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Effect of poly(ADP-ribosyl)ation inhibitors on the genotoxic effects of the boron neutron capture reaction

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Abstract

The boron neutron capture (BNC) reaction results from the interaction of ^{10}B with low-energy thermal neutrons and gives rise to highly damaging lithium and alpha-particles. In this work the genotoxicity caused by the BNC reaction in V79 Chinese hamster cells was evaluated in the presence of poly(ADP-ribosyl)ation inhibitors. Poly(ADP-ribose) polymerase-1 (PARP-1), the most important member of the PARP enzyme family, is considered to be a constitutive factor of the DNA damage surveillance network present in eukaryotic cells, acting through a DNA break sensor function. Inhibition of poly(ADP-ribosyl)ation was achieved with the classical compound 3-aminobenzamide (3-AB), and with two novel and very potent inhibitors, 5-aminoisoquinolinone (5-AIQ) and PJ-34. Dose-response increases in the frequencies of aberrant cells excluding gaps (%ACEG) and chromosomal aberrations excluding gaps per cell (CAEG/cell) were observed for increasing exposures to the BNC reaction. The presence of 3-AB did not increase the %ACEG or CAEG/cell, nor did it change the pattern of the induced chromosomal aberrations. Results with 5-AIQ and PJ-34 were in agreement with the results obtained with 3-AB. We further studied the combined effect of a PARP inhibitor and a DNA-dependent protein kinase (DNA-PK) inhibitors (3-AB and wortmannin, respectively) on the genotoxicity of the BNC reaction, by use of the cytokinesis-block micronucleus assay. DNA-PK is also activated by DNA breaks and binds DNA ends, playing a role of utmost importance in the repair of double-strand breaks. Our results show that the inhibition of poly(ADP-ribosyl)ation does not particularly modify the genotoxicity of the BNC reaction, and that PARP inhibition together with a concomitant inhibition of DNA-PK revealed barely the same sensitizing effect as DNA-PK inhibition per se.

Keywords: α-Particles; Boron neutron capture reaction; DNA-PK; Poly(ADP-ribosyl)ation inhibitors; PARP-1

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1. Introduction

The boron neutron capture (BNC) reaction results from the interaction of ^{10}B with low-energy thermal neutrons and gives rise to lithium and α-particles. These particles have a high linear energy transfer (LET) and a short range, which renders them highly damaging to cells. Accordingly, the BNC reaction has been used in clinical trials [boron neutron capture therapy (BNCT)] to treat some aggressive types of neoplasia [1–4]. Mechanistic knowledge on DNA and cell damage induced by high-LET radiation, in particular by α-particles, remains limited [5]. DNA single-strand breaks (SSB) or DNA double-strand breaks (DSB) are strongly related to high-LET radiation [6,7], the latter being frequently associated with chromosomal aberrations, mutations, cell death and cancer [8,9]. The evaluation of the involvement of key enzymes for DNA damage surveillance and repair has not yet been fully addressed for high-LET radiation in general and for the BNC reaction in particular, and can be valuable in understanding high-LET radiation effects.

Poly(ADP-ribosyl)ation is a posttranslational modification of proteins carried out by a family of NAD^+ ADP-ribosyltransferases, the poly(ADP-ribose) polymerases (PARPs) [10]. It is now clear that poly(ADP-ribose) polymerase-1 (PARP-1, formerly known as PARP, E.C. 2.4.2.30), the most important member of the PARP family, is a constitutive factor of the DNA damage surveillance network present in eukaryotic cells [11–13], acting through a DNA break sensor function [14]. PARP-1 activity is strongly dependent on the presence of strand breaks in DNA (nicks and double-strand breaks), which are recognized by the two zinc-finger motifs within the DNA-binding domain of the enzyme [15]. The exposure of PARP-1-deficient cells to DNA damaging agents, namely alkylating agents and γ-radiation, increases recombination observed as an increase in sister chromatid exchange (SCE) and chromosome aberrations [12,16,17]. These findings, obtained with PARP-$1^{-/-}$ mice and their cells, have generally recapitulated former results achieved with studies using PARP inhibitors, and confirmed their main conclusions (reviewed by Shall and de Murcia [12]).

Another component of the DNA damage surveillance network is the DNA-dependent protein kinase (DNA-PK), which is likewise activated by DNA breaks. DNA-PK is involved in double-strand break (DSB) repair and V(D)J recombination [9,18,19].

Recently, various studies implying the interaction of both these enzymes have been published, suggesting that DNA strand-break recognition and repair occurs in a concerted action involving a functional interplay between them [13,20,21]. The exact interaction is unknown, but some studies suggest a mutual regulation and modification between PARP and DNA-PK [21–23].

This work aims at assessing the role of the inhibition of poly(ADP-ribosyl)ation on the genotoxicity of the BNC reaction in V79 Chinese hamster cells. This cell line has been used in a number of previous studies to evaluate the involvement of PARP-1 in the genotoxicity and/or cytotoxicity of different agents [20,24–27]. The chromosomal aberration assay was used and the BNC reaction was carried out with different concentrations of 4-borono-L-phenylalanine (BPA; ^{10}B-enriched) and different periods of irradiation with low-energy thermal neutrons. For the inhibition of poly(ADP-ribosyl)ation, the standard inhibitor 3-aminobenzamide (3-AB) [28] as well as two novel, very potent, highly specific and water-soluble inhibitors were used, viz. 5-aminoisoquinolinone (5-AIQ) [29] and PJ-34 [30]. The combined inhibition of PARP and DNA-PK was also evaluated in this study by use of the cytokinesis-block micronucleus assay (CBMN) as a test for genotoxicity. The inhibition of DNA-PK was achieved with wortmannin (WM), a fungal metabolite that is a potent, irreversible and non-competitive inhibitor of the catalytic sub-unit of this enzyme (DNA-PKcs) [31].

2. Material and methods

2.1. Chemicals and culture medium

Foetal calf serum, RPMI medium, 3-aminobenzamide (CAS number 3544-24-9), wortmannin (CAS number 19545-26-7), cytochalasin-B (CAS number 14930-96-2) and *N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(*N*,*N*-dimethylamino)acetamide, hydrochloride (PJ-34, CAS number 344458-15-7) were purchased from Sigma (St. Louis, MO). 4-Borono-L-phenylalanine ^{10}B-enriched (BPA, 99.7%; CAS number 80994-59-8) was obtained from KatChem

(Prague, Czech Republic). Methanol, acetic acid, potassium chloride and Giemsa dye were obtained from Merck (Darmstadt, Germany). Colchicine was purchased from Fluka (Buchs, Switzerland) and trypsin from Difco Laboratories (Detroit, Mich). 5-Aminoisoquinolin-1-one, hydrochloride (5-AIQ, CAS number 1125-60-6) was kindly supplied by Dr M.D. Threadgill (Bath, UK) and synthesized as described in [32].

2.2. BNC reaction: cell culture and BPA incubation

Wild-type V79 Chinese hamster cells (MZ) were kindly provided by Prof. H.R. Glatt (Mainz and Potsdam). These cells were cultured in RPMI medium supplemented with 10% foetal calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml) and incubated at 37 °C under an atmosphere containing 5% carbon dioxide.

For BPA treatment of cells, a BPA stock supplemented medium was prepared with a final BPA concentration of 2.4 mM (500 μg/ml; 24.0 ppm of ^{10}B). Cells were seeded (approximately 1.0×10^5) in 25-cm^2 tissue culture flasks (Greiner; Frickenhausen, Germany) and incubated either with 5 ml of BPA medium (0.48, 1.2 and 2.4 mM) or with BPA-free culture medium. The cells were grown as monolayers for 48 h and then irradiated with thermal neutrons (low-energy neutrons, average value of 0.025 eV). Two independent experiments were performed.

