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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  $^{10}\text{B}$  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.

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**Keywords:**  $\alpha$ -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  $^{10}\text{B}$  with low-energy thermal neutrons and gives rise to lithium and  $\alpha$ -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  $\alpha$ -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  $\text{NAD}^+$  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  $\gamma$ -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;  $^{10}\text{B}$ -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  $^{10}\text{B}$ -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 <sup>10</sup>B). Cells were seeded (approximately  $1.0 \times 10^5$ ) in 25-cm<sup>2</sup> 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 ( $\phi$ ) between  $1.1 \times 10^{11}$  and  $4.4 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>. 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  $D_B = 8.66 \times 10^{-8} [^{10}\text{B}] \phi$ . The absorbed doses from α- and lithium particles for the fluences ( $\phi$ ) and BPA (<sup>10</sup>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 cri-

teria [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:

$$\text{NDI} = [M1 + 2(M2) + 3(M3) + 4(M4)]/N,$$
 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 ( $1.1 \times 10^{11}$ ,  $2.1 \times 10^{11}$  and  $4.4 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>) 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<br>(n <sub>th</sub> cm <sup>-2</sup> ) | BPA<br>(mM) | 3-AB<br>(1.5 mM) | Chromosomal aberrations<br>per 100 cells <sup>a</sup> |     |      |      |     |       |       | MA<br>(%) | CAEG/cell     | %ACEG      | MI (%)    |
|--|-------------|------------------|---|-----|------|------|-----|-------|-------|-----------|---------------|------------|-----------|
|  |             |                  | Ctg   | Csg | Ctb  | Csb  | Dic | Rings | Rearr |           |               |            |           |
| 0 <sup>#</sup>                                 | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>+</sup>                                 | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>+</sup>                                 | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>+</sup>                                 | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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.

<sup>a</sup> 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<br>(n <sub>th</sub> cm <sup>-2</sup> ) | BPA<br>(mM) | 3-AB<br>(mM) | Chromosomal aberrations<br>per 100 cells <sup>a</sup> |     |     |      |     |       |       | MA<br>(%) | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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.

<sup>a</sup> 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  $\alpha$ - 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.

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

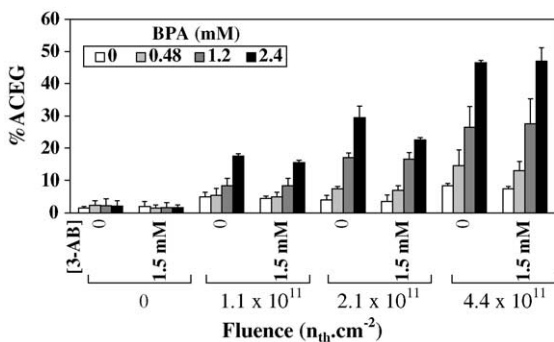


Fig. 1. Effect of 3-aminobenzamide (3-AB, 1.5 mM) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean ± S.D.). The BNC reaction was performed using different concentrations of 4-borono-L-phenylalanine (BPA, 0.48–2.4 mM) and different fluences of thermal neutrons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

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<br>(n <sub>th</sub> cm <sup>-2</sup> ) | BPA<br>(mM) | Chromosomal aberrations<br>per 100 cells <sup>a</sup> |     |     |     |     |       |       | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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.

<sup>a</sup> These results are average values from two independent experiments (100 metaphases analysed per experiment).



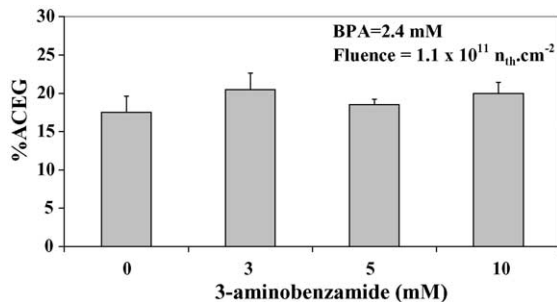


Fig. 2. Effect of different concentrations of 3-aminobenzamide (3-AB, 3–10 mM) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean  $\pm$  S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence of  $1.1 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>. 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  $1.1 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>). 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  $\mu$ M) and PJ-34 (10  $\mu$ M) on the induction of chromosomal aberrations by the BNC reaction (BPA concentration of 2.4 mM and thermal neutrons fluence of  $1.2 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>). 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

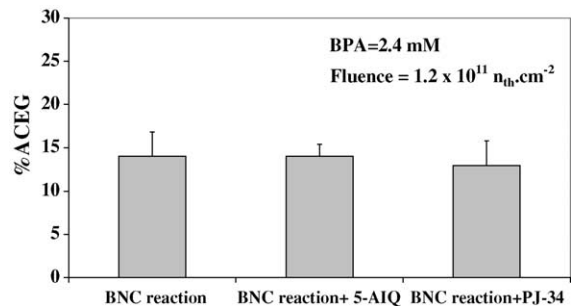


Fig. 3. Effect of 5-AIQ (100  $\mu$ M) and PJ-34 (10  $\mu$ M) on the induction of aberrant cells excluding gaps (%ACEG) by the BNC reaction in V79 cells (mean  $\pm$  S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence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

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  $\sim 10\%$  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  $1.2 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>) in the presence of a fixed concentration of 3-AB (5.0 mM) per se or with WM (5.0  $\mu$ M) 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  $\geq 5$ ). 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<br>(n <sub>th</sub> cm <sup>-2</sup> ) | BPA<br>(mM) | 3-AB<br>(mM) | WM<br>(μM) | Number of MN-distribution<br>(2000 cells) |     |    |    |   |    | MN/BN         | %MNBN <sup>a</sup> | %BN <sup>a</sup> | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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 <sup>11</sup>                         | 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.).

<sup>a</sup> These results are average values from two independent experiments (1000 binucleated cells analysed per experiment).

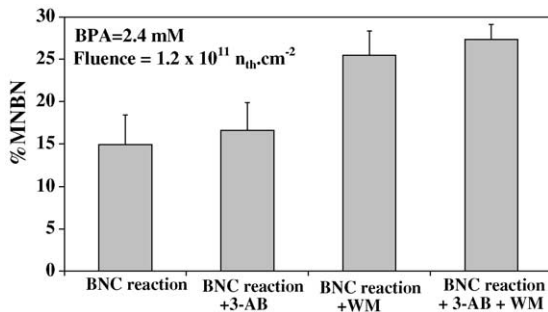


Fig. 4. Effect of 3-aminobenzamide (3-AB) and wortmannin (WM), alone or in combination, on the frequency of micronucleated binucleated cells (%MNBN) induced by the BNC reaction (mean  $\pm$  S.D.). The BNC reaction was performed using a 4-borono-L-phenylalanine (BPA) concentration of 2.4 mM and thermal neutrons at a fluence of  $1.2 \times 10^{11}$  n<sub>th</sub> cm<sup>-2</sup>. 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  $\alpha$ - 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 (IC<sub>50</sub>) of less than 0.5  $\mu$ M in cell-free experiments, whereas a value of 33  $\mu$ M 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 IC<sub>50</sub> of 0.24  $\mu$ M in cell-free experiments [48] and an IC<sub>50</sub> of 4.5  $\mu$ M 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 IC<sub>50</sub> in the range of 0.1–1  $\mu$ M in intact cells [30]. The two inhibitors were used in appropriate inhibitory concentrations as reported previously, 100  $\mu$ M for 5-AIQ [29] and 10  $\mu$ M 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  $\alpha$ -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 com-

pound (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  $\mu$ M 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  $\alpha$ -particles and high-LET radiation therapies, such as BNC therapy.

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