Original Research

Mitotic recombination: a genotoxic effect of the antidepressant citalopram in *Aspergillus nidulans*

Claudinéia C S Franco, Juliana Castro-Prado, Lúcia J Rosada, Juliane R Sant'Anna and Marialba A A Castro-Prado

Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Avenida Colombo 5790, Bloco H67. Maringá, Paraná 87020-900, Brazil
Corresponding author: Marialba A A de Castro-Prado. Email: maacprado@uem.br

Abstract

This report evaluates the potential of the antidepressant drug citalopram to induce homozygotization of genes previously present in a heterozygous condition, by homologous recombination. In order to address this question, a heterozygous diploid strain of the filamentous fungus *Aspergillus nidulans* and the homozygotization assay were utilized. Non-cytotoxic concentrations of citalopram (50, 75 and 100 μmol/L) showed a strong recombinogenic effect in *A. nidulans*, inducing homozygosis of the diploid strain's nutritional markers. The genetic markers exhibited homozygotization index (HI) rates higher than 2.0 and significantly different from HI control ones. Since citalopram has been previously characterized as a DNA synthesis inhibitor, the recombinogenic potential of this antidepressant in *A. nidulans* may be associated with the recombinational repair of citalopram-induced DNA strand breaks.

Keywords: cancer patients, citalopram, tumor promoters, second malignancy, somatic recombination

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Introduction

Citalopram is a selective serotonin reuptake inhibitor (SSRI) commonly prescribed in the treatment of various forms of psychiatric disorders, such as depression, obsessive-compulsive disorder, panic disorder, bulimia nervosa and anorexia. It is the most selective serotonin antidepressant with a low potential for interactions with other concomitant medication. Among currently available SSRIs, citalopram is a good choice for the treatment of many illnesses, including anxiety disorders and depression in children and adolescents, partly due to its pharmacokinetic profile, low toxicity in comparison to older tricyclic antidepressants (TCA) and favorable adverse-effect profile.[1–4] Although information on the genotoxic effect of citalopram are scarce, SSRI antidepressants, in concentrations relevant to the treatment of human depression, were found to promote fibrosarcomas, melanomas and mammary carcinogenesis in rodent models.[5–7] Epidemiologic studies, however, have shown conflicting results. The positive association among breast cancer risk and SSRIs users observed in some studies[5,8–10] has been regarded inconsistent in other investigations.[11,12]

Cancer is a progressive process that results from environmental and endogenous agents' toxic effects. The disease develops from a single oncogenic mutation-bearer cell (either a proto-oncogene or a tumor suppressor gene), whose primary mutagenic event ('first hit') is called initiation. The promotion of cancer ('second hit') occurs when the initiated cell is exposed to a cancer-promoting condition (or agent) which triggers the rapid progress of cancer. Tumor promoters are not themselves carcinogenic, but may promote tumors in systems already exposed to carcinogens.[13–15] Homologous mitotic recombination, the exchange of genetic material between homologous chromosomes, is an essential mechanism for faithful DNA replication in vertebrate cells and is an important repair pathway of DNA double-strand breaks. This recombinational repair, however, may result in the loss of the functional allele ($s+$) in a heterozygous cell of a defective tumor suppressor gene ($s+/s$).[16] The loss of heterozygosity (LOH), triggered by mitotic recombination, has been described as the somatic 'second hit' in initiated cells bearing germline mutations in the *APC* (adenomatous polyposis coli) gene (chromosome 5q21), which is a tumor suppressor gene.[16,17] The role of mitotic recombination in cancer development was also demonstrated in Bloom syndrome patients, whose cells increased in sister chromatid exchange as well as in the exchange between homologous chromosomes.[18–20] An increase in the frequency of mitotic

recombination may occur in response to several DNA damaging agents, such as DNA synthesis inhibitors and DNA strand breaks inducers.