2.3. BNC reaction: thermal neutrons irradiation

The irradiation of V79 cells took place at the vertical access of the thermal column of the Portuguese Research Reactor (RPI). The characterization of the radiation field and the reduction of the background γ-radiation from the reactor were essential for these radiobiological experiments and have been described elsewhere [33,34]. Thermal neutron irradiations between 30 and 120 min were considered in this study, corresponding to average fluences (ϕ) between 1.1×10^{11} and 4.4×10^{11} n_{th} cm^{-2}. The neutron flux was monitored in each irradiation by using gold foil detectors. The absorbed dose (expressed in Gy) for the α- and lithium particles from the BNC reaction was calculated as previously described [35] using the formula $D_B = 8.66 \times 10^{-8}[^{10}B]\phi$. The absorbed doses from α- and lithium particles for the fluences (ϕ) and BPA (^{10}B) concentrations studied in this report are thus in the range of 0.05–0.9 Gy.

2.4. BNC reaction controls

Controls included cells irradiated with the low-energy thermal neutrons without BPA incubation (thermal neutron controls). Thermal neutron controls assess the genotoxicity of the γ-ray background of the reactor, and also of the reactions with other nuclides, namely hydrogen and nitrogen [34,35]. These control cultures were exposed to a mixed field of high (protons) and low (γ-rays) LET radiation that could contribute to a small extent to the total dose. Other controls included cells incubated with BPA without thermal neutron irradiation (BPA controls), and non-irradiated cells without BPA incubation (background V79 controls).

2.5. Chemical inhibitors

The poly(ADP-ribosyl)ation inhibitors (3-AB, 5-AIQ and PJ-34), and the DNA-PK inhibitor (WM, stock solution prepared in DMSO) were added, respectively, at 4 and 3.5 h before the irradiation with thermal neutrons and remained for a further 6 h (CBMN assay, 3-AB and WM) or 14.5 h (chromosomal aberration assay, 3-AB, 5-AIQ and PJ-34).

The final concentrations of the inhibitors studied were in the range of 1.5–10 mM for 3-AB, 100 μM for 5-AIQ, 10 μM for PJ-34 and 5.0 μM for WM. In the experiments using WM, DMSO was added to the negative, neutron-only and BPA-only controls, and its concentration did not exceed 0.2% (v/v).

2.6. Chromosomal aberration assay

At 14.5 h after the irradiation, the medium was removed and colchicine added in BPA-free culture medium at a final concentration of 0.6 μg/ml. Cells were grown for a further 2.5 h and then harvested by trypsinization. After a 3-min hypotonic treatment with 75 mM KCl at 37 °C, the cells were fixed with methanol/acetic acid (3:1), and slides were prepared and stained with Giemsa (4% (v/v) in 0.01 M phosphate buffer, pH 6.8) for 10 min.

For each individual experiment, 100 well-spread metaphases were observed using a 1250× magnification on a light microscope. Scoring of the different types of aberrations followed described criteria [36,37] and two standard indices were used:

(1) %ACEG: percent of aberrant cells excluding gaps, which represents the frequency of metaphases of V79 cells containing chromosomal aberrations. The types of aberrations considered for this index were: breaks (chromatid and chromosome), dicentric chromosomes and rings, chromatid-type rearrangements (triradial, quadriradial), other complex rearrangements and multi-aberrant cells (MA, cells with more than 10 aberrations, including heavily damaged pulverized cells).
(2) CAEG/cell: number of chromosomal aberrations excluding gaps per cell, which represents the average number of chromosomal aberrations per V79 cell metaphase. This index is the sum of the individual aberrations mentioned in (1), divided by the total number of metaphases analysed. The individual aberrations presented in multi-aberrant cells (MA) were not included in the CAEG/cell index.

Gaps were recorded and are mentioned in Tables 1–3, but were excluded from the statistical analysis [37]. The paired Student's *t*-test was used in order to compare the %ACEG and the CAEG/cell of the enzyme-inhibited cultures versus controls.

2.7. Cytokinesis-block micronucleus assay

Six hours after the irradiation the culture medium was removed, the cells washed and fresh culture medium added. Cytochalasin-B (Cyt-B) was added at a final concentration of 6 μg/ml [38]. The cells were grown for a further 16 h for recovery of binucleated V79 cells. The cells were then harvested by trypsinization, rinsed and submitted to a mild hypotonic treatment as described elsewhere [34,38]. The centrifuged cells were placed on dry slides and smears were made. After air-drying the slides were fixed with cold methanol (30 min). One day later the slides were stained with Giemsa (4% (v/v) in 0.01 M phosphate buffer, pH 6.8) for 10 min.

For each experimental point, 1000 binucleated V79 cells (BN) with well-preserved cytoplasm were scored. Micronuclei were identified according to described criteria [39] using a 1250× magnification on a light microscope. Two indices were evaluated, the MN/BN which represents the average number of micronuclei per binucleated cell, and the %MNBN which represents the fraction of cytokinesis-blocked (binucleated) cells with micronuclei, regardless of the number of micronuclei per BN cell [34,35]. The paired Student's *t*-test was used in order to compare the %MNBN and MN/BN of the study cultures versus controls.

2.8. Cytotoxicity/cell proliferation

For the chromosomal aberration assay, cell proliferation was assessed using the mitotic index (MI). For this index, 1000 V79 cells from two independent experiments were scored and the number of metaphases recorded [36,37]. For the CBMN assay, the decrease in cell proliferation for the experiments described above was assessed using the two standard methods described elsewhere [39]: (1) the frequency of binucleated cells (%BN) and (2) the nuclear division index (NDI). The NDI was calculated as follows:

$$\mathrm{NDI} = [\mathrm{M1} + 2(\mathrm{M2}) + 3(\mathrm{M3}) + 4(\mathrm{M4})]/N,$$

where M1–M4 represent the number of cells with 1–4 nuclei, and N the total number of cells scored. For these indices, 500 cells with well-preserved cytoplasm from two independent experiments were analysed according to number of nuclei, using a 500× magnification.

3. Results

3.1. Induction of chromosomal aberrations

The induction of chromosomal aberrations by the BNC reaction in V79 cells in the presence or absence of a fixed concentration of 3-AB (1.5 mM) is presented in Table 1 and Fig. 1. In these experiments, three different BPA concentrations (0.48, 1.2 and 2.4 mM) as well as three different fluences of thermal neutrons (1.1×10^{11}, 2.1×10^{11} and 4.4×10^{11} n_{th} cm^{-2}) were studied.

Fig. 1 and Table 1 show that the BNC reaction is clearly clastogenic, increasing the frequency of DNA-damaged cells in a dose-dependent manner for both BPA concentrations and thermal neutron fluences. All the different types of individual aberrations were increased in cells exposed to the BNC reaction (Table 1),

Table 1
Induction of chromosomal aberrations in V79 cells by the boron neutron capture reaction in the presence of 3-aminobenzamide (1.5 mM)

