Benznidazole-induced genotoxicity in diploid cells of
Aspergillus nidulans

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Genotoxic effects of benznidazole were studied by the induction of homozygosis of genes previously present in heterozygous. UT448/A757 diploid strain was used in the benznidazole’s recombinagenesis test. Although toxic effects on growth of colonies were not observed, 75 and 100 µM benznidazole induced an increasing of mitotic recombination events in diploid strain. Results were related to the induction of chromosomal breaks by the anti-parasitic drug.

Key words: benznidazole - somatic recombination - genotoxicity - homozigotization index

Chagas disease, caused by Trypanosoma cruzi, is one of the most serious parasitic diseases of Latin America, where some 16 - 18 million people are infected by the T. cruzi (WHO 1991). Although in Brazil the disease is under strict control, there are still about 4 million people infected by the parasite (Dias 1998). Benznidazole is used in the treatment of Chagas disease in the acute phase, at the start of the chronic phase, in congenital cases and accidents. On the other hand, although Teixeira et al. (1994) observed a high incidence of lymphoblastic lymphoma in mice treated with benznidazole, Andrade et al. (2003) verified that the administration of benznidazole in immunosuppressed mice and chronically infected by T. cruzi did not result in the emergence of lymphomas or other neoplasms.

In spite of these contradictory results, the clastogenic effect of benznidazole (Lacava & Luna 1994) suggest its participation in the carcinogenic process due to the occurrence of mitotic crossing-over that induces the loss of heterozygosity of tumor suppressor genes (Zimmermann 1971, Weinberg 1991, Beumer et al. 1998). The evaluation of the recombinagenic potential of benznidazol in heterozygous cells of Aspergillus nidulans will be provided. Information on the participation of the anti-parasitic drug in the carcinogenesis process will be thus ensued.

MATERIALS AND METHODS

Strains and culture media - A. nidulans strains used are described in Table I. Minimum medium (MM) was Czape-Dox with 1% (w/v) glucose. Complete medium (CM) has previously been described by Pontecorvo et al. (1953) and Van De Vate and Jansen (1978). Supplemented medium (SM) consisted of MM plus nutrients required by each strain. Solid medium contained 1.5% agar. Incubation occurred at 37°C.

Methods - General methodology followed previous reports (Roper 1952, Pontecorvo et al. 1953). Heterokaryons were prepared in liquid MM plus 2% CM. Cleistothecia were obtained from heterokaryons after 21 days of incubation in sealed petri dishes containing MM, supplemented according to the requirements of the crossed strains. Diploids were prepared by method described by Roper (1952).

Evaluation of drug toxicity - Filter-sterilized aqueous solutions of benznidazole (Roche), 99.8% pure, was added

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RESULTS AND DISCUSSION

Three different benznidazole concentrations were assayed for their ability to induce cytotoxic effects and somatic segregation in the A. nidulans mould. Benznidazole had no effect on colonies’ morphologies and micelial growth of diploid UT448//A757 and B211//A837 strains at the three tested concentrations (results not shown).

Induction of aneuploidy and mitotic crossing-over was studied in heterozygous diploid B211//A837 strain exposed to benznidazole. Treatment of B211//A837 with benznidazole 100 mM in CM allowed the isolation of a mitotic segregant named R1. Phenotypic analyses of R1 showed it was recombinant for Acr-w interval of chromosome II (Fig. 2). Segregant was grown in CM + benzylpenyl (2 µg/ml) so that its mitotic stability could be analyzed. R1 did not produce new mitotic sectors with the haploidization agent and was classified as haploid segregant (results not shown). In fact, when submitted to the sexual cycle, R1 produced normal frequencies of meiotic recombination for markers of chromosomes I, IV, and VIII (Table II).

Prototrophic diploid segregates were isolated from UT448//A757 colonies, after treatment with 50, 75, and 100 µM of benznidazole in MM. Diploids (D1 to D9) were submitted to spontaneous haploidization in CM (Fig. 3a,b) and the selected mitotic segregates were tested for their mitotic stability in CM + benzylpenyl (Fig. 3c,d). Only segregants that failed to produce new mitotic sectors, demonstrating genetic stability, were selected for HI determination.

Although HI values obtained from benznidazole 50 µM were lower than 2.0 (results not shown), results obtained with 75 and 100 µM demonstrate that benznidazole is effective in inducing mitotic crossing-over in A. nidulans diploid strain. HI values obtained from benznidazole 75 and 100 µM (D2, D4, D5, and D6) were higher than 2.0 and statistically different from control (Table III).

