resulting supernatant with added 1.0 mM PMSF was used for enzymatic analysis. Protein concentration was determined according to the method of Bradford. All the spectrophotometric measurements were performed using a Hitachi-557 spectrophotometer at 25 °C in 0.1 M K-phosphate (pH 7.0) containing 1 mM EDTA. The activity of catalase was determined following the decomposition of 10 mM $H_2O_2$ at 240 nm according to $\Delta\varepsilon_{240} = 0.04$ mM$^{-1}$ cm$^{-1}$ [18]. The activity of superoxide dismutase was determined from the inhibition of reduction of 100 μM nitrobluetetrazolium by a xanthine oxidase / xanthine system monitored at 560 nm. The activity of NAD(P)H: oxidases was determined according to the rate of oxidation of 100 μM NAD(P)H ($\Delta\varepsilon_{340} = 6.2$ mM$^{-1}$ cm$^{-1}$). The activity of NAD(P)H: cytochrome *c* reductases was determined according to the rate of reduction of 50 μM cytochrome *c* ($\Delta\varepsilon_{550} = 20$ mM$^{-1}$ cm$^{-1}$) in the presence of 100 μM NAD(P)H. The activity of glutathione reductase was determined according to the rate of oxidation of 100 μM NADPH in the presence of 1.0 mM GSSG. The activity of NAD(P)H: quinone oxidoreductase (NQO1) was determined following the rate of reduction of 50 μM cytochrome *c* in the presence of 10 μM menadione (2-methyl-1,4-naphthoquinone) and 100 μM NADPH, as a difference between the reduction

rate in the absence of dicumarol and its presence (20 μM). In this assay, Tween 20 (0.01%) and bovine serum albumin (0.25 mg × $ml^{-1}$) were used as activators of NQO1.

Statistical analysis was performed using Statistica (version 4.3, StatSoft Inc., 1993).

## RESULTS AND DISCUSSION

The mammalian cell cytotoxicity of quinones frequently increases with an increase in their single-electron reduction potential ($E^1_7$) with the coefficient $\Delta \log cL_{50} / \Delta E^1_7 \sim -10\ V^{-1}$, where $cL_{50}$ is the compound concentration for 50% cell survival [4, 7, 19, 20]. This type of dependence points to the dominating role of the oxidative stress-type cytotoxicity because it reflects the ease of the single-electron reduction of quinones by P-450R or other single-electron transferring flavoenzymes, i. e. a linear or parabolic log (rate constant) vs. $E^1_7$ relationship ([4, 7], and references therein), which initiates their redox cycling. The 24 h cytotoxicity studies of the model aziridinyl-unsubstituted quinones ($n$ = 10) in MH22a cells show that their cytotoxicity also increased with an increase in their $E^1_7$ (Table 1, Fig. 2). Importantly, aziridinyl-substituted quinones DZQ, RH1, MeDZQ, trimethyl-2-aziridinyl-1,4-benzoquinone, and BZQ, and redox active and DNA-intercalating anticancer anthracycline, daunorubicin, displayed higher cytotoxicity than may be expected from their $E^1_7$ values (Fig. 2). It points to the additional mechanisms of their action.

Table 1. **Redox potentials of quinone / semiquinone couples of quinones at pH 7.0 ($E^1_7$) [1, 21] and their concentrations for 50% survival of MH22a cells during 24 h incubation ($cL_{50}$)**