Fluence (n_{th} cm^{-2})	BPA (mM)	3-AB (1.5 mM)	Chromosomal aberrations per 100 cells[a]							MA (%)	CAEG/cell	%ACEG	MI (%)
			Ctg	Csg	Ctb	Csb	Dic	Rings	Rearr				
0#	0	–	1.2	0.8	0.5	0	0.3	0	0	0.7	0.008 ± 0.007	1.5 ± 0.5	8.0 ± 1.0
	0	+	1.0	1.2	0.2	0.7	0.3	0	0	1.0	0.012 ± 0.010	2.0 ± 1.5	7.2 ± 1.3
1.1×10^{11}	0	–	0.5	0	0	2.0	1.0	0	0	2.5	0.030 ± 0.014	5.0 ± 1.4	8.6 ± 2.5
	0	+	0.5	2.0	0.5	3.5	0	0	0	0.5	0.040 ± 0.014	4.5 ± 0.7	5.1 ± 2.1
2.1×10^{11}	0	–	2.0	1.0	2.0	1.0	0	0	0	1.0	0.030 ± 0.000	4.0 ± 1.4	9.0 ± 1.1
	0	+	1.5	2.0	0.5	1.0	1.0	0.5	0	0.5	0.030 ± 0.014	3.5 ± 2.1	6.7 ± 1.1
4.4×10^{11}	0	–	4.0	1.0	5.0	2.0	2.0	0	1.5	0	0.105 ± 0.021	8.5 ± 0.7	8.7 ± 0.7
	0	+	1.0	3.5	1.0	3.5	1.0	0.5	0.5	1.0	0.065 ± 0.021	7.5 ± 0.7	5.2 ± 2.0
0+	0.48	–	1.2	1.0	0.5	0.5	0.3	0	0	1.0	0.013 ± 0.012	2.3 ± 1.5	8.2 ± 1.7
	0.48	+	0.7	1.5	0.7	0.3	0.3	0	0	0.2	0.013 ± 0.012	1.5 ± 1.0	7.1 ± 1.0
1.1×10^{11}	0.48	–	2.5	1.0	1.0	1.0	1.0	0	1.0	1.5	0.040 ± 0.028	5.5 ± 2.1	6.5 ± 0.9
	0.48	+	1.5	2.0	1.0	3.5	0.5	0	0	0.5	0.050 ± 0.014	5.0 ± 1.4	5.2 ± 0.6
2.1×10^{11}	0.48	–	1.5	3.5	1.0	4.5	0.5	0.5	0.5	1.0	0.070 ± 0.014	7.5 ± 0.7	6.3 ± 2.3
	0.48	+	2.0	3.0	1.5	5.0	1.0	0.5	0	0	0.080 ± 0.014	7.0 ± 1.4	4.3 ± 0.6
4.4×10^{11}	0.48	–	2.5	1.5	3.5	6.5	2.5	0.5	2.0	2.5	0.150 ± 0.042	14.5 ± 4.9	5.0 ± 0.3
	0.48	+	0.5	4.0	3.5	5.5	3.0	1.0	3.5	2.0	0.165 ± 0.035	13.0 ± 2.8	3.7 ± 0.9
0+	1.2	–	1.6	0.8	0.6	0.2	0.2	0	0	1.2	0.010 ± 0.012	2.2 ± 2.2	8.3 ± 1.3
	1.2	+	2.3	1.3	0.5	0.3	0	0	0.2	0.7	0.010 ± 0.011	1.7 ± 1.5	7.6 ± 1.0
1.1×10^{11}	1.2	–	2.0	1.5	1.5	2.0	0.5	1.0	1.5	3.0	0.065 ± 0.049	8.5 ± 2.1	6.1 ± 0.8
	1.2	+	2.0	1.0	1.0	2.5	1.5	0.5	1.5	2.5	0.070 ± 0.028	8.5 ± 2.1	4.0 ± 2.1
2.1×10^{11}	1.2	–	1.0	2.5	6.5	8.5	1.0	1.5	2.5	2.0	0.200 ± 0.014	17.0 ± 1.4	5.6 ± 0.6
	1.2	+	1.5	2.0	3.0	6.5	4.0	1.0	5.0	1.5	0.195 ± 0.007	16.5 ± 2.1	4.6 ± 1.0
4.4×10^{11}	1.2	–	4.5	0.5	11.0	7.0	7.0	2.0	8.5	3.0	0.355 ± 0.035	26.5 ± 6.4	5.3 ± 1.7
	1.2	+	2.0	1.0	8.0	11.0	3.0	1.0	10.0	2.5	0.330 ± 0.085	27.5 ± 7.8	4.3 ± 1.4
0+	2.4	–	1.2	1.2	0.5	0.7	0.2	0	0.2	1.0	0.015 ± 0.014	2.2 ± 1.6	9.1 ± 1.0
	2.4	+	0.8	2.0	0.5	0.7	0	0.2	0	0.3	0.013 ± 0.010	1.7 ± 0.8	7.6 ± 0.9
1.1×10^{11}	2.4	–	4.0	2.0	4.5	5.0	2.5	0	3.5	5.0	0.155 ± 0.049	17.5 ± 0.7	5.3 ± 2.2
	2.4	+	1.5	3.5	3.5	5.0	3.0	1.0	3.0	1.5	0.155 ± 0.007	15.5 ± 0.7	4.1 ± 2.1
2.1×10^{11}	2.4	–	0.5	1.5	7.5	11.5	6.5	1.0	8.0	3.5	0.345 ± 0.007	29.5 ± 3.5	4.7 ± 0.6
	2.4	+	1.0	1.0	6.0	16.0	2.0	0.5	6.5	2.0	0.310 ± 0.057	22.5 ± 0.7	5.0 ± 0.3
4.4×10^{11}	2.4	–	3.5	3.5	19.5	24.5	7.5	2.0	25.0	4.0	0.785 ± 0.049	46.5 ± 0.7	5.1 ± 1.1
	2.4	+	0.5	2.5	18.0	28.5	5.0	2.5	19.0	6.5	0.730 ± 0.071	47.0 ± 4.2	5.2 ± 0.2

BPA, 4-borono-L-phenylalanine; 3-AB, 3-aminobenzamide; Ctg, chromatid gap; Csg, chromosome gap; Ctb, chromatid break; Csb, chromosome break; Dic, dicentric chromosome; Rearr, rearrangements (triradial, quadriradial, and other complex rearrangements); CAEG/cell, chromosomal aberrations excluding gaps per cell, corresponding to the sum of Ctb, Csb, Dic, Rings and Rearr per cell (average ± S.D.); %ACEG, percent of aberrant cells excluding gaps (average ± S.D.); MA, multi-aberrant cells, corresponding to cells with more than 10 aberrations. MA are included in the index %ACEG; MI, mitotic index.

[a] These results are average values from two independent experiments (100 metaphases analysed per experiment) for all the points, except for negative V79 cells controls (#) and BPA controls (+). In these cases the results are average values from six independent experiments (100 metaphases analysed per experiment).

Table 2
Induction of chromosomal aberrations in V79 cells by the boron neutron capture reaction in the presence of 3-aminobenzamide (3, 5 and 10 mM)

Fluence (n_{th} cm^{-2})	BPA (mM)	3-AB (mM)	Chromosomal aberrations per 100 cells[a]							MA (%)	CAEG/cell	%ACEG	MI (%)
			Ctg	Csg	Ctb	Csb	Dic	Rings	Rearr				
0	0	0	2.5	1.0	1.0	1.0	0	0	0	0	0.020 ± 0.028	1.5 ± 2.1	6.3 ± 0.5
0	0	10	0.5	1.5	0.5	1.5	0	0.5	0	0	0.025 ± 0.007	2.5 ± 0.7	5.7 ± 0.4
0	2.4	0	1.0	0.5	1.0	0.5	0.5	0	0	0	0.020 ± 0.000	2.0 ± 0.0	6.8 ± 0.1
0	2.4	3	3.0	2.0	1.0	0.5	0	0	0	0	0.015 ± 0.007	1.5 ± 0.7	7.0 ± 0.1
0	2.4	5	2.5	2.5	1.5	0.5	1.0	0	0	0	0.030 ± 0.014	3.0 ± 1.4	8.3 ± 1.1
0	2.4	10	2.5	4.0	1.5	3.5	0	0.5	0	0	0.055 ± 0.021	5.5 ± 2.1	6.1 ± 2.4
1.1×10^{11}	0	0	1.5	2.0	2.0	2.0	0.5	0	0	0	0.045 ± 0.021	4.0 ± 1.4	6.4 ± 0.7
1.1×10^{11}	0	10	1.5	4.5	3.5	3.0	0.5	0.5	0.5	0	0.080 ± 0.000	8.0 ± 0.0	5.0 ± 1.3
1.1×10^{11}	2.4	0	0	1.0	4.5	7.0	2.0	1.0	5.0	2.0	0.195 ± 0.021	17.5 ± 2.1	7.3 ± 0.8
1.1×10^{11}	2.4	3	0.5	1.0	6.0	8.0	2.5	2.5	4.5	1.5	0.235 ± 0.049	20.5 ± 2.1	5.7 ± 0.9
1.1×10^{11}	2.4	5	0.5	4.0	5.5	12.5	2.5	0.5	5.0	0.5	0.260 ± 0.014	18.5 ± 0.7	5.9 ± 2.8
1.1×10^{11}	2.4	10	1.5	1.5	7.5	4.0	3.5	0.5	7.5	0.5	0.230 ± 0.042	20.0 ± 1.4	3.9 ± 0.7