The recombinogenic effect of benznidazole may be related to the induction of chromosomal breaks, such as has been observed in cytogenetic analyses of human lym-
phocytes and of mice’s peritoneal macrophages (Moya & Trombott 1988, Lacava & Luna 1994).

Multiple genetic alterations, such as point mutations, chromosomal translocations and loss of heterozygosity (LOH), are involved in the cellular carcinogenesis (Barrett 1993, Ramel et al. 1996). In human retinoblastoma, LOH is the most common mechanism by which the normal wild-type allele at the RB1 locus is lost in a heterozygous retinal cell for a null mutation. Possible chromosomal mechanisms triggering LOH would include: mitotic non-disjunction with loss of chromosome bearing the wild-allele or mitotic recombination between the RB1 locus and the centromere, resulting in homozygosity of defective allele (Cavenee et al. 1991, Hagstrom & Dryja 1999).

Somatic recombination consists of exchange events between homologous chromosomes that, following chromosome segregation and cell division, may result in homozygosity of distal genes to the point of exchange (Lasko et al. 1991, Zimmermann 1992, Beumer et al. 1998). Although the rate of spontaneous mitotic recombination in dividing cells of mammals is very low (Morley et al. 1990), it is known that mitotic recombination may occur during the repair of chromosomal double-strand and single-strand break (Galli & Schiestl 1998, Hagstrom & Dryja 1999, Helleday 2003, Stark & Jasin 2003).

Benznidazole in vitro increased the frequency of the sister chromatid exchanges in human cells of hepatoma and in lymphocytes of patients treated with the drug. Drug is further capable of increasing the micronuclei frequency in the hepatoma cells (Santos et al. 1994). The clastogenic effect of the anti-parasitic agent was also observed in peripheral lymphocytes of chagasic children (Gorla 1988).

Although the mutagenic effect of benznidazole at 50 µM has been observed in S. typhimurium assays (Nagel & Nepomnaschy 1983), this dose does not show recombinogenic effect in A. nidulans (results not shown).

Our results demonstrate that benznidazole recombinogenic effect is dose-dependent. Since somatic recombination may trigger neoplasms, current analysis suggests that the carcinogenic potential of the anti-parasitic drug may be conducted by loss of heterozygosity mediated by mitotic crossing-over.

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**Fig. 1:** origin of recombinant diploid segregants through mitotic crossing-over.

**Fig. 2:** schematic representation of chromosomes I, II, IV, and VIII of R1 mitotic segregant.
TABLE II
Frequencies of meiotic recombination between markers from I, IV, and VIII chromosomes obtained in R1 x A507 cross

<table>
<thead>
<tr>
<th>Genetic interval</th>
<th>Control crosses</th>
<th>R1 x A507</th>
</tr>
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<tbody>
<tr>
<td>paba-y</td>
<td>15.9 (21/132)</td>
<td>14.0 (28/200)</td>
</tr>
<tr>
<td>paba-bi</td>
<td>21.2 (28/132)</td>
<td>17.5 (35/200)</td>
</tr>
<tr>
<td>paba-pyro</td>
<td>58.3 (77/132)</td>
<td>40.0 (80/200)</td>
</tr>
<tr>
<td>paba-cha</td>
<td>48.7 (37/76)</td>
<td>40.0 (80/200)</td>
</tr>
<tr>
<td>bi-y</td>
<td>5.3 (7/132)</td>
<td>5.5 (11/200)</td>
</tr>
<tr>
<td>bi-pyro</td>
<td>43.9 (58/132)</td>
<td>35.5 (71/200)</td>
</tr>
<tr>
<td>bi-cha</td>
<td>43.4 (33/76)</td>
<td>38.5 (77/200)</td>
</tr>
<tr>
<td>y-pyro</td>
<td>53.0 (70/132)</td>
<td>34.0 (68/200)</td>
</tr>
<tr>
<td>y-cha</td>
<td>46.1 (35/76)</td>
<td>42.0 (84/200)</td>
</tr>
<tr>
<td>pyro-cha</td>
<td>42.1 (32/76)</td>
<td>39.0 (78/200)</td>
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a: UT448 x A757 cross; b: B1 x UT184 cross

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REFERENCES


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