| No. | Compound | $E^1_7$, V | $cL_{50}$, μM |
|---|---|---|---|
| 1. | 2,3-Dichloro-1,4-naphthoquinone | –0.035 | 2.7 ± 0.1 |
| 2. | DZQ | –0.054 | 0.25 ± 0.05 |
| 3. | 5-Hydroxy-1,4-naphthoquinone | –0.09 | 2.5 ± 0.04 |
| 4. | 5,8-Dihydroxy-1,4-naphthoquinone | –0.11 | 0.58 ± 0.05 |
| 5. | 9,10-Phenanthrene quinone | –0.12 | 4.6 ± 0.3 |
| 6. | Trimethyl-1,4-benzoquinone | –0.15 | 25.0 ± 8.0 |
| 7. | 1.4-Naphthoquinone | –0.15 | 3.1 ± 0.05 |
| 8. | 2-Methyl-1,4-naphthoquinone | –0.20 | 18.0 ± 1.3 |
| 9. | Trimethyl-aziridinyl-1,4-benzoquinone | –0.23 | 1.3 ± 0.3 |
| 10. | MeDZQ | –0.23 | 0.31 ± 0.05 |
| 11. | RH1 | –0.23 | 0.12 ± 0.02 |
| 12. | Tetramethyl-1,4-benzoquinone | –0.26 | 59.0 ± 5.0 |
| 13. | 1,8-Dihydroxy-1,4-anthraquinone | –0.325 | 120 ± 15.0 |
| 14. | Daunorubicin | –0.34 | 5.9 ± 0.5 |
| 15. | BZQ | –0.38 | 28.0 ± 4.0 |
| 16. | 2-Hydroxy-1,4-naphthoquinone | –0.41 | 500 ± 80 |

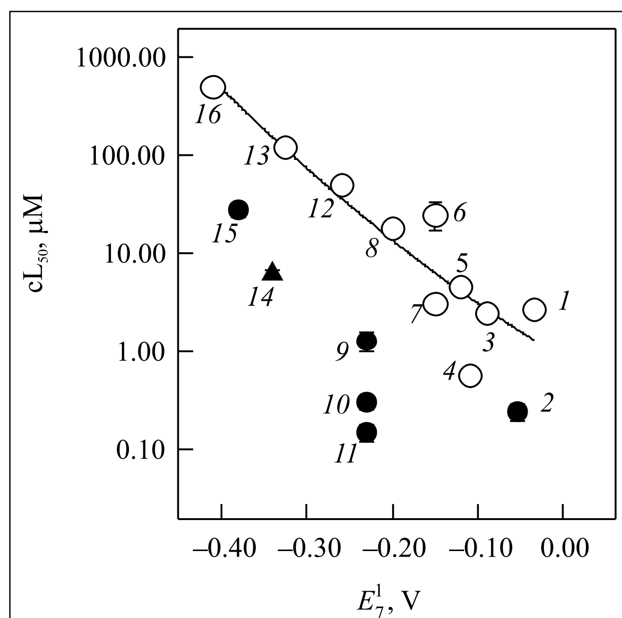


**Fig. 2.** The dependences of $cL_{50}$ of aziridinyl-unsybstituted quinones (blank circles), aziridinyl-substituted quinones (solid circles), and daunorubicin (solid triangle) in MH22a cells on the redox potentials of quinone / semiquinone couples of quinones at pH 7.0 ($E^1_7$). The numbers of compounds correspond to those given in Table 1. The line corresponds to the second order (parabolic) regression drawn through the $cL_{50}$ values of aziridinyl-unsubstituted quinones

Next, we compared the action of RH1 and its aziridinyl-unsubstituted analogue duroquinone (DQ, tetramethyl-1,4-benzoquinone), which are characterized by similar $E^1_7$ values (Table 1), by similar redox cycling activity in P-450R-catalyzed reactions, and by similar reactivity towards the two-electron transferring NQO1 [4] (Fig. 3A, B). In this case, the cytotoxicity of DQ and RH1 was partly prevented by the antioxidants DPPD and desferrioxamine, and potentiated by the prooxidant BCNU, the latter inactivating the antioxidant enzyme NADPH: glutathione reductase, and depleting the reduced glutathione, GSH [22] (Fig. 3A, B). It points to an involvement of the oxidative stress-type cytotoxicity in their action. However, an inhibitor of NQO1, dicumarol, did not affect the cytotoxicity of DQ (Fig. 3B), whereas it partly protected against the cytotoxicity of RH1 (Fig. 3A). The analogous results were obtained in the studies of cytotoxicity of MeDZQ (data not shown). It points to the additional involvement of NQO1 in the cytotoxicity of RH1 and MeDZQ, which may enhance their cytotoxicity with respect to aziridinyl-unsubstituted quinones (Fig. 2).

