Arch Toxicol (1994) 68: 255–260

Archives of
Toxicology

Quantitative structure activity relationship for the acute cytotoxicity of 13 (bis)aziridinyl-benzoquinones: relation to cellular ATP depletion

Bram Prins[1], Wim P. Dartee[1], Willem Verboom[2], David N. Reinhoudt[2], Andries Sj. Koster[1]

[1] Department of Pharmacology, Faculty of Pharmacy, University of Utrecht, PO Box 80.082, NL-3508 TB Utrecht, The Netherlands
[2] Department of Organic Chemistry, University of Twente, PO Box 217, NL-7500 AE Enschede, The Netherlands

Received: 23 June 1993/Accepted: 9 November 1993

Abstract. This study was performed to establish relationships between the structure of 2,5-bis(1-aziridinyl)-1,4-benzoquinones (BABQs) bearing different substituents at the 3- and 6-position and their acute toxic effects in rat hepatocytes. The cell viability, loss of cellular glutathione (GSH+GSSG) and loss of ATP were followed during 4 h of incubation of freshly isolated hepatocytes. The toxicity of these compounds (100 μM) was predicted better by their reactivity with GSH than by their redox cycling in rat liver microsomes. The time of 50% loss of viability (LT_{50}) correlated very well with the time of 50% depletion of ATP (AT_{50}). LT_{50} could be adequately predicted by using the electronic field parameter (F_{total}) describing the electron withdrawing or donating properties for all the substituents on the quinone-nucleus. 7-(Di)halogen-substituted BABQs that all very rapidly depleted cellular glutathione showed significant differences in AT_{50} as well as in LT_{50}. This suggests that alterations in ATP levels are important for explaining the differences in cytotoxicity of these compounds.

Key words: 2,5-Bis(1-aziridinyl)-1,4-benzoquinones – Carboquone – Diaziquone – Hepatocytes – Structure activity relationship – Toxicity in vitro – Triaziquone

Introduction

Quinones may exert their toxic effects by a number of different mechanisms (Koster 1991) including oxidative stress resulting from redox cycling (Kappus and Sies 1981), inhibition of mitochondrial electron transport (Phelps and Crane 1985) and interaction with the thiol groups of cellular macromolecules. In hepatocytes exposed to naphthoquinones, such as menadione, redox cycling is thought to play an important role in acute toxicity (DiMonte et al. 1982; Thor et al. 1985). For naphthoquinones depletion of cellular glutathione may result indirectly from redox cycling or from direct arylation of GSH (Rossi et al. 1986; Gant et al. 1988). However, quinones can also affect the energy status of hepatocytes by compromising mitochondrial ATP synthesis (Meredith and Reed 1982) or by inducing loss of reduced pyridine nucleotides (Kappus and Sies 1981). We have recently observed that the time of onset of menadione-induced cytotoxicity correlated better with ATP depletion than with GSH depletion (Redegeld et al. 1990) and that GSH depletion leads to cytotoxicity only when accompanied by ATP depletion (Redegeld et al. 1992). This suggests that ATP depletion, rather than redox cycling and/or GSH depletion, is a primary cause of acute quinone-induced cytotoxicity.

For the cytostatic agent diaziquone (2,5-bis(1-aziridinyl)-3,6-bis(ethoxy-carbonylamino)-1,4-benzoquinone) also, it was suggested that redox cycling with the formation of H_2O_2 plays an important role in the toxicity of this compound (Silva and O'Brien 1989). We have investigated redox cycling capacity of 22 different 3,6-disubstituted 2,5-bis(1-aziridinyl)-1,4-benzoquinones (BABQs) and found a 200-fold difference in superoxide anion production rate (measured in rat liver microsomes) of different compounds (Prins et al. 1989). In order to investigate whether acute cytotoxicity of these compounds can be correlated to redox cycling capacity we investigated in the present study the toxicity of 13 different BABQ derivatives. Compounds spanning the earlier observed range of redox cycling capacity were chosen. In addition, ATP and GSH depletion were measured to establish a possible role of ATP and/or GSH depletion.