BPA, 4-borono-L-phenylalanine; 3-AB, 3-aminobenzamide; Ctg, chromatid gap; Csg, chromosome gap; Ctb, chromatid break; Csb, chromosome break; Dic, dicentric chromosome; Rearr, rearrangements (triradial, quadriradial, and other complex rearrangements); CAEG/cell, chromosomal aberrations excluding gaps per cell, corresponding to the sum of Ctb, Csb, Dic, Rings and Rearr per cell (average ± S.D.); %ACEG, percent of aberrant cells excluding gaps (average ± S.D.); MA, multi-aberrant cells, corresponding to cells with more than 10 aberrations. MA are included in the index %ACEG; MI, mitotic index.

[a] These results are average values from two independent experiments (100 metaphases analysed per experiment).

with a special reference to chromosome rearrangements (triradial, quadriradial and complex rearrangements). This type of aberration was relatively more frequent for the higher fluences of thermal neutrons and the higher concentrations of BPA, that is for higher doses of α- and lithium particles. Multi-aberrant cells (MA%) were also increased for higher doses of high-LET radiation. It is clear that the incubation of the V79 cells with 1.5 mM 3-AB did not increase the %ACEG (Table 1, Fig. 1) presented by the BNC reaction per se. Moreover, neither the CAEG/cell (Table 1) nor the pattern of the individual aberrations (Table 1) was modified after incubation with 3-AB.

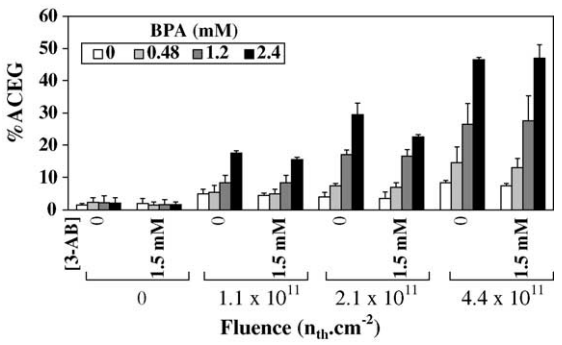

Fig. 1. Effect of 3-aminobenzamide (3-AB, 1.5 mM) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean ± S.D.). The BNC reaction was performed using different concentrations of 4-borono-L-phenylalanine (BPA, 0.48–2.4 mM) and different fluences of thermal neutrons (1.1×10^{11} to 4.4×10^{11} n_{th} cm^{-2}). Two independent experiments were performed. In each experiment, 100 metaphases were analysed for chromosomal aberrations.

The genotoxicity of thermal neutrons can also be observed in Fig. 1 and Table 1 and corresponds to the chromosomal aberrations of cells from cultures that were irradiated with thermal neutrons, but without BPA pre-incubation. The genotoxicity of thermal neutrons is low when compared with the genotoxicity of the BNC reaction, and the presence of 3-AB (1.5 mM) did not modify this index. In addition, the presence of 3-AB alone was not genotoxic and BPA did not induce genotoxicity, alone or in the presence of 3-AB.

The MI of 3-AB(1.5 mM)-treated cultures was generally lower than that of non-treated cultures. This decrease was present especially for the BNC reaction, although mild decreases were also found for the controls (V79, BPA and thermal neutrons) (Table 1).

Table 2 and Fig. 2 present the effect of different concentrations of 3-AB (3.0, 5.0 and 10.0 mM) on the

Table 3
Induction of chromosomal aberrations in V79 cells by the boron neutron capture reaction in the presence of 5-AIQ and PJ-34

Conditions	Inhibitor	Fluence (n_{th} cm^{-2})	BPA (mM)	Chromosomal aberrations per 100 cells[a]							MA (%)	CAEG/cell	%ACEG	MI (%)
				Ctg	Csg	Ctb	Csb	Dic	Rings	Rearr				
V79 cells controls	–	0	0	2.5	1.0	1.5	0	0	0	0	0	0.015 ± 0.007	1.5 ± 0.7	8.5 ± 1.3
	5-AIQ	0	0	4.5	1.0	0	0.5	1.0	0	0	0	0.015 ± 0.007	1.0 ± 0.0	8.2 ± 1.0
	PJ-34	0	0	0.5	1.5	0.5	0.5	0	0	0	0.5	0.010 ± 0.014	1.5 ± 2.1	7.2 ± 1.3
BPA controls	–	0	2.4	1.0	1.0	0.5	0	1.0	0	0	0	0.015 ± 0.021	1.5 ± 2.1	8.0 ± 0.1
	5-AIQ	0	2.4	3.5	0.5	0.5	0.5	0	0	0	0	0.010 ± 0.000	1.0 ± 0.0	7.6 ± 0.4
	PJ-34	0	2.4	4.0	0.5	1.5	0	0	0	0	1.0	0.015 ± 0.007	2.5 ± 2.1	7.9 ± 0.4
Neutrons controls	–	1.2×10^{11}	0	2.0	2.0	2.5	0.5	0.5	0	0.5	0	0.040 ± 0.014	3.5 ± 0.7	7.3 ± 0.1
	5-AIQ	1.2×10^{11}	0	2.0	2.5	1.5	2.0	1.0	0	0	0	0.045 ± 0.021	4.0 ± 1.4	8.3 ± 1.1
	PJ-34	1.2×10^{11}	0	2.0	1.0	2.0	0.5	2.5	0	0.5	0	0.055 ± 0.021	5.0 ± 1.4	7.9 ± 0.1
BNC reaction	–	1.2×10^{11}	2.4	1.5	1.0	1.0	4.5	5.0	0.5	4.5	1.0	0.155 ± 0.035	14.0 ± 2.8	7.9 ± 0.8
	5-AIQ	1.2×10^{11}	2.4	1.0	0	5.5	5.0	2.0	1.0	1.0	1.0	0.145 ± 0.007	14.0 ± 1.4	7.0 ± 0.6
	PJ-34	1.2×10^{11}	2.4	2.0	3.5	4.5	1.5	1.0	1.0	2.5	3.0	0.105 ± 0.007	13.0 ± 2.8	7.1 ± 0.6

5-AIQ, 5-aminoisoquinolin-1-one (100 μM); PJ-34 (10 μM); BPA, 4-borono-L-phenylalanine; Ctg, chromatid gap; Csg, chromosome gap; Ctb, chromatid break; Csb, chromosome break; Dic, dicentric chromosome; Rearr, rearrangements (triradial, quadriradial, and other complex rearrangements); CAEG/cell, chromosomal aberrations excluding gaps per cell, corresponding to the sum of Ctb, Csb, Dic, Rings and Rearr per cell (average ± S.D.); %ACEG, percent of aberrant cells excluding gaps (average ± S.D.); MA, multi-aberrant cells, corresponding to cells with more than 10 aberrations. MA are included in the index %ACEG; MI, mitotic index.