According to our best knowledge, the RH1-resistant cell sublines have not been obtained so far. We derived a subline of MH22a hepatoma cells (MH22a-R), which was resistant to RH1 by 16.6 times (Table 2). Importantly, this subline was not cross-resistant to DQ and to another quinone, anticancer agent daunorubicin, which exhibits both prooxidant and DNA-intercalating properties [23] (Table 2).

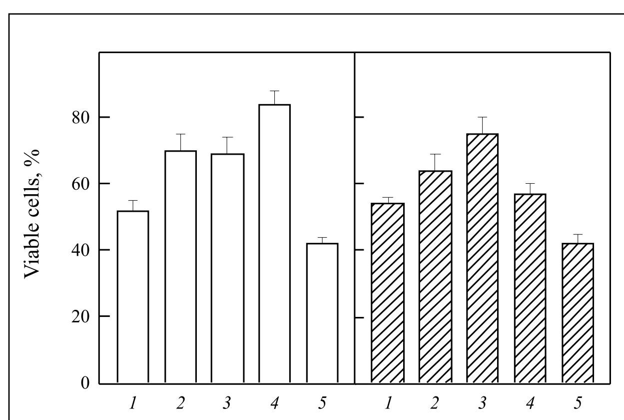


**Fig. 3.** Viability of MH22a cells in the presence of 0.12 μM RH1 (A) and 50 μM duroquinone (B). Additions: quinone (*1*), quinone + 3.0 μM DPPD (*2*), quinone + 300 μM desferrioxamine (*3*), quinone + 20 μM dicumarol (*4*), and quinone + 20 μM BCNU (*5*) (n = 3). In the experiments with RH1 (A), $p < 0.03$ for *1* against *2–4*, and $p < 0.04$ for *1* against *5*. In the experiments with duroquinone (B), $p < 0.03$ for *1* against *2*, *3*, *5*, and $p > 0.05$ for *1* against *4*

Table 2. **The concentrations for 50% cell survival ($cL_{50}$) of RH1, tetramethyl-1,4-benzoquinone (duroquinone), and daunorubicin in MH22a cells and their RH1-resistant subline (n = 3)**

| No. | Cell line | $cL_{50}$, μM | | |
|---|---|---|---|---|
| | | a) RH1 | b) Duroquinone | c) Daunorubicin |
| 1. | MH22a | 0.12 ± 0.02 | 59.0 ± 5.0 | 5.9 ± 0.5 |
| 2. | MH22a-R | 2.0 ± 0.10 | 25.5 ± 3.0 | 5.0 ± 0.5 |

Subsequently, we examined the activities of the relevant redox enzymes in both cell lines. The activity of NQO1 in RH1-resistant MH22a cells was decreased by 24 times (Table 3), which points to the leading role of NQO1 in the manifestation of the cytotoxicity of RH1 in this case. Similarly, the 10–20-fold decreased activity of NQO1 was observed in human erythroleukemic K562 sublines resistant to another aziridinyl-substituted quinone, AZQ (2,5-bis(carboethoxy-amino)-3,6-diaziridinyl-1,4-benzoquinone) [24]. On the other hand, there also exist differences in the expression of other redox enzymes in RH1-resistant MH22a cells and AZQ-resistant K562 cells, which point to the different resistance mechanisms: i) The activities of both antioxidant enzymes (superoxide dismutase, catalase, and glutathione reductase), and the prooxidant ones (NAD(P)H: oxidases and NAD(P)H: cytochrome *c* reductases) were decreased insignificantly, by 1.5–2 times in RH1-resistant MH22a cells (Table 3). It shows that in RH1-resistant MH22a cells the balance between the prooxidant activities and the antioxidant defence was disturbed insignificantly. Probably for this reason, the RH1-resistant cells were not cross-resistant to other prooxidant quinones, duroquinone and daunorubicin (Table 2); ii) In contrast, the AZQ-resistant K562 sublines may be regarded as more antioxidant ones as compared to the parent cells because apart from a 2–2.5-fold decrease in the activity of P-450R, they possessed a 2–2.5-fold increased activity of the antioxidant enzyme superoxide dismutase [24]. It may partly explain their increased resistance to another redox cycling quinone, adriamycin.