Based on diaziquone and related compounds (Driscoll et al. 1979) a large series of 3,6-disubstituted BABQs was synthesized to evaluate structure-activity relationships for DNA alkylation and cytotoxic activity (Driebergen et al. 1986; Lusthof et al. 1990). The ability for redox cycling

Correspondence to: A.Sj. Koster

Table 1. Acute toxicity of 13 bis(aziridinyl)benzoquinones (100 μM) in relation to physicochemical properties

nr	Substituents						
	R_1	R_2	F_{total}[a]	MR_{total}[b]	SAP[c]	GT_{90}[d]	AT_{50}[e]	LT_{50}[f]
TW19	F	F	+ 0.76	1.45	53	2	13	20
TW14	Cl	Cl	+ 0.72	1.96	545	4	15	50
TW26	Az[g]	F	+ 0.28	2.08	52	2	15	90
TW22	Br	CH_3	+ 0.30	2.09	279	30	60	120
TW25	Br	C_2H_5	+ 0.29	2.32	260	35	90	150
TW50	Br	$C_2H_4OCONH_2$	+ 0.32	2.87	304	15	45	110
TW81	Cl	CH_3	+ 0.27	1.95	260	60	90	160
TW13	H	H	− 0.10	1.46	123	65	60	240
triaziquone	Az[g]	H	− 0.15	2.08	21	140	150	240
TW82	OCH_3	OCH_3	− 0.30	2.15	112	185	180	>240
TW39	CH_3	$C_2H_4OCONH_2$	− 0.18	2.71	10.4	80	180	>240
diaziquone	$NHCO_2C_2H_5$	$NHCO_2C_2H_5$	+ 0.18	3.48	36	>240	180	>240
carboquone	CH_3	$CH(OCH_3)CH_2OCONH_2$	− 0.16	3.05	3.7	120	170	>240

[a] F_{total}, summated electronic field constant, from Driebergen (1987)
[b] MR_{total}, summated molecular refraction, from Driebergen (1987)
[c] SAP, superoxide anion production rate (nmole/min per mg microsomal protein), from Prins et al. (1989)
[d] GT_{90}, time needed for 90% loss of cellular glutathione (min)
[e] AT_{50}, time needed for 50% loss of ATP in hepatocytes (min)
[f] LT_{50}, time needed for 50% loss of cellular viability (min)
[g] Az, aziridinyl group

and thiol reactivity of these compounds could be reasonably predicted from the electron donating and withdrawing effects of substituents in the 3- and 6-positions (Prins et al. 1989; Wilson et al. 1987; Wardman 1990). In the present study quantitative structure activity relationships for cytotoxicity of BABQ-derivatives in rat isolated hepatocytes were established.

Materials and methods

Compounds. The compounds that are used in this study are designated by TW numbers and presented in Table 1. BABQ derivatives were prepared as described before (Prins et al. 1989). Diaziquone [2,5-bis(1-aziridinyl)-3,6-bis(ethoxy-carbonylamino)-1,4-benzoquinone] was a gift from the Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Md., USA and carboquone [2,5-bis(1-aziridinyl)-3-{2-(carbamoyloxy)-1-(methoxyethyl)}-6-methyl-1,4-benzoquinone] was a gift from the Chemical Research Laboratories, Sankyo Co. Ltd, Tokyo, Japan. Triaziquone (Trenimon) was a gift from Bayer AG, Pharma Research Center, Wuppertal, Germany. Stock solutions of the quinone compounds (10 mM) were prepared in dimethylsulphoxide (DMSO, from Merck, scintillation grade).

Chemicals. Collagenase (E.C. 3.4.24.8), ATP and NADH were obtained from Boehringer-Mannheim GmbH. Glutathione reductase (E.C. 1.6.4.2), NADPH (type X) and glutathione disulfide (grade IV) were purchased from Sigma. Menadione, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Merck (Munich, Germany). All other chemicals were of analytical grade.

Isolation and incubation of hepatocytes. Male Wistar rats weighing 240–280 g (Hsd/CPB:Wu, Harlan-CPB, Zeist, The Netherlands) were used throughout the study. Rats were allowed to consume food and water ad libitum. Hepatocytes were isolated by collagenase perfusion according to Berry and Friend (1969) as modified by Groen et al. (1982). The hepatocytes were washed and resuspended in Krebs-Henseleit buffer, containing 25 mM HEPES and 10 mM glucose, pH 7.4. Initial cell viability was greater than 85% as judged by Trypan Blue exclusion. Hepatocytes (0.9×10^6/ml) were incubated in closed polypropylene flasks in a shaking water bath at 37° C under frequent gassing with 95% O_2 plus 5% CO_2. After 20 min of preincubation the quinone compounds (final concentrations 100 μM) were added in

