Biochemical Pharmacology, Vol. 37, No. 9, pp. 1837–1838, 1988.
Printed in Great Britain.

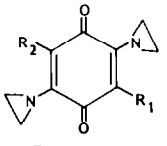
0006-2952/88 $3.00 + 0.00
Pergamon Press plc

DNA MODIFICATIONS BY POTENTIAL ANTITUMOUR BISAZIRIDINYLQUINONES

Nicolaas J. de Mol, Klaas J. Lusthof and Jean Decuyper
Section Pharmaceutical Chemistry, Faculty of Pharmacy
University of Utrecht, Catharijnesingel 60,
3511 GH Utrecht, The Netherlands

The concept of bioreductive activation has been applied to explain the activity of quinone-containing antitumour antibiotics and has been used to develop drugs with selectivity for hypoxic solid tumour cells [1]. Based on AZQ [2] a series of bisaziridinyl quinones was synthesized. These compounds contain two active parts: the quinone moiety and the alkylating aziridinyl groups. The reduction mechanism has been studied in our laboratory by characterization of electrochemical parameters [3]. Reduction changes the quinone ring from relatively electron deficient to electron rich. This facilitates protonation and ring opening of the aziridine ring, forming an electrophilic alkylating species.

We studied the effects of the bisaziridinyl compounds on DNA in two different systems. Firstly, a DNA repair test in which the colony forming ability of a DNA repair deficient strain (*rec* A, *uvr* B) of E.coli K12 is compared with that of a strain with wild type DNA repair. Reductive activation was achieved endogenously by bacterial reductases. Secondly, we used the inactivation of ss M13 mp19 DNA after exposure to quinones reduced by sodium borohydride. The inactivation is assayed by transfection in E.coli JM 105.



Compound	R_1	R_2	E. coli DNA repair test	ss-phage DNA inactivation	-log ID_{75}^{**} in L_{1210} cells	-log D_{125}^{***} in vivo with L_{1210} cells	-log D_{125}^{***} in vivo with B16 cells
AZQ	$R_1 = R_2 =$	$NH\text{-}CO\text{-}OC_2H_5$	0.03	0.03	-0.57	2.26	2.32
TW73*	$R_1 = R_2 =$	$NH\text{-}CO\text{-}OC_2H_5$	0.03	0.04	-1.00	1.59	-
TW53*	CH_3	Br	0.04	0.06	2.66	1.79	1.71
TW87*	CH_3	$(CH_2)_2\text{-}O\text{-}CO\text{-}NH_2$	0.07	0.13	-0.15	1.89	-
CbQ	CH_3	$CH(OCH_3)CH_2O\text{-}CO\text{-}NH_2$	0.22	0.22	1.05	3.21	3.32
TW32	CH_3	C_2H_5	0.49	0.43	-0.31	-	3.25
TW25	Br	C_2H_5	0.51	0.35	2.82	1.41	-
TW39	CH_3	$(CH_2)_2\text{-}O\text{-}CO\text{-}NH_2$	1.00	1.00	1.82	2.86	3.49
TW13	H	H	2.36	1.32	1.68	2.77	3.16
TW26	Az	F	3.76	1.29	2.32	1.95	-

*contains methyl aziridinyl

**ID_{75} dose (n mol/l) at which 75% of the colonies is inhibited

***D_{125} dose (n mol/kg) which increases life span with 25%

The results of these two tests are included in the table together with the cytotoxicity in tumour models which were performed elsewhere [4]. L1210 cells are murine tumour cells; B16 is a solid tumour and serves as a model for the hypoxic cytotoxicity. The results of the two assays for DNA damage agree remarkably despite differences in the two test systems. (i.e. effects on bacterial DNA *in situ* vs isolated ss DNA, enzymatic reductive activation vs chemical reduction).

In both tests we see that the parent compound TW13 shows a high activity. Introduction of an additional aziridinyl group as in TW26 increases the activity. Introduction of other substituents decreases the DNA modifying activity. Therefore we expect that sterical factors play a role in the DNA alkylating activity of aziridinylbenzoquinones. Methylation of the aziridinyl substituents strongly decreases the DNA modifying activity. In comparing the DNA modifying activity with the cytotoxicity in tumour models we see many differences in activity. In the more complicated tumour models the lipophilicity of the quinones is likely to play an important role, as the measured activity is a result of the intrinsic activity and accessibility of the cellular targets, including membrane passage. The activity of the bismethylaziridinyl compound TW 53 against L1210 cells suggests that in the antitumour action other cellular targets than DNA may also be involved; TW 53 has a very low DNA modifying activity.

The pH dependence of DNA alkylation by TW 13 was studied. After electrochemical reduction DNA alkylation occurs at pH < 7. Without reduction alkylation occurs to a lesser extent at pH < 5.5. The pH dependence of the inactivation of ss M13mp19 DNA is in agreement with this alkylation profile. These results support the concept that reduction increases the pK_a value of the aziridine group to physiologically relevant values.

As the aziridinyl quinones contain two aziridine rings, the possibility exists that interstrand cross links are formed in ds DNA. We studied the formation of interstrand cross links in calf thymus DNA with the ethidium bromide fluorescence assay. The pH dependence for TW 13 was essentially the same as that of the alkylation; after reduction cross links are formed at higher pH compared to the oxidized form of the quinones. There is no straight correlation between the ability to form cross links and the DNA modifying activity as observed in our two tests.

The reductively activated quinones are expected to modify DNA not only by alkylation but also by generation of reactive oxygen species which are formed by redox cycling [5]. Addition of superoxide dismutase only slightly decreases the inactivation of E.coli in the DNA repair test.

Catalase and superoxide dismutase have no effect on the inactivation of ss M13 mp19 DNA when we add electrochemically reduced quinones. The lack of cycling of reactive intermediates may strongly limit the generation of reactive oxygen species. Until now we find no indications for a major role of reactive oxygen species in the inactivation of DNA by the aziridinylquinones, however, experiments will be performed in cycling activation systems.

References

1. H.S. Schwartz, in *Molecular aspects of anticancer drug action* (Eds. S. Neidle and M.J. Waring), p.93, Verlag Chemie, Weinheim (1983).
2. J.S. Driscoll, L Dudeck, G. Congleton and R.I. Geran, *J. Pharm Sci* **68,** 185 (1979).
3. R.J. Driebergen, W.J. van Oort, S.J. Postma-Kelder, D.N. Reinhoudt and W. Verboom, *Proc. Am. Ass. Cancer Res.* **26,** 332 (1985).
4. R.J. Driebergen, *Thesis,* University of Utrecht, The Netherlands (1987).
5. H. Kappus, *Biochem. Pharmacol,* **35,** 1 (1986).