## CONCLUSIONS

The cytotoxicity of aziridinyl-substituted quinone RH1 in MH22a cells is attributed to two main factors, the oxidative stress exerted by its redox cycling under the action of single-electron transferring flavoenzymes, and the two-electron reduction by flavoenzyme NQO1 into DNA-alkylating hydroquinone. However, the enzymatic properties of the RH1-resistant MH22a subline point to the leading role of NQO1 in the manifestation of the cytotoxicity of RH1. These data do not entirely match the previously reported enzymatic properties of the cells resistant to other diaziridinyl-benzoquinone AZQ, and point to an existence of different resistance mechanisms.

Table 3. **The activities of antioxidant and prooxidant enzymes in MH22a cells and their RH1-resistant subline (n = 3)**

| No. | Enzyme | Activity | |
|---|---|---|---|
| | | a) MH22a | b) MH22a-R |
| 1. | Superoxide dismutase[a] | 2.0 ± 0.40 | 1.5 ± 0.3 |
| 2. | Catalase[b] | 59.0 ± 7.0 | 25.7 ± 3.5 |
| 3. | Glutathione reductase[c] | 48.9 ± 5.5 | 28.2 ± 7.7 |
| 4. | NADH:oxidase[c] | 1.5 ± 0.3 | 0.8 ± 0.1 |
| 5. | NADPH:oxidase[c] | 1.1 ± 0.2 | 0.6 ± 0.1 |
| 6. | NADH:cytochrome *c* reductase[c] | 21.3 ± 3.7 | 10.4 ± 2.2 |
| 7. | NADPH:cytochrome *c* reductase[c] | 6.2 ± 1.2 | 2.9 ± 0.4 |
| 8. | NAD(P)H:quinone oxidoreductase (NQO1)[c] | 79.5 ± 7.5 | 3.3 ± 1.0 |

[a] Units/mg protein; one unit of enzyme activity is defined as amount of protein needed to inhibit the reduction of nitroblue tetrazolium by 50%;

[b] $\mu mol \times mg\ protein^{-1} \times min^{-1}$;

[c] $nmol \times mg\ protein^{-1} \times min^{-1}$.

## ACKNOWLEDGEMENTS

This research was funded by the European Social Fund under Global Grant Measure No. VP1-3.1-ŠMM-07-K01-103 (Scientific Council of Lithuania).

Received 21 November 2014
Accepted 12 December 2014

## References

1. A. M. DiFrancesco, T. H. Ward, J. Butler, *Methods Enzymol.*, **382B**, 174 (2004).
2. S. A. Fitzsimmons, P. Workman, M. Grever, K. Paull, R. Camalier, A. D. Lewis, *J. Natl. Cancer Inst.*, **88**, 259 (1996).
3. J. Butler, B. M. Hoey, T. H. Ward, *Biochem. Pharmacol.*, **36**, 923 (1989).
4. A. Nemeikaitė-Čėnienė, J. Šarlauskas, Ž. Anusevičius, H. Nivinskas, N. Čėnas, *Arch. Biochem. Biophys.*, **416**, 110 (2003).
5. A. Begleiter, M. K. Leith, D. Patel, B. B. Hasinoff, *Cancer Chemother. Pharmacol.*, **60**, 713 (2007).
6. C. Yan, J. K. Kepa, D. Siegel, I. J. Stratford, D. Ross, *Mol. Pharmacol.*, **74**, 1657 (2008).
7. P. J. O'Brien, *Chem.-Biol. Interact.*, **80**, 1 (1991).
8. T. H. Ward, S. Danson, A. T. McGown, et al., *Clin. Cancer Res.*, **11**, 2695 (2005).
9. D. Hussein, S. V. Holt, K. E. Brookes, et al., *Br. J. Cancer*, **101**, 55 (2009).
10. S. J. Danson, P. Johnson, T. H. Ward, et al., *Ann. Oncol.*, **22**, 1653 (2011).
11. S. L. Winski, E. Swann, R. H. J. Hargreaves, et al., *Biochem. Pharmacol.*, **61**, 1509 (2001).
12. G. Tudor, M. Alley, C. M. Nelson, et al., *Anti-Cancer Drugs*, **16**, 381 (2005).
13. S. Petersen, W. Gauss, E. Urbschat, *Angew. Chem.*, **67**, 217 (1955).
14. D. W. Cameron, R. F. G. Gilles, *J. Chem. Soc. C*, 1461 (1968).
15. F. Chou, A. H. Khan, J. S. Driscoll, *J. Med. Chem.*, **28**, 1302 (1976).
16. S. L. Winski, R. H. J. Hargreaves, J. Butler, D. Ross, *Clin. Cancer Res.*, **4**, 3083 (1998).
17. I. N. Shvemberger, S. A. Alexandrova, *Int. Rev. Cytol.*, **199**, 117 (2000).
18. H. U. Bergmeyer (ed.), *Methods of Enzymatic Analysis*, Verlag Chemistry Acad., New York (1965).
19. P. Wardman, *Free Rad. Res. Commun.*, **8**, 219 (1990).
20. E. Dičkancaitė, N. Čėnas, A. Kalvelytė, N. Serapinienė, *Biochem. Mol. Biol. Int.*, **41**, 987 (1997).
21. P. Wardman, *J. Phys. Chem. Ref. Data*, **18**, 1637 (1989).
22. K. Őllinger, A. Brunmark, *J. Biol. Chem.*, **266**, 21496 (1991).
23. D. A. Gewirtz, *Biochem. Pharmacol.*, **57**, 727 (1999).
24. T. H. Ward, M. S. Haran, D. Whittaker, A. J. Watson, T. D. Howard, J. Butler, *Biochem. Pharmacol.*, **50**, 459 (1995).