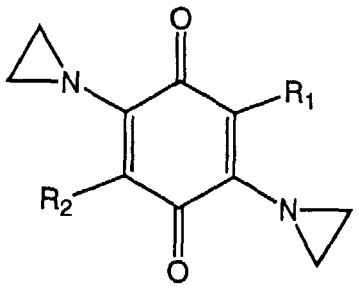

Fig. 1. Structural formula of 2,5-bis(1-aziridinyl)-1,4-benzoquinones (BABQs) with different substituents at the R_1- and R_2-positions

DMSO (1.0% v/v). GT_{90}, AT_{50} and LT_{50} were defined as the time of incubation after which the cells demonstrated a 90% loss of total glutathione, a 50% loss of ATP and a 50% loss of initial viability, respectively.

Biochemical assays. Cell viability was monitored by the the leakage of the cytosolic lactate dehydrogenase (LDH) according to the method described by Moldeus et al. (1978). At various times an aliquot of the cell suspension was taken and centrifuged at 500 *g* for 1 min. The LDH activity was determined in the cell-free supernatant. A separate sample was treated with Triton X-100 (final concentration 0.5% w/v) for the measurement of total LDH activity. No significant differences were found between viability measurements as determined by LDH activity or by Trypan Blue exclusion. For the measurement of cellular glutathione (GSH+GSSG) and ATP a 0.40-ml aliquot of the cell suspension was centrifuged at 500 *g* and 0.30 ml of 3% (w/v) $HClO_4$ was added to the cell pellet. After centrifugation at 3000 *g* the supernatant fraction was neutralized with potassium phosphate (0.8 M, pH 7.4). The insoluble potassium chlorate was removed by centrifugation at 12000 *g* and in the supernatant cellular glutathione and ATP were measured. Total glutathione (GSH+GSSG) was measured according to Tietze et al. (1969) by the recycling reaction of GSH with DTNB, catalyzed by glutathione reductase. The concentration of glutathione was calculated by comparison with GSSG standards. Intracellular ATP levels were determined by a modification of the HPLC method of Jones (1981), as described by Redegeld et al. (1989). Cell protein was determined according to Lowry et al. (1951) using bovine serum albumin as a standard.