[a] These results are average values from two independent experiments (100 metaphases analysed per experiment).

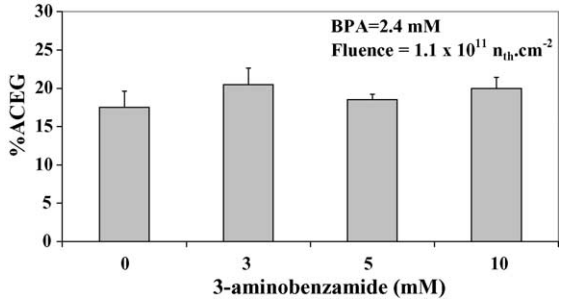

Fig. 2. Effect of different concentrations of 3-aminobenzamide (3-AB, 3–10 mM) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean ± S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence of 1.1×10^{11} n_{th} cm^{-2}. Two independent experiments were performed. In each experiment, 100 metaphases were analysed for chromosomal aberrations.

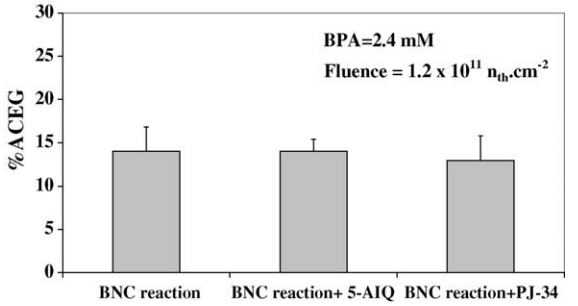

Fig. 3. Effect of 5-AIQ (100 μM) and PJ-34 (10 μM) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean ± S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence of 1.2×10^{11} n_{th} cm^{-2}. Two independent experiments were performed. In each experiment, 100 metaphases were analysed for chromosomal aberrations.

induction of chromosomal aberrations by the BNC reaction (BPA concentration of 2.4 mM and thermal neutrons fluence of 1.1×10^{11} n_{th} cm^{-2}). Both Fig. 2 and Table 2 show the clastogenic effect of the BNC reaction. This genotoxicity is not affected by the inhibition of poly(ADP-ribosyl)ation even when high doses of 3-AB are used. No differences were observed for the %ACEG (Table 2, Fig. 2). In addition, although the CAEG/cell (Table 2) was slightly higher in 3-AB-treated cultures these increases were not statistically significant. Also, the pattern of the aberrations observed was not modified in the presence of 3-AB. Regarding the genotoxicity of the control cultures exposed to thermal neutrons, an approximately two-fold increase in the CAEG/cell and %ACEG (without statistical significance) was observed in the presence of 10 mM 3-AB. This concentration of 3-AB also increased the %ACEG of BPA-treated controls and background V79 controls, albeit not significantly. The MI was generally lower in 3-AB-treated cultures, especially for the 10 mM concentration of 3-AB.

Table 3 and Fig. 3 present the effect 5-AIQ (100 μM) and PJ-34 (10 μM) on the induction of chromosomal aberrations by the BNC reaction (BPA concentration of 2.4 mM and thermal neutrons fluence of 1.2×10^{11} n_{th} cm^{-2}). The presence of these potent inhibitors did not increase the indices %ACEG and CAEG/cell induced by the BNC reaction and did not change the pattern of the aberrations presented. No differences were observed in the genotoxicity presented by the controls of the BNC reaction (background V79 cells, BPA-treated V79 cells and neutron-irradiated V79 cells) in the presence of the same concentrations of 5-AIQ and PJ-34. No marked differences in the MI were observed in 5-AIQ- or PJ-34-treated cells compared with non-treated cultures, although in some experimental points a slight decrease of ∼10% was found in PARP-inhibited cultures.

3.2. Induction of micronuclei

The induction of micronuclei by the BNC reaction (2.4 mM of BPA and 1.2×10^{11} n_{th} cm^{-2}) in the presence of a fixed concentration of 3-AB (5.0 mM) per se or with WM (5.0 μM) is presented in Table 4 and Fig. 4. Table 4 shows the frequency of micronucleated binucleated cells (%MNBN), the number of micronuclei per binucleated cell (MN/BN) as well as the distribution of the BN cells scored according to the number of MN (0, 1, 2, 3, 4 and ≥5). This table presents data not only for the BNC reaction but also for the controls (V79 cells, BPA and thermal neutrons) in the presence or absence of the inhibitors. The %BN cells and NDI are also presented in the same table as indices of cell proliferation.

The effect of 3-AB and WM, alone or in combination, on the genotoxicity of the BNC reaction is depicted in Fig. 4. The presence of 3-AB slightly increased the %MNBN induced by the BNC reaction (1.1-fold, $p < 0.05$). The comparison between the BNC

Table 4
Effect of the association of 3-aminobenzamide and wortmannin on the genotoxicity of the boron neutron capture reaction

Conditions	Fluence (n_{th} cm^{-2})	BPA (mM)	3-AB (mM)	WM (μM)	Number of MN-distribution (2000 cells)						MN/BN	%MNBN[a]	%BN[a]	NDI
					0	1	2	3	4	≥5				
V79 cells controls	0	0	0	0	1970	28	1	1	0	0	0.017 ± 0.002	1.5 ± 0.0	66.3 ± 0.7	1.69 ± 0.01
	0	0	5	0	1969	28	3	0	0	0	0.017 ± 0.004	1.6 ± 0.4	62.8 ± 7.9	1.66 ± 0.13
	0	0	0	5	1967	28	4	1	0	0	0.020 ± 0.004	1.7 ± 0.1	39.8 ± 7.6	1.43 ± 0.09
	0	0	5	5	1969	28	3	0	0	0	0.017 ± 0.006	1.6 ± 0.4	52.3 ± 4.9	1.55 ± 0.01
BPA controls	0	2.4	0	0	1960	40	0	0	0	0	0.020 ± 0.008	2.0 ± 0.8	70.3 ± 1.6	1.69 ± 0.03
	0	2.4	5	0	1968	30	2	0	0	0	0.017 ± 0.000	1.6 ± 0.0	72.3 ± 1.6	1.75 ± 0.04
	0	2.4	0	5	1964	33	2	1	0	0	0.020 ± 0.000	1.8 ± 0.3	47.0 ± 12.4	1.50 ± 0.16
	0	2.4	5	5	1964	32	2	1	1	0	0.022 ± 0.008	1.8 ± 0.4	65.1 ± 10.0	1.64 ± 0.18
Neutrons controls	1.2×10^{11}	0	0	0	1913	80	7	0	0	0	0.047 ± 0.006	4.4 ± 0.8	66.3 ± 6.6	1.66 ± 0.06
	1.2×10^{11}	0	5	0	1912	83	5	0	0	0	0.047 ± 0.002	4.4 ± 0.3	62.7 ± 8.1	1.63 ± 0.07
	1.2×10^{11}	0	0	5	1864	130	6	0	0	0	0.071 ± 0.004	6.8 ± 0.3	33.2 ± 3.7	1.33 ± 0.05
	1.2×10^{11}	0	5	5	1884	110	5	1	0	0	0.062 ± 0.008	5.8 ± 0.8	50.8 ± 1.1	1.48 ± 0.04
BNC reaction	1.2×10^{11}	2.4	0	0	1702	268	29	1	0	0	0.165 ± 0.045	14.9 ± 3.5	59.7 ± 4.9	1.61 ± 0.02
	1.2×10^{11}	2.4	5	0	1668	289	34	9	0	0	0.192 ± 0.030	16.6 ± 3.3	60.4 ± 5.4	1.60 ± 0.09
	1.2×10^{11}	2.4	0	5	1491	405	79	22	2	1	0.321 ± 0.047	25.5 ± 2.9	44.3 ± 4.1	1.45 ± 0.07
	1.2×10^{11}	2.4	5	5	1453	436	89	19	3	0	0.342 ± 0.011	27.4 ± 1.8	50.3 ± 6.1	1.53 ± 0.06

BPA, 4-borono-L-phenylalanine; 3-AB, 3-aminobenzamide; WM, wortmannin; MN, micronuclei; MN/BN, number of micronuclei per binucleated cell (average ± S.D.); %MNBN, percent of micronucleated binucleated cells (average ± S.D.); %BN, percent of binucleated cells (average ± S.D.).