Aspergillus nidulans is a filamentous fungus with an excellent model system for the study of mitotic crossing-over due to the fact that its cells pass the greater part of their cell cycle in the G2 phase.[21] Since chromosomes in this phase are in duplicate, they significantly favor mitotic recombination. Actually, *A. nidulans* diploid strains have been used to evaluate the recombinogenic potential of chemical compounds.[22–24] Doxorubicin and cytosine arabinoside, previously described as genotoxic in human cells,[25–27] were characterized as effective inducers of homozygotization of heterozygous-conditioned genes in *A. nidulans* diploid strains.[24,28]

Since the potential of chemical compounds to affect the carcinogenic process may be investigated through their effects on mitotic recombination,[25] this study evaluates the recombinogenic potential of citalopram for its ability to induce homozygosity of genes previously present in heterozygous condition in *A. nidulans.*

Materials and methods

Strain and culture media

The *A. nidulans* strain used is described in Table 1. The UT448//A757 diploid strain, with green conidia and obtained according to Roper[29] (Figure 1A and B), is heterozygous for nutritional markers and for conidia color markers *yA2* (yellow) and *wA2* (white). Mitotic segregants were observed as normally growing yellow, green or white sectors on UT448//A757 diploid green colonies. Minimal medium (MM) consisted of Czapek-Dox medium supplemented with 1% (w/v) glucose, while complete medium (CM) has been previously described.[28] Supplemented medium (SM) consisted of MM plus nutrients required by each strain. Solid medium contained 1.5% (w/v) agar.

Drug treatments

Filter-sterilized aqueous solutions of racemic citalopram hydrobromide ($C_{20}H21FN_2OHBr$, FW 405.3, Catalog # C 7861, 100% pure, Sigma-Aldrich Co, Steinheim, Germany) were added to molten MM. In the present study, non-cytotoxic concentrations of citalopram (50, 75 and 100 μmol/L), which inhibited DNA synthesis in biopsy-like Burkitt lymphoma cells (line L3055),[30] were used for recombinogenesis tests. In the case of toxicity measurements, UT448//A757 diploid colonies' diameters were determined during six days after incubation, at 37°C. Rates in the presence and in the absence (control) of the antidepressant drug were compared by one-way variance analysis and by Bonferroni post-test, for $P < 0.05$ (results not shown). The antichagasic agent benznidazole ($C_{12}H_{12}N_4O_3$, FW 260.25, 99.8% pure, Roche, Rio de Janeiro, Brazil), previously characterized as recombinogenic in human blood lymphocytes, human hepatoma cell line (Hep G2), and in *A. nidulans* diploid cells, was used as a positive control.[31,32]

Table 1 Genotype and origin of *Aspergillus nidulans* strains

Strains	Genotype	Origin
A757	*yA2* (I), *methA17* (II), *pyroA4* (IV)	FGSC*
UT448	*riboA1* (I), *pabaA124* (I), *biA1* (I), *AcrA1* (II), *wA2* (II)	Utrecht, Holand

Requirements for: riboflavin, *ribo*A1; *p*-aminobenzoic acid, *paba*A124; biotin, *bi*A1; methionine, *meth*A17; pyridoxine, *pyro*A4. Conidia color: white, *wA2*; yellow, *yA2. AcrA1*, resistance to acriflavine

*FGSC, Fungal Genetic Stock Center, University of Kansas Medical Center, Kansas, USA

Homozygotization assay

Colonies of UT448//A757 diploid strain of *A. nidulans* were grown onto Petri plates containing MM (negative control, named D1), MM + benznidazole (100 μmol/L, positive control, named D2) and MM + citalopram (50, 75 and 100 μmol/L, treatment). Plates were incubated for six days at 37°C and then visually inspected for diploid sectors arising on the diploid strains' colonies. The first treatment (MM + 100 μmol/L citalopram) produced three morphologically identifiable diploid sectors, named D3–D5. The second treatment (MM + 75 μmol/L citalopram) produced three diploid sectors that were sequentially numbered D6–D8. Finally, the third treatment (MM + 50 μmol/L citalopram) produced three diploid segregants, named D9–D11. Diploids (D1–D11) were homozygous (+/+) or heterozygous (+/− or −/+) for nutritional markers but never recessive homozygotes (−/−) since the latter cannot grow on MM (Table 2). Untreated (D1) and treated (D2–D11) diploid strains were purified on MM, individually transferred to CM plates and processed by spontaneous haploidization. Haploidization, the loss of one member of each chromosome pair through successive mitotic divisions, results in the haploid condition of the nuclei. After haploidization, each diploid sector (D1–D11) produced haploid mitotic segregants (Figure 1C–F). They were purified in CM and their mitotic stability was evaluated in CM + benomyl (0.2 μg/mL). Only mitotically stable haploid segregants at the final stage were selected for the recombinogenesis test. In the case of phenotypic analyses, the haploid segregants were individually transferred to different supplemented media: MM supplemented with all nutritional requirements of the master strains except one, in each medium type. Mitotic crossing-over causes homozygotization of heterozygous-conditioned genes. If the antidepressant induces mitotic crossing-over in the original diploid strain, only heterozygote (+/− or −/+) or homozygote (+/+) diploids will develop in MM and the nutritional markers will segregate among the haploids in the proportion of 4+ to 2−. However, if the drug fails to induce crossing-over, the proportion will be 4+ to 4−. This is due to the fact that the initial selection process limits the growth of −/− diploids (Table 2). The ratio of prototrophic to auxotrophic segregants is described by the homozygosity index (HI), or rather, an HI equal to or higher than 2.0 indicates recombinogenic effects of the antidepressant drug. The recombinogenic potential of citalopram was assessed by