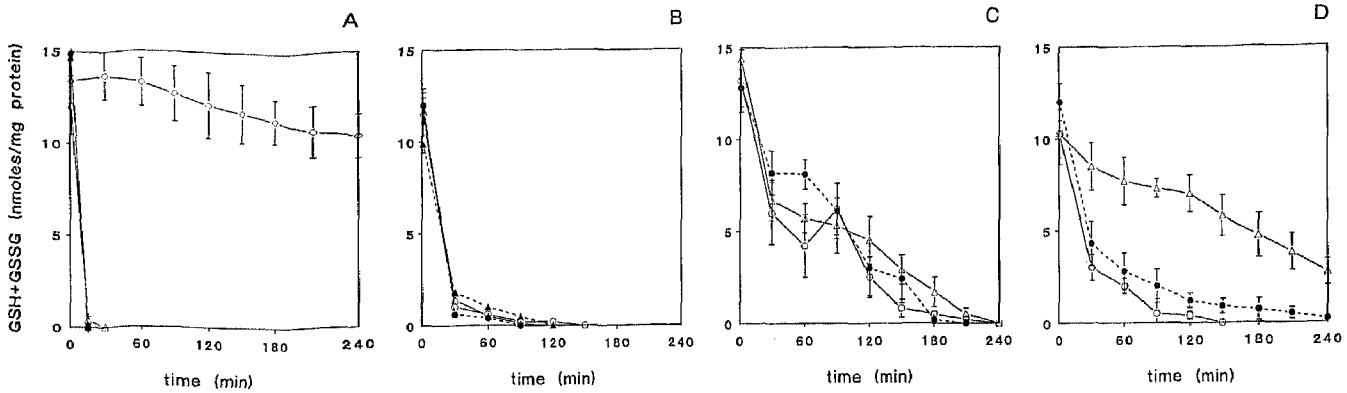

Fig. 2. A–D. Time course of total glutathione levels (nmol/mg protein) in freshly isolated rat hepatocytes in the presence of various BABQs (100 μM). **A** Dihalogenated BABQs TW19 (*closed triangles*), TW14 (*open triangles*) and the fluorinated TW26 (*closed circles*). Control values are also indicated (*open diamonds*). **B** Mono-halogen substituted BABQs TW22 (*open circles*), TW25 (*closed circles*), TW50 (*open triangles*) and TW81 (*closed triangles*). **C** Hydrogen- or methoxy-substituted BABQs TW13 (*open circles*), triaziquone (*closed circles*) and TW82 (*open triangles*). **D** BABQs with electron-donating substituents: TW39 (*open circles*), carboquone (*closed circles*) and triaziquone (*open triangles*). Data points represent the mean ±SEM of duplicate incubations from three separate cell preparations. If no SEM is shown the value is smaller than the symbol used. In Table 1 the substituents for all compounds are described

Statistical analysis. Statistical evaluations were made using a multivariate least squares regression analysis from SYSTAT (1980). Physicochemical properties of the compounds were summated over all substituents on the quinone nucleus: electronic field parameter = F_{total}, and steric parameter = MR (Hansch et al. 1973 and Driebergen 1987). Results were expressed as means ±SEM unless otherwise indicated. Statistical significance was evaluated on the basis of regression coefficients (r) and variance ratios (F).

Results

Depletion of cellular glutathione (Fig. 2, Table 1)

Incubation of rat hepatocytes in the presence of BABQs bearing one or two halogen substituents resulted in very fast depletion of cellular glutathione. Compounds that reacted with GSH within seconds (TW14, TW19 and TW26) in a nonenzymatic reaction (Lusthof et al. 1990) depleted all of the cellular glutathione within 15–30 min (Fig. 2A). BABQs bearing one chloro- or bromo-substituent (TW22, TW25, TW50 and TW81) depleted 90% of cellular glutathione in 15–60 min (Fig. 2B, Table 1). Triaziquone and the unsubstituted TW13 that also showed a chemical reaction with GSH (Lusthof et al. 1990) depleted 90% of intracellular glutathione only after 140–165 min (Table 1). The compounds bearing only electron-donating groups at the R_1- and R_2-positions (TW82, TW39, caboquone and triaziquone) that did not show a nonenzymatic reaction with GSH caused different rates of glutathione depletion, with GT_{90} ranging from 80 to >240 min (Table 1).

Loss of intracellular ATP (Fig. 3, Table 1)

BABQs with two halogen substituents (TW14 and TW19, Fig. 3A) caused very fast ATP depletion: more than 50% of cellular ATP was lost within 15 min. For the dihalogen-substituted compounds no ATP could be measured after 60 min while for TW26, bearing one fluoro substituent, no ATP was detectable after 150 min (Fig. 3A). The four other BABQs with one chloro or bromo substituent (TW22, TW25, TW50 and TW81) caused a 50% loss of cellular

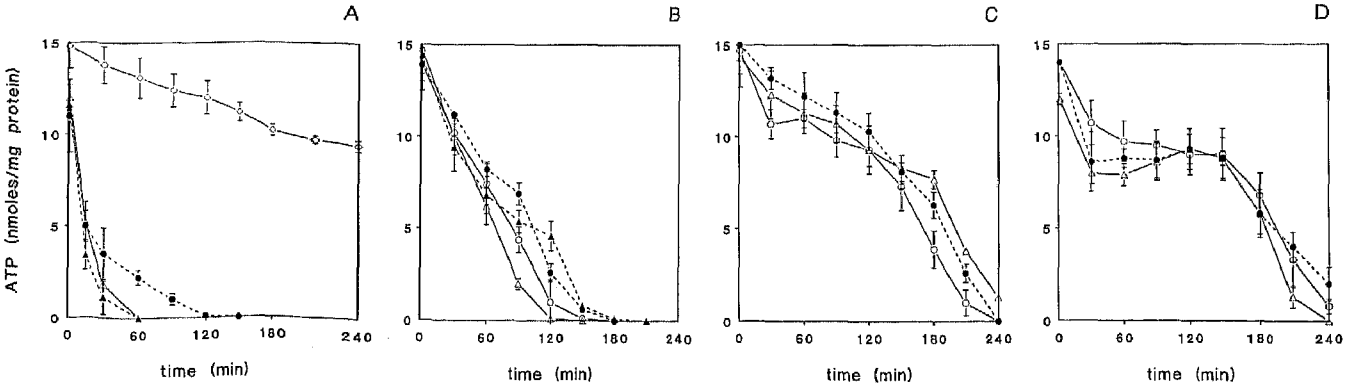

Fig. 3. A–D. Time course of ATP levels (nmol/mg protein) of freshly isolated rat hepatocytes in the presence of various BABQs (100 μM). See Fig. 2 for an explanation of the different symbols used