[a] These results are average values from two independent experiments (1000 binucleated cells analysed per experiment).

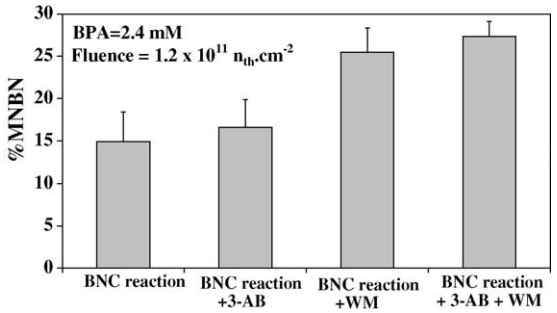

Fig. 4. Effect of 3-aminobenzamide (3-AB) and wortmannin (WM), alone or in combination, on the frequency of micronucleated binucleated cells (%MNBN) induced by the BNC reaction (mean ± S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence of 1.2×10^{11} n_{th} cm^{-2}. Two independent experiments were performed. In each experiment, 1000 binucleated cells were analysed for the presence of micronuclei.

reaction in the presence and absence of 3-AB for the MN/BN index (Table 4) revealed no statistically significant increase. It is also clear in Fig. 4 and in Table 4, that the effect of the concomitant incubation of WM and 3-AB on the increase of micronucleated cells induced by the BNC reaction (1.8–2.1-fold, $p < 0.05$) was in the same range as the effect observed for the incubation of WM alone and the BNC reaction (1.7–1.9-fold; $p < 0.05$).

The presence of WM significantly enhanced the genotoxicity of thermal neutrons (Table 4, 1.5-fold, $p < 0.05$) but the incubation with 3-AB had no effect. Other controls (BPA-treated V79 cells and background V79 cells) were not affected by the two enzyme inhibitors.

4. Discussion

The information available on the role of poly(ADP-ribosyl)ation and PARP enzymes on the genotoxicity of high-LET radiation is sparse. The work presented here addresses the effect of the poly(ADP-ribosyl)ation inhibitors 3-AB, 5-AIQ and PJ-34, alone or in combination with WM, a DNA-PK inhibitor, on the modulation of the genotoxic damage induced by the α- and lithium particles generated during the BNC reaction.

The benzamide derivative 3-AB is a prototype inhibitor that binds to the C-terminal nicotinamide-binding site of PARP-1 [20,40]. The ability of PARP-1 to recognize and bind DNA interruptions is not hampered in the presence of 3-AB [41]. According to some authors, 3-AB remains an important tool to understand the role of PARP-1 in different exposure scenarios if used in appropriate pharmacological concentrations, that is, concentrations in the low millimolar range (reviewed in [28]). The present study shows that a concentration of 1.5 mM 3-AB did not increase or modify the pattern of genotoxicity presented by a wide range of doses of the BNC reaction (Table 1, Fig. 1). The pattern of chromosomal aberrations induced by the BNC reaction, with or without 3-AB, included not only breaks, dicentric chromosomes and rings, but also an important number of chromosomal rearrangements, which can be regarded as typical features of high-LET radiation (reviewed in [42]) and were formed probably due to the erroneous rejoining of DSBs [43].

3-AB was tested with different doses of high-LET radiation that were genotoxic to up to ∼50% of cells, in order to enable a more complete view of the poly(ADP-ribosyl)ation inhibition on different degrees of DNA damage. In fact, it is well known that PARP-1 has a dual role in the genotoxic response of mammalian cells, acting as a caretaker in situations of mild genotoxic burden to DNA and, conversely, as an inducer of cell death in situations where severe DNA damage is found [10,30].

Higher concentrations of 3-AB (3–10 mM) were also included in this study. Some authors found quantitative differences in the cellular effects of 3-AB, dependent on its concentration. Utsumi and Elkind [24] found that post-irradiation (X-rays) treatment with up to 1 mM 3-AB inhibited the slow type of potentially lethal damage (PLD) repair in V79 cells, whereas the fast type of PLD repair was only inhibited by 3-AB concentrations of 2 mM and above. Other studies also showed dose-dependent sensitizing effects of 3-AB [44,45]. The results in terms of genotoxicity observed by us in the dose-response curve of 3-AB up to 10 mM (Table 2, Fig. 2) were similar to the previous ones with 1.5 mM of 3-AB.

The mitotic indices were, in general, lower in 3-AB-treated cultures even for the 1.5 mM concentration. This effect on cell division was highly variable within the experimental points studied and was especially, although not always, observed in cultures exposed to the BNC reaction. This effect could be due to non-specific

effects of 3-AB, usually described for concentrations of about 5 mM or higher, namely interference with the glucose metabolism or even with DNA synthesis, as reported by Milan and Cleaver [46].

A major caveat of 3-AB, and usually the one most often referred to, is its relatively weak inhibitory potency compared with that of other PARP inhibitors, namely with the isoquinolinone derivatives. In fact, as identified by Banasik et al. [47], some isoquinolinone derivatives and analogues were indeed very strong inhibitors of PARP-1, presenting 50% inhibitory concentrations (IC50) of less than 0.5 μM in cell-free experiments, whereas a value of 33 μM was found for 3-AB in the same experimental protocol. In view of this, we have tested two promising and structurally different poly(ADP-ribosyl)ation inhibitors, 5-AIQ and PJ-34. 5-AIQ is a water-soluble 5-aminosubstituted isoquinolin-1-one described by Suto et al. [48], with an IC50 of 0.24 μM in cell-free experiments [48] and an IC50 of 4.5 μM in experiments with intact cells [29]. On the other hand, PJ-34 is a phenanthridinone substitute and also a highly potent inhibitor of PARP-1 with an IC50 in the range of 0.1–1 μM in intact cells [30]. The two inhibitors were used in appropriate inhibitory concentrations as reported previously, 100 μM for 5-AIQ [29] and 10 μM for PJ-34 [30], but the results obtained confirm the previous ones with 3-AB, where no increase in the genotoxic burden induced by the BNC reaction was observed.

Our results are in agreement with a previous report where a normal sensitivity of human lymphocytes irradiated with high-LET fission neutrons (1.2–1.3 MeV) was observed in the presence of 5.0 mM of 3-AB [49]. According to the authors, the specific nature of base damage and of the structure of the DNA breaks induced by high-LET radiation might be responsible for such a response. In fact, DNA damage induced by high-LET radiations, such as α-particles, has a complex clustered nature [50] which can indeed be critical for the efficacy of PARP-1 and/or for the subsequent processes of DNA repair.