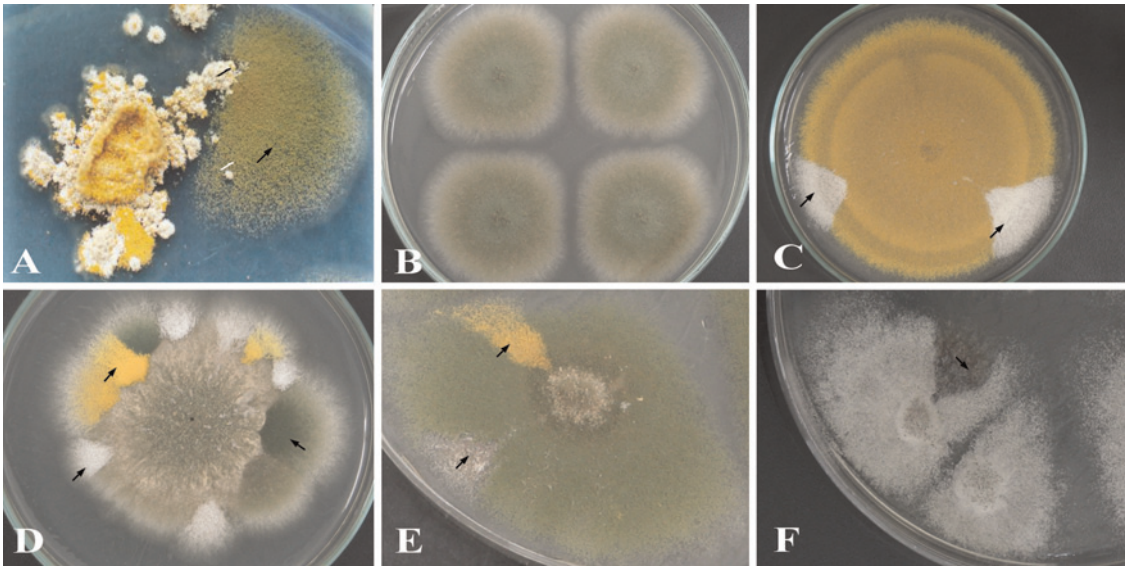


Figure 1 (A) Origin of the UT448//A757 diploid strain (arrow) from the heterokaryon formed between the haploid UT448 (with white conidia) and A757 (with yellow conidia) strains. (B) Growth of UT448//A757 diploid strain in the absence of citalopram. Diploid D9, yellow conidia (C); Diploid D6, green conidia (D, E); Diploid D3, white conidia (F), respectively, obtained from 50, 75 and 100 μmol/L of citalopram. Arrows indicate the origin of mitotic yellow, green or white segregants (sectors) on treated diploid strains. (A color version of this figure is available in the online journal)

comparing the HIs of the nutritional markers with an Yates corrected χ^2 test, $P < 0.05$.