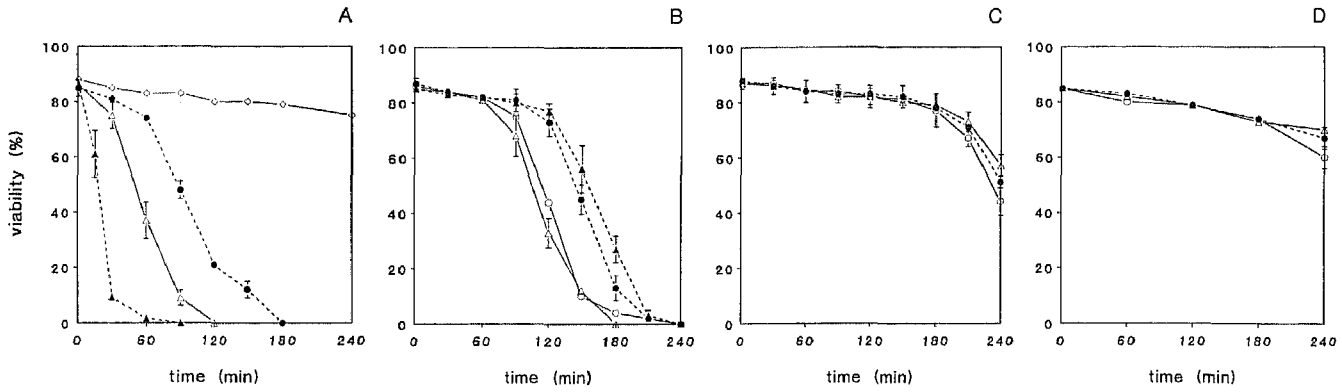

Fig. 4. A–D. Time course of loss of viability (%) of freshly isolated rat hepatocytes in the presence of various BABQs (100 μM). See Fig. 2 for an explanation of the different symbols used

ATP in 45–90 min (Fig. 3B). All of the aforementioned compounds depleted ATP faster than did menadione (150 μM) which caused a 50% loss of cellular ATP within 120 min. Triaziquone, TW13 and the compounds bearing only electron-donating groups at R_1- and R_2-positions (TW39, TW82, carboquone and diaziquone) depleted 50% of cellular ATP within 150–180 min (Figs 3C and 3D).

Cellular viability (Fig. 4, Table 1)

Hepatocyte viability in control incubations was 75±3% after 3 h. Loss of cellular viability occurred very fast for the dihalogen-substituted BABQs. Difluoro substitution (TW19) caused a faster loss than did dichloro substitution (TW14), the LT_{50}s being 20 and 50 min, respectively. For TW26 bearing one fluoro substituent the LT_{50} was 90 min (Fig. 4A). The four other BABQs with one chloro or bromo substituent caused a 50% loss of viability after 110–120 min for TW22 and TW50 and after 150–160 min for TW25 and TW81 (Fig. 4B). Triaziquone and TW13 were equally toxic in rat hepatocytes: they caused 50% loss of viability in 240 min (Fig. 4C). The compounds bearing only electron-donating groups (TW82, TW39, diaziquone and carboquone) did not significantly alter cellular viability within 240 min (Fig. 4C,D).