Recently, we have found that the fungal metabolite WM significantly increased the frequency of micronuclei induced by the BNC reaction [35]. Although WM is not a specific inhibitor for DNA-PK [51], some authors have demonstrated with cell lines defective in DNA-PK or ATM, that the DNA-PK is most likely the key-target for the radio-sensitizing effects induced by this compound (reviewed in [35]). WM strongly inhibits DNA-PK, and thus interferes in the non-homologous end-joining (NHEJ) repair, which is a process of utmost importance for double-strand break repair in eukaryotes [9,18,19]. Several reports have indicated an interaction between PARP-1 and DNA-PK [13,20–23,52], possibly in order to minimize aberrant chromosomal recombination. Accordingly, our further aim was to evaluate the effect of 3-AB on the sensitizing effect of WM for the BNC reaction, using 5 μM of WM, a concentration used previously for this inhibitor [35,53]. The results obtained show that the induction of micronuclei by the BNC reaction in the presence of 5.0 mM 3-AB revealed a minor increase of about 1.1-fold and that PARP inhibition together with a concomitant inhibition of DNA-PK revealed barely the same sensitizing effect as did DNA-PK inhibition per se.

The mechanisms by which PARP-1 and PARP-2, another DNA repair enzyme [54] with a DNA-dependent ADP-ribosylation activity, function as DNA repair enzymes are complex and not completely known (reviewed in [30]). The three poly(ADP-ribosyl)ation inhibitors used in our study are likely to interfere with all PARP enzymes since they have catalytical domains highly homologous to that of PARP-1 [27,28,30,55]. However, besides PARP-2 [30,56], the information on the effect of poly(ADP-ribosyl)ation inhibitors on other PARP enzymes is very limited. Hence, the lack of increase in the genotoxicity of the BNC reaction could be related to the activity of a back-up enzyme.

Altogether, these results suggest that the poly(ADP-ribosyl)ation inhibitors, which are undoubtedly promising drugs for various pathologies, are not good candidates for sensitizing the effect of α-particles and high-LET radiation therapies, such as BNC therapy.

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References

[1] H. Hatanaka, Y. Nakagawa, Clinical results of long-surviving brain tumor patients who underwent boron neutron capture therapy, Int. J. Radiat. Oncol. Biol. Phys. 28 (1994) 1061–1066.

[2] Y. Mishima, Melanoma and nonmelanoma neutron capture therapy using gene therapy: overview, in: B. Larsson, J. Crawford, R. Weinreich (Eds.), Advances in Neutron Capture Therapy. Medicine and Physics, vol. I, Elsevier Science B.V., Amsterdam, 1997, pp. 10–25.

[3] S.J. González, M.R. Bonomi, G.A. Santa Cruz, H.R. Blaumann, O.A. Calzetta Larrieu, P. Menéndez, R. Jiménez Rebagliati, J. Longhino, D.B. Feld, M.A. Dagrosa, C. Argerich, S.G. Castiglia, D.A. Batistoni, S.J. Liberman, B.M.C. Roth, First BNCT treatment of a skin melanoma in Argentina: dosimetric analysis and clinical outcome, Appl. Radiat. Isotopes 61 (2004) 1101–1105.

[4] I. Kato, K. Ono, Y. Sakurai, M. Ohmae, A. Maruhashi, Y. Imahori, M. Kirihata, M. Nakazawa, Y. Yura, Effectiveness of BNCT for recurrent head and neck malignancies, Appl. Radiat. Isotopes 61 (2004) 1069–1073.

[5] NRC (National Research Council), Committee on Heath Effects of Exposure to Radon (BEIR VI), and Commission on Life Sciences, Heath Effects of Exposure to Radon, National Academy Press, Washington, D.C., 1999.

[6] T.J. Jenner, C.M. deLara, P. O'Neill, D.L. Stevens, Induction and rejoining of DNA double-strand breaks in V79-4 mammalian cells following gamma- and alpha-irradiation, Int. J. Radiat. Biol. 64 (1993) 265–273.

[7] H.C. Newman, K.M. Prise, M. Folkard, B.D. Michael, DNA double-strand break distributions in X-ray and alpha-particle irradiated V79 cells: evidence for non-random breakage, Int. J. Radiat. Biol. 71 (1997) 347–363.

[8] G. Obe, C. Johannes, D. Schulte-Frohlinde, DNA double-strand breaks induced by sparsely ionizing radiation and endonucleases as critical lesions for cell death, chromosomal aberrations, mutations and oncogenic transformation, Mutagenesis 7 (1992) 3–12.

[9] S.E. Critchlow, S.P. Jackson, DNA end-joining: from yeast to man, Trends Biochem. Sci. 23 (1998) 394–398.

[10] S. Beneke, J. Diefenbach, A. Burkle, Poly(ADP-ribosyl)ation inhibitors: promising drug candidates for a wide variety of pathophysiologic conditions, Int. J. Cancer 116 (2004) 813–818.

[11] F. Dantzer, H.P. Nasheuer, J.L. Vonesch, G. de Murcia, J. Menissier-de Murcia, Functional association of poly(ADP-ribose) polymerase with DNA polymerase alpha-primase complex: a link between DNA strand break detection and DNA replication, Nucl. Acids Res. 26 (1998) 1891–1898.

[12] S. Shall, G. de Murcia, Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? Mutat. Res. 460 (2000) 1–15.

[13] J.M. Pleschke, H.E. Kleczkowska, M. Strohm, F.R. Althaus, Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins, J. Biol. Chem. 275 (2000) 40974–40980.

[14] F.R. Althaus, H.E. Kleczkowska, M. Malanga, C.R. Muntener, J.M. Pleschke, M. Ebner, B. Auer, Poly ADP-ribosylation: a DNA break signal mechanism, Mol. Cell. Biochem. 193 (1999) 5–11.

[15] A. Burkle, Poly(ADP-ribosyl)ation, a DNA damage-driven protein modification and regulator of genomic instability, Cancer Lett. 163 (2001) 1–5.

[16] J. Ménissier-de Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F.J. Oliver, M. Masson, A. Dierich, M. LeMeur, C. Walztinger, P. Chambon, G. de Murcia, Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 7303–7307.

[17] M. Masutani, T. Nozaki, K. Nakamoto, H. Nakagama, H. Suzuki, O. Kusuoka, M. Tsutsumi, T. Sugimura, The response of Parp knockout mice against DNA damaging agents, Mutat. Res. 462 (2000) 159–166.

[18] C. Featherstone, S.P. Jackson, DNA double-strand break repair, Curr. Biol. 9 (1999) R759–R761.

[19] P. Pfeiffer, W. Goedecke, G. Obe, Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations, Mutagenesis 15 (2000) 289–302.

[20] M. Fernet, V. Ponette, E. Deniaud-Alexandre, J. Menissier-De Murcia, G. De Murcia, N. Giocanti, F. Megnin-Chanet, V. Favaudon, Poly(ADP-ribose) polymerase, a major determinant of early cell response to ionizing radiation, Int. J. Radiat. Biol. 76 (2000) 1621–1629.

[21] W.M. Tong, U. Cortes, Z.Q. Wang, Poly(ADP-ribose) polymerase: a guardian angel protecting the genome and suppressing tumorigenesis, Biochim. Biophys. Acta 1552 (2001) 27–37.

[22] Y. Ariumi, M. Masutani, T.D. Copeland, T. Mimori, T. Sugimura, K. Shimotohno, K. Ueda, M. Hatanaka, M. Noda, Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase *in vitro*, Oncogene 18 (1999) 4616–4625.

[23] T. Ruscetti, B.E. Lehnert, J. Halbrook, H. Le Trong, M.F. Hoekstra, D.J. Chen, S.R. Peterson, Stimulation of the DNA-dependent protein kinase by poly(ADP-ribose) polymerase, J. Biol. Chem. 273 (1998) 14461–14467.

[24] H. Utsumi, M.M. Elkind, Inhibitors of poly (ADP-ribose) synthesis inhibit the two types of repair of potentially lethal damage, Int. J. Radiat. Oncol. Biol. Phys. 29 (1994) 577–578.