**Aušra Nemeikaitė-Čėnienė, Rasa Jarašienė, Henrikas Nivinskas, Jonas Šarlauskas, Narimantas Čėnas**

## PRIEŠNAVIKINIŲ AZIRIDINIL-BENZOCHINONŲ CITOTOKSIŠKUMAS PELIŲ HEPATOMOS MH22A LĄSTELĖSE: RH1 ATSPARIOS SUBLINIJOS SAVYBĖS

*Santrauka*

2,5-diaziridinil-3-(hidroksimetil)-6-metil-1,4-benzochinonas (RH1) yra potencialus priešnavikinis agentas, charakterizuotas ikiklinikiniais ir 1-osios fazės klinikiniais tyrimais. Ankstesnių tyrimų duomenys nepakankamai atskleidžia jo pagrindinių citotoksiškumo mechanizmų, t. y. DT-diaforazės (NQO1), atliekamos bioredukcinės aktyvacijos arba ciklinių redoks virsmų, veikiant vieną elektroną pernešantiems fermentams, santykinę svarbą. Siekdami detalizuoti šių mechanizmų vaidmenis, ištyrėme RH1 ir kitų chinonų citotoksiškumą pelių hepatomos ląstelėse MH22a ir RH1 nejautrioje MH22a sublinijoje. Aziridinil-nepavaduotų chinonų citotoksiškumas MH22a ląstelėse augo didėjant junginių vienelektroninės redukcijos potencialui ($E^1_7$). Papildomai remiantis apsauginiu antioksidantų poveikiu galima manyti, kad pagrindinis šių junginių citotoksiškumo mechanizmas yra oksidacinis stresas. RH1 ir kitų aziridinil-benzochinonų citotoksiškumas buvo didesnis nei galima tikėtis iš jų $E^1_7$ reikšmių. Apsaugantis dikumarolo poveikis rodo, kad NQO1 turi papildomą įtaką RH1 citotoksiškumui. Mūsų darbe gauta 16,6 kartų atsparesnė RH1 MH22a sublinija nebuvo atspari durochinonui ir daunorubicinui. NQO1 aktyvumas šioje sublinijoje buvo sumažėjęs 24 kartus, o NAD(P)H:citochromo *c* reduktazių, katalazės, superoksiddismutazės ir katalazės aktyvumai buvo sumažėję nežymiai. Vadinasi, RH1 citotoksiškumas yra iš esmės priklausomas nuo NQO1 poveikio.