Structure-activity relations

In Table 1 the toxicity of the 13 BABQs in freshly isolated rat hepatocytes is summarized by their GT_{90}-, AT_{50}-, and LT_{50} values. LT_{50} correlated very well with AT_{50} (n = 13, r = 0.87, $F_{1,11}$ = 74.9, $p = 3\times10^{-6}$), suggesting that cytotoxicity is critically dependent on ATP depletion. Correlations were not improved by including GT_{90} or the earlier published microsomal superoxide production rates (Prins et al. 1989) in regression equations, either alone or in combination. Correlation between LT_{50} and microsomal redox cycling had a comparatively low significance (n = 13, r = 0.57, $F_{1,11}$ = 5.4, p = 0.04). Prediction of LT_{50} values from the physicochemical substituent constant F (summated over all the substituents on the quinone nucleus) was excellent (n = 13, r = 0.90, $F_{1,11}$ = 49.1, $p = 2.3\times10^{-5}$). Inclusion of MR substituent constants slightly improved the correlation, resulting in the following Hansch equation (values ±SD) for the prediction of cytotoxicity of BABQs (Fig. 5):

$$LT_{50} = 117\ (\pm46) - 250\ (\pm34)\ F + 48\ (\pm19)\ MR$$

$$n = 13 \quad r = 0.94 \quad F_{2,10} = 39.9 \quad p = 1.7\times10^{-5}$$

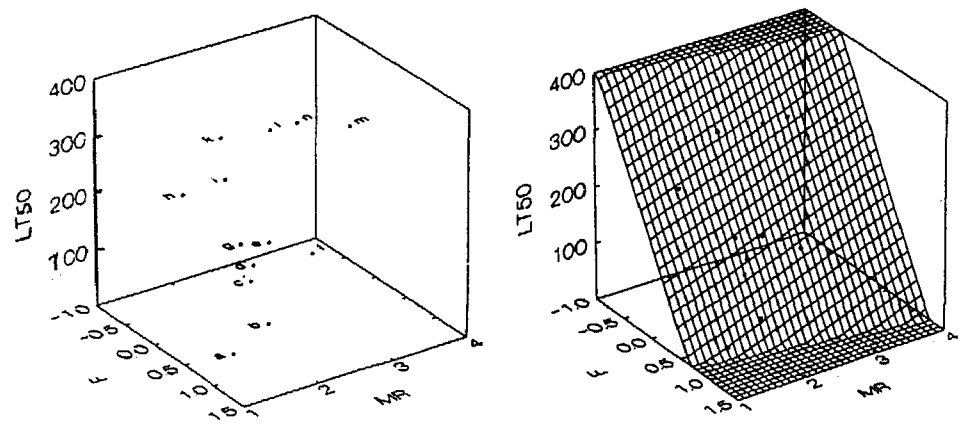

Fig. 5. Prediction of cytotoxicity of BABQs from their physicochemical parameters F_{total} and MR_{total}. In the left figure the experimentally obtained datapoints are indicated (*a*, TW19; *b*, TW14; *c*, TW26; *d*, TW22; *e*, TW25; *f*, TW50; *g*, TW81; *h*, TW13; *i*, triaziquone; *k*, TW82; *l*, TW39; *m*, diaziquone; *n*, carboquone). In the *right* figure the regression surface, calculated by multiple linear least squares regression, is represented

Discussion

The cytotoxic effects of many quinones are thought to be mediated through their one-electron reduction to semiquinone radicals. These radicals subsequently can enter redox cycles with molecular oxygen to produce active oxygen species and oxidative stress (Kappus and Sies 1981). The compounds that were used in this study, BABQs with substituents at the 3- and 6-position, contain the quinone moiety. Initially the concept of redox cycling leading to oxidative stress was assumed for predicting differences in acute cellular toxicity of BABQs. Therefore we determined the ability of 22 BABQs for redox cycling by measuring superoxide anion production and NADPH oxidation in rat liver microsomes (Prins et al. 1989). In that study a good correlation was found between the parameters for redox cycling and the electronic parameters of the substituents. Subsequently we wanted to establish whether the same properties of the substituents could account for acute cytotoxic effects in isolated hepatocytes. For this purpose we used 13 compounds covering the entire range of redox cycling (see Table 1). The nonenzymatic reaction of BABQs with GSH was not prevented by the two aziridinyl groups and it mainly depended on the number and on the nature of halogen substituents (Lusthof et al. 1990).