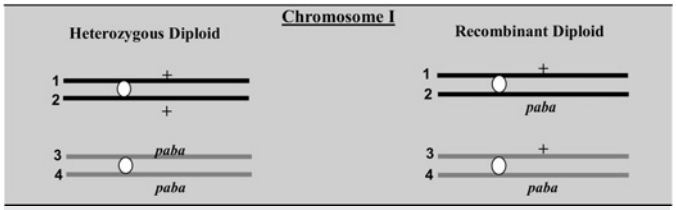
Results

The recombinogenic potential of citalopram towards UT448//A757 diploid strain was evaluated by using the HI of various nutritional markers from chromosomes I, II and IV. HIs for the *ribo*, *paba*, *bi* and *pyro* genes were significantly higher in diploid strains treated with citalopram (strains D3, D4, D7 and D9) when compared with untreated control strains (D1) (Table 3). Although the citalopram (50, 75 and 100 μmol/L) treatment of UT448//A757 diploid strains in MM impaired the growth of auxotrophic diploids ($-/-$), most of the recovered prototrophic diploids were homozygous for the recessive conidia color markers (*w* or *y* genes). Treatment produced nine diploid segregants: three (D6, D10 and D11) with green conidia ($y+/y$), two (D3 and D7) with white conidia (w/w) and four (D4, D5, D8 and D9) with yellow conidia (y/y) (Figure 1C–F). Diploids D3 and D7 obtained, respectively, with 100 and 75 μmol/L citalopram showed HI values higher than 2.0 and significantly different from the negative control (D1) for the *ribo*, *bi*, *paba* and *pyro* genes. In addition, diploids D3 and D7, homozygous for the *w* gene, are recombinant for the *w*-centromere interval from chromosome II (Tables 1 and 3). The diploid D7, although heterozygous for the *ribo* and *bi* genes, is homozygous for two other genes, *paba* and *pyro* genes, since auxotrophic *paba* or *pyro* segregants were not recovered among their haploid-derived mitotic segregants. Results indicated that D7 is also recombinant for the centromere-*paba* and centromere-*pyro* intervals from chromosomes I and IV, respectively (Table 3).

The recombinant diploid strains with yellow conidia, D4 and D9, showed HI values higher than 2.0 and significantly different from the negative control (D1) for the *bi* marker from chromosome I. The diploid D8, recombinant for the *paba-y* interval, as well as other yellow diploid strains (D4 and D9) also showed HI values higher than 2.0 for the *bi* marker, but the HI value of diploid D8 was not statistically significant.

HI values for *paba* and *bi* genes could not be determined in the analysis of the recombinant diploid D5 because it is homozygous for the *paba+* (similar to diploids D7 and D9) and *bi+* alleles (Table 3).

Table 2 Mitotic segregation of *paba* gene among haploid segregants derived from heterozygous *paba+/paba* diploid strains in the presence and in the absence of crossing-over



No crossing-over		Crossing-over in the centromere-*paba* interval	
Chromatid segregation	**Diploid genotypes**	**Chromatid segregation**	**Diploid genotypes**
1+3	*paba+ / paba*	1+3	*paba+ / paba+*
1+4	*paba+ / paba*	1+4	*paba+ / paba*
2+3	*paba+ / paba*	2+3	*paba / paba+*
2+4	*paba+ / paba*	2+4	*paba / paba**
Segregation after haploidization	4 *paba+*: 4 *paba*		4 *paba+*: 2 *paba*

*The homozygous diploid *(paba / paba)* will not grow in MM plates

Table 3 Homozygotization index (HI) values for markers from UT448//A757 diploid strains after treatment with 100 μmol/L (D3–D5), 75 μmol/L (D6–D8) and 50 μmol/L (D9–D11) of citalopram

	D1‡		D2¶		D3		D4		D5		D6		D7		D8		D9		D10		D11	
Markers†	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI	NS#	HI
ribo+	42	1.3	69	**3.0***	21	**7.0***	53	1.1	61	1.0	36	1.2	36	**9.0***	29	1.1	71	1.0	46	1.8	47	1.1
ribo	32		23		03		47		60		29		04		26		70		26		44	
paba+	40	1.2	69	**3.0***	21	**7.0***	49	1.0	121	–	35	1.2	40	–	26	0.9	141	–	46	1.8	43	0.9
paba	34		23		03		51		0		30		0		29		0		26		48	
bi+	45	1.6	72	**3.6***	13	1.2	83	**4.9***	121	–	32	1.0	35	**7.0***	40	2.5	113	**4.0***	45	1.7	44	0.9
bi	29		20		11		17		0		33		05		14		28		27		47	
pyro+	32	0.8	68	**2.8***	21	**7.0***	47	0.9	60	1.0	32	1.0	40	–	30	1.2	70	1.0	34	0.9	54	1.5
pyro	42		24		03		53		61		33		0		25		71		38		37	