[25] B. Koberle, G. Speit, Molecular characterization of mutations at the hprt locus in V79 Chinese hamster cells induced by bleomycin in the presence of inhibitors of DNA repair, Mutat. Res. 249 (1991) 161–167.

[26] R. Helbig, E. Gerland, G. Speit, The molecular nature of mutations induced by adriamycin at the hprt locus of V79 cells, Mutagenesis 9 (1994) 113–116.

[27] A. Chalmers, P. Johnston, M. Woodcock, M. Joiner, B. Marples, PARP-1, PARP-2, and the cellular response to low doses of ionizing radiation, Int. J. Radiat. Oncol. Biol. Phys. 58 (2004) 410–419.

[28] C. Cosi, New inhibitors of poly(ADP-ribose) polymerase and their potential therapeutic targets, Expert Opin. Ther. Patents 12 (2002) 1047–1071.

[29] N. Wayman, M.C. McDonald, A.S. Thompson, M.D. Threadgill, C. Thiemermann, 5-Aminoisoquinolinone, a potent inhibitor

of poly (adenosine 5′-diphosphate ribose) polymerase, reduces myocardial infarct size, Eur. J. Pharmacol. 430 (2001) 93–100.
[30] L. Virag, C. Szabo, The therapeutic potential of poly(ADP-ribose) polymerase inhibitors, Pharmacol. Rev. 54 (2002) 375–429.
[31] R.A. Izzard, S.P. Jackson, G.C. Smith, Competitive and non-competitive inhibition of the DNA-dependent protein kinase, Cancer Res. 59 (1999) 2581–2586.
[32] M.C. McDonald, H. Mota-Filipe, J.A. Wright, M. Abdelrahman, M.D. Threadgill, A.S. Thompson, C. Thiemermann, Effects of 5-aminoisoquinolinone, a water-soluble, potent inhibitor of the activity of poly (ADP-ribose) polymerase on the organ injury and dysfunction caused by haemorrhagic shock, Br. J. Pharmacol. 130 (2000) 843–850.
[33] I.C. Gonçalves, A.G. Ramalho, I.F. Gonçalves, J. Salgado, J. Pertusa, A. Irles, F. Mascarenhas, M. Castro, F. Vale, J. Toscano-Rico, V. Alcober, Work in the field of BNCT using the Portuguese Research Reactor, J. Radioanal. Nucl. Chem. 240 (1999) 431–432.
[34] N.G. Oliveira, M. Castro, A.S. Rodrigues, I.C. Gonçalves, R. Cassapo, A.P. Fernandes, T. Chaveca, J.M. Toscano-Rico, J. Rueff, Evaluation of the genotoxic effects of the boron neutron capture reaction in human melanoma cells using the cytokinesis block micronucleus assay, Mutagenesis 16 (2001) 369–375.
[35] N.G. Oliveira, M. Castro, A.S. Rodrigues, I.C. Gonçalves, O.M. Gil, A.P. Fernandes, J.M. Toscano-Rico, J. Rueff, Wortmannin enhances the induction of micronuclei by low and high let radiation, Mutagenesis 18 (2003) 37–44.
[36] B.J. Dean, N. Danford, Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells, in: S. Venitt, J.M. Parry (Eds.), Mutagenicity Testing. A Practical Approach, IRL Press, Oxford, 1984, pp. 187–232.
[37] S.H. Swierenga, J.A. Heddle, E.A. Sigal, J.P. Gilman, R.L. Brillinger, G.R. Douglas, E.R. Nestmann, Recommended protocols based on a survey of current practice in genotoxicity testing laboratories. IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures, Mutat. Res. 246 (1991) 285–300.
[38] P. Van Hummelen, M. Kirsh-Volders, An improved method for the ‘in vitro’ micronucleus using human lymphocytes, Mutagenesis 5 (1990) 203–204.
[39] M. Fenech, The in vitro micronucleus technique, Mutat. Res. 455 (2000) 81–95.
[40] A. Ruf, J. Mennissier de Murcia, G. de Murcia, G.E. Schulz, Structure of the catalytic fragment of poly(ADP-ribose) polymerase from chicken, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 7481–7485.
[41] M.S. Satoh, T. Lindahl, Role of poly(ADP-ribose) formation in DNA repair, Nature 356 (1992) 356–358.
[42] E.A. Blakely, A. Kronenberg, Heavy-ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness, Radiat. Res. 150 (1998) 126–145.
[43] K. Rothkamm, M. Lobrich, Misrepair of radiation-induced DNA double-strand breaks and its relevance for tumorigenesis and cancer treatment (Review), Int. J. Oncol. 21 (2002) 433–440.
[44] P. Thraves, K.L. Mossman, T. Brennan, A. Dritschilo, Radiosensitization of human fibroblasts by 3-aminobenzamide: an inhibitor of poly(ADP-ribosylation), Radiat. Res. 104 (1985) 119–127.
[45] F. Darroudi, A.T. Natarajan, Cytological characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. I. Induction of chromosomal aberrations by X-irradiation and its modulation with 3-aminobenzamide and caffeine, Mutat. Res. 177 (1987) 133–148.
[46] K.M. Milam, J.E. Cleaver, Inhibitors of poly(adenosine diphosphate-ribose) synthesis: effect on other metabolic processes, Science 223 (1984) 589–591.
[47] M. Banasik, H. Komura, M. Shimoyama, K. Ueda, Specific inhibitors of poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase, J. Biol. Chem. 267 (1992) 1569–1575.
[48] M.J. Suto, W.R. Turner, C.M. Arundel-Suto, L.M. Werbel, J.S. Sebolt-Leopold, Dihydroisoquinolinones: the design and synthesis of a new series of potent inhibitors of poly(ADP-ribose) polymerase, Anticancer Drug Des. 6 (1991) 107–117.
[49] M.W. Heartlein, R.J. Preston, The effect of 3-aminobenzamide on the frequency of X-ray- or neutron-induced chromosome aberrations in cycling or non-cycling human lymphocytes, Mutat. Res. 148 (1985) 91–97.
[50] D.T. Goodhead, Mechanisms for the biological effectiveness of high-LET radiations, J. Radiat. Res. 40 (1999) 1–13.
[51] J.N. Sarkaria, R.S. Tibbetts, E.C. Busby, A.P. Kennedy, D.E. Hill, R.T. Abraham, Inhibition of phosphoinositide 3-kinase related kinases by the radiosensitizing agent wortmannin, Cancer Res. 58 (1998) 4375–4382.
[52] M. Weinfeld, M.A. Chaudhry, D. D’Amours, J.D. Pelletier, G.G. Poirier, L.F. Povirk, S.P. Lees-Miller, Interaction of DNA-dependent protein kinase and poly(ADP-ribose) polymerase with radiation-induced DNA strand breaks, Radiat. Res. 148 (1997) 22–28.
[53] N.G. Oliveira, M. Castro, A.S. Rodrigues, O.M. Gil, J.M. Toscano-Rico, J. Rueff, DNA-PK inhibitor wortmannin enhances DNA damage induced by bleomycin in V79 Chinese hamster cells, Teratogen. Carcinogen. Mutagen. 22 (2002) 343–351.
[54] R.D. Wood, M. Mitchell, J. Sgouros, T. Lindahl, Human DNA repair genes, Science 291 (2002) 1284–1289.
[55] S. Smith, The world according to PARP, Trends Biochem. Sci. 26 (2001) 174–179.
[56] J.C. Amé, V. Rolli, V. Schreiber, C. Niedergang, F. Apiou, P. Decker, S. Muller, T. Hoger, J. Menissier de Murcia, G. de Murcia, PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase, J. Biol. Chem. 274 (1999) 17860–17868.