The toxicity of BABQs for rat hepatocytes at a concentration of 100 μM was very different among the 13 compounds (Table 1). Over a 4-h time period halogen-substituted BABQs were significantly more toxic than other compounds. Halogen substitution by two fluoro atoms was most effective in promoting acute toxicity, even more than dichloro substitution. The difluoro-substituted TW19 shows a relatively low redox cycling (Prins et al. 1989) but a fast nonenzymatic reaction with GSH was observed (Lusthof et al. 1990). The same observations were made for the mono-fluorinated TW26, which was the most toxic of the mono-halogenated compounds. The differences in toxicity of other mono-halogen-substituted BABQs were only small. Despite minor differences in depletion of cellular glutathione by halogenated BABQs these compounds demonstrate marked differences in their effects on cellular ATP levels and viability. Depletion of the mitochondrial pool of glutathione (between 10 and 15% of total glutathione in isolated hepatocytes) by the use of ethacrynic acid has been shown to be important in causing toxicity in isolated hepatocytes (Meredith and Reed 1982; Redegeld et al. 1992). For halogenated BABQs more than 90% of total glutathione was rapidly depleted. It therefore appears likely that the mitochondrial glutathione pool is also disturbed. An explanation of the differences in toxicity by BABQs could also be found in different effects on the mitochondrial respiration (Prins et al., submitted). The importance of possible mitochondrial effects of these quinones is supported by the fact that cellular ATP levels, in contrast to glutathione levels, seem to be important for maintaining cell viability in hepatocytes exposed to redox cycling compounds (Redegeld et al. 1992). In accordance with this is the fact that AT_{50} values always preceded LT_{50} values by 60–90 min for mono-halogenated and hydrogen-substituted BABQs.

Non-halogenated BABQs are considerably less toxic at 100 μM than halogenated BABQs. Even the non-substituted TW13, a compound that shows redox cycling comparable to (mono)-halogenated BABQs (Prins et al. 1989) and that also reacts nonenzymatically with GSH (Lusthof et al. 1990) only causes a gradual decrease of cellular glutathione levels followed by a slow loss of ATP (Figs 2C, 3C). BABQs with only electron-donating substituents (TW39, TW82 and carboquone) cause a comparable loss of cellular glutathione in the first 30 min. As these compounds do not directly react with glutathione nonenzymatically (Lusthof et al. 1990), the loss of cellular glutathione must be explained by the effects of redox cycling or by the action of glutathione transferases. Diaziquone, in contrast to the other compounds with only electron donating substituents, has little effect on total glutathione levels. As diaziquone is a relatively active redox cycling compound (Gutierrez et al. 1986; Prins et al. 1989) and produces H_2O_2 in hepatocytes (Silva and O'Brien 1989) some GSH can be expected to be oxidized to GSSG.

In conclusion, it can be said that the BABQs that were synthesized as analogues of diaziquone and carboquone differ widely in their acute toxic effects on freshly isolated rat hepatocytes. The loss of viability (LT_{50}) and the loss of ATP (AT_{50}) caused by compounds that are toxic within 4 h at a concentration of 100 μM cannot be predicted by their redox cycling in rat liver microsomes. A much better prediction of acute cytotoxicity is possible by using the electronic field parameter (F_{total}) describing the electron withdrawing or donating properties for all the substituents to the quinone nucleus. Inclusion of the molecular refraction parameter in the regression equation gave a small improvement. These results strongly indicate that cellular ATP depletion rather than microsomal redox cycling of these BABQs is of critical importance for affecting cell viability. We have recently shown that menadione-induced cytotoxicity is dependent on ATP depletion (Redegeld et al. 1989, 1990) and that depletion of mitochondrial glutathione – in contrast to non-mitochondrial glutathione – results in ATP depletion and subsequent loss of viability (Redegeld et al. 1992). We suggest, therefore, that interference of BABQs with mitochondrial ATP production (either by GSH adduct formation and/or other uncoupling effects; Prins et al., submitted) is the major cause of cytotoxicity of these compounds.

Acknowledgements. We thank Dr. F.A.M. Redegeld and P.G.F. van de Loo for their assistance in these experiments.

Results

Berry MN, Friend DS (1969) High yield preparation of isolated rat liver parenchymal cells. J Cell Biol 43: 506–520

DiMonte D, Ross D, Bellomo G, Eklow L, Orrenius S (1984) Alterations in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. Arch Biochem Biophys 235: 334–342

Driebergen RJ (1987) Qualitative and quantitative aspects of structure-electrochemistry-cytotoxicity relationships of aziridinylquinones. PhD Thesis, University of Utrecht

Driebergen RJ, Holthuis JJM, Postma-Kelder SJ, Verboom W, Reinhoudt DN, Lelieveld P (1986) Electrochemistry of potential bioreductive alkylating quinones: its use in the development of new aziridinylquinones. Anticancer Res 6: 605–620