†*ribo*, riboflavin; *paba*, *p*-aminobenzoic acid; *bi*, biotin; *meth*, methionine; *pyro*, pyridoxine
‡Negative control, not treated with citalopram or benznidazole
¶Positive control, treated with benznidazole at 100 μmol/L
#Number of mitotic segregants
*Significantly different from negative control‡ ($P < 0.05$)

Discussion

An *in vivo* assay concerning the ability of the citalopram to induce homozygosis of genetic markers in diploid cells of *A. nidulans* was applied in this study. Citalopram in non-toxic concentrations (50, 75 and 100 μmol/L) was found to be an effective inducer of mitotic recombination, promoting homozygosis of both nutritional markers and conidia color markers. The recombinogenic test identified HI rates higher than 2.0 and significantly different from the HI control ones for the genetic markers of the UT448//A757 diploid strain. Likewise, phenotypic analysis of mitotic segregants derived from the citalopram-treated diploids D5, D7 and D9 revealed that they were homozygous for the auxotrophic markers of chromosomes I (*paba* and *bi*) and IV (*pyro*).

Recombination between homologous chromosomes during mitosis will result in LOH at loci located in distal positions to the recombination break point. Therefore, LOH is a mechanism by which mutations, germline or sporadic, in tumor suppressor genes may become homozygous and lead towards a tumor predisposition in the affected cells. The TP53 tumor suppressor gene encodes a nuclear phosphoprotein (p53), which controls the decision of the cell to replicate at the G1/S checkpoint of the cell cycle. Mutations in p53, observed frequently in human cancers, lead to genomic instability including increased spontaneous homologous recombination.[33] Various genetic mechanisms of TP53 inactivation have been described, such as point mutations, homozygous deletions and LOH. LOH of the TP53 locus is implicated in the initiation and progression of various human malignancies and has been observed in both early or in advanced-stage neoplasms.[34–37]

Emerging evidences suggest that homologous recombination is critical in the repair of damages encountered during normal DNA replication. Defects in this recombinational repair have long been known to contribute to the genomic instability often found in cancers. Individuals with defects in either recombination mediators BRCA1 and BRCA2 are in fact predisposed to breast, ovarian and other types of cancer.[38–40]

In rhabdoid tumors, the highly malignant pediatric cancers, mitotic recombination has been described as one of the chromosomal mechanisms involved in the homozygotization of the *hSNF5/INI1* tumor suppressor gene, occurring in close to one-half of the cases.[41] In addition, non-disjunction and recombination at the G2 phase of the cell cycle have been described as chromosomal mechanisms leading to retinoblastoma and Wilms' tumors.[42,43] Since inhibitors of DNA synthesis, such as cytosine arabinoside and hydroxyurea, have been described as the most potent inducers of homologous recombination in mammalian cells,[25] and taking into account that citalopram has been found to promote growth cessation and DNA synthesis inhibition in Burkitt lymphoma cells in a concentration-dependent manner,[30] the recombinogenic effect of this antidepressant in *A. nidulans* diploid cells may be associated with the recombinational repair of citalopram-induced DNA strand breaks.

Depressive disorders in adults occur in about 15% of the general population and are at least two or three times more

common in cancer patients. Both TCA and SSRI antidepressants, including citalopram, are effective in reducing cases of depression and in improving depressive symptoms in cancer patients receiving chemotherapy at any stage of the illness trajectory.[44–46] Furthermore, SSRI medications, including citalopram, have been shown to be preferentially selected drugs in the treatment of depression in pediatric oncology patients.[1,47] Although previous studies, based on citalopram's ability to induce apoptosis in a human leukemic cell line,[48] have demonstrated its antineoplastic effect, the recombinogenic potential of citalopram described in the current study points to possible risks of this antidepressant to act as a secondary malignance promoter in cancer patients or as a tumor-promoter agent (the second-hit) in the two-step model for carcinogenesis.

Whereas recent epidemiologic evidences suggest no association between long-term use of SSRI antidepressants and cancer risk,[49] further studies are needed to investigate the genotoxic potential of citalopram in mammalian cells, including rodents, at clinically relevant doses, owing to the high prevalence of use of this drug to treat depressive disorders.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; CCSF, JC-P, LJR, JRS and MAAC-P conducted the experiments; and MAAC-P wrote the manuscript.

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