Driscoll JS, Dudeck L, Congleton G, Geran RI (1979) Potential CNS antitumour agents. J Pharm Sci 68: 185–188

Gant TW, Rao DNR, Mason RP, Cohen GM (1988) Redox cycling and sulphydryl arylation: their relative importance in the mechanism of quinone cytotoxicity to isolated rat hepatocytes. Chem Biol Interact 65: 157–173

Groen AK, Sips HJ, Vervoorn RC, Tager JM (1982) Intracellular compartmentation and control of alanine metabolism in rat liver parenchymal cells. Eur J Biochem 122: 8793–8799

Gutierrez PL, Biswal S, Nardino R, Biswal N (1986) Reductive activation of diaziquone and possible involvement of free radicals and the hydroquinone dianion. Cancer Res 46: 5779–5785

Hansch C, Leo A, Unger SH, Kim KH, Nikaitani D, Lien EJ (1973) Aromatic substituent constants for structure-activity correlations. J Med Chem 16: 1207–1216

Jones DP (1981) Determination of pyridine nucleotides in cell extracts by high-performance liquid chromatography. J Chromatogr 225: 446–449

Kappus H, Sies H (1981) Toxic effects assiociated with oxygen metabolism: redox cycling and lipid peroxidation. Experientia 11: 1233–1241

Koster AS (1991) Bioreductive activation of quinones: a mixed blessing. Pharm Weekbl [Sci] 13: 123–126

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Lusthof KJ, De Mol NJ, Janssen LHM, Prins B, Verboom W, Reinhoudt DN (1990) Interactions between potential antitumour 2,5-bis(1-aziridinyl)-1,4-benzoquinone derivatives and glutathione: reductive activation, conjugation and DNA damage. Anti-Cancer Drug Des 5: 283–290

Meredith MJ, Reed J (1982) Status of the mitochondrial pool of glutathione in the isolated hepatocyte. J Biol Chem 257: 3743–3753

Moldeus P, Mögberg J, Orrenius S (1978) Isolation and use of liver cells. Meth Enzymol 52: 60–71

Phelps OC, Crane FL (1975) Inhibition of mitochondrial electron transport by hydroxy-substituted 1,4-quinones. Biochemistry 14: 116–122

Prins B, Koster ASj, Verboom W, Reinhoudt DN (1989) Microsomal superoxide anion production and NADPH-oxidation in a series of 22 aziridinylbenzoquinones. Biochem Pharmacol 38: 3753–3757

Redegeld FAM, Moison RMW, Koster ASj, Noordhoek J (1989) Alterations in energy status by menadione metabolism in hepatocytes from fasted and fed rats. Arch Biochem Biophys 273: 215–222

Redegeld FAM, Moison RMW, Barensten HM, Koster ASj, Noordhoek J (1990) Interaction with cellular ATP generating pathways mediates menadione-induced cytotoxicity in isolated rat hepatocytes. Arch Biochem Biophys 280: 130–136

Redegeld FAM, Moison RWM, Koster ASj, Noordhoek J (1992) Depletion of ATP but not of GSH affects viability of rat hepatocytes. Eur J Pharmacol 228: 229–236

Rossi L, Moore GA, Orrenius S, O'Brien PJ (1986) Quinone toxicity in hepatocytes without oxidative stress. Arch Biochem Biophys 251: 25–35

Silva JML, O'Brien PJ (1989) Diaziquone-induced cytotoxicity in isolated rat hepatocytes. Cancer Res 49: 5550–5554

SYSTAT, the System for Statistics (1980) Systat. Inc., Evanston, Ill.

Thor H, Hartzell P, Svensson S-A, Orrenius S, Mirabelli F, Marinoni V, Bellomo G (1985) On the role of thiol groups in the inhibition of liver microsomal Ca^{2+} sequestration by toxic agents. Biochem Pharmacol 34: 3717–3723

Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian and other tissue. Anal Biochem 27: 502–522

Wardman P (1990) Bioreductive activation of quinones: redox properties and thiol reactivity. Free Rad Res Commun 8: 219–229

Wilson I, Wardman P, Lin T-S, Sartorelli AC (1987) Reactivity of thiols towards derivatives of 2-methyl and 6-methyl-1,4-naphthoquinone bioreductive alkylating agents. Chem Biol Interact 61: 229–240