Cytotoxic effects of aripiprazole on MKN45 and NIH3T3 cell lines and genotoxic effects on human peripheral blood lymphocytes

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ABSTRACT – **Background** – Gastric cancer is known as the fourth most common cancer. Current treatments for cancer have damaged the sensitive tissues of the healthy body, and in many cases, cancer will be recurrent. Therefore, need for treatments that are more effective is well felt. Researchers have recently shifted their attention towards antipsychotic dopamine antagonists to treat cancer. The anticancer activities of aripiprazole remain unknown. **Objective** – This study aimed to evaluate the efficacy and safety of aripiprazole on gastric cancer and normal cell lines. **Methods** – In this regard, the cytotoxicity and genotoxicity of aripiprazole were investigated in MKN45 and NIH3T3 cell lines by methyl tetrazolium assay and on peripheral blood lymphocytes by micronucleus assay. For this purpose, cells were cultured in 96 wells plate. Stock solutions of aripiprazole and cisplatin were prepared. After cell incubation with different concentrations of aripiprazole (1, 10, 25, 50, 100 and 200 µL), methyl tetrazolium solution was added. For micronucleus assay fresh blood was added to RPMI culture medium 1640 supplemented, and different concentrations of aripiprazole (50, 100 and 200 µL) were added. **Results** – The finding of present study showed that the IC_{50} of aripiprazole in the cancer cell line (21.36 µg/mL) was lower than that in the normal cell line (54.17 µg/mL). Moreover, the micronucleus assay showed that the frequency of micronuclei of aripiprazole at concentrations below 200 µM was much less than cisplatin. **Conclusion** – Aripiprazole can be a good cytotoxic compound and good candidate for further studies of cancer therapy.

HEADINGS – Stomach neoplasms. Dopamine antagonists. Aripiprazole, therapeutic use. Tetrazolium salts. Micronucleus tests. Mutagenicity tests.

INTRODUCTION

Cancer is now believed to rank among the three major causes of death and among them gastric cancer is known as the third leading cause of cancer-related death worldwide; however, the incidence of gastric cancer varies greatly and this number can change across populations[1,2]. Although there are various treatment options to treat localized gastric cancer, ranging from minimally invasive EMR to aggressive extended lymphadenectomy and perioperative empirical adjuvant chemoradiation, gastric cancer still poses a major clinical challenge. Because most disease is detected at a late stage, where there are limited treatment options for that and patients have a median survival of only 6~9 months[3]. Recently, important data was published on the potential genotoxic or carcinogenic effects of antipsychotics, as well as their cytotoxic properties on cancer cells. Thus, researchers have shifted their attention towards antipsychotic dopamine antagonists to treat cancers. Aripiprazole, an atypical antipsychotic drug with mixed antagonism and agonism on dopamine D_2 and serotonin receptors, is usually prescribed to cure psychosis such as schizophrenia, episodes associated with bipolar disorder, delayed sleep phase syndrome, and irritability in children with autism[4]. Aripiprazole has shown growth inhibitions against some cancer cell lines such as the glioma cell lines U251 and LN428, the gastric cancer cell line MKN-1, the breast cancer cell line MDA-MB-231, the colon carcinoma cell line CT26, the human embryonic kidney cell line HEK293, serum-cultured cancer cells and cancer stem cells[5,6]. In this study, anti-cancer effects of aripiprazole on MKN45 cancer cell line and NIH3T3 cell line were carefully examined by using methyl tetrazolium (MTT) assay. This assay is one of the most common employed for the detection of cytotoxicity or cell viability following exposure to toxic substances and is one of the most sensitive cytotoxicity assay. This assay was shown to have a very wide applicability for measuring survival and/or proliferation of various cells and can potentially be applied to any assay in which living cells must be distinguished from dead cells or a lack of cells. Besides, it showed the potential value for quantitative and rapid measurement of cell death[7-9]. Moreover, we used micronucleus assay to assess genetic damage of aripiprazole in human peripheral blood lymphocytes. The development of the cytokinesis-block (CB) technique has transformed the human-lymphocyte micronucleus (MN) assay into a reliable and precise method for assessing chromosome damage[10,11]. On the other hand, cisplatin based combination chemotherapies have been used since the 1980's. However, the results of cisplatin-based regimens have not been satisfactory, and they have shown a response rate of less than 50%[12]. Such problems along with many side effects still remain for anti-cancer drugs such as Docetaxel, Paclitaxel, Doxorubicin, and etc. as well[13-17]. Therefore, the need for new agents remains

essential. Actually, a favorable profile of safety and tolerability and good clinical effectiveness are among the advantages of aripiprazole[18,19]. However, the anticancer effects of aripiprazole on gastric cancer MKN45 cell line, and the possible genotoxicity of it in human cells has not been confirmed yet. This study aimed to evaluate the efficacy and safety of aripiprazole on gastric cancer and normal cell lines. In this regard, the cytotoxicity and genotoxicity of aripiprazole were investigated in MKN45 and NIH3T3 cell lines by MTT assay and on peripheral blood lymphocytes by MN assay.

METHODS

Cell culture

NIH3T3 and MKN45 Cell lines (Pasteur Institute, Tehran, Iran) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO, Berlin, Germany) with 10% fetal bovine serum (Gibco-BRL, Germany) and 100 µg/mL streptomycin (Gibco-BRL, Germany) and 100 IU/mL penicillin (Gibco-BRL, Germany). Cell cultures were adjusted to allow for exponential growth.

MTT assay

NIH3T3 and MKN45 Cell lines (10^4 cells) were cultured in 200 µL DMEM/F12 medium containing 10% bovine serum in 96 wells plate and incubated at 37°C for 24h. Stock solutions of aripiprazole and cisplatin were prepared in 1% DMSO and phosphate buffered saline (PBS), respectively. Twenty-µL of MTT solution (5 mg/mL) was added to each well following 48h incubation with different concentrations of aripiprazole (1, 10, 25, 50, 100 and 200 µL). The optical density (OD) of the MTT reaction was measured on a microplate ELISA reader at 570 nm. All experiments were repeated two times and each treatment was run in triplicate. The percentage of cell viability was calculated using the equation: [mean (OD) of treated cells/mean OD of control cells (1%DMSO)] ×100.

Micronucleus assay (CBMN assay)

Fresh blood was collected from 10 healthy, no smoking, no alcoholic, male donors aged between 25-35 years by venepuncture in heparinized falcons. 0.5 mL of whole blood was added to 4.5 mL of Roswell Park Memorial Institute (RPMI) culture medium 1640 supplemented with 10% fetal bovine serum containing L-glutamine, antibiotics, and phytohemagglutinin (PHA), and different doses of duloxetine (1, 10, 25, 50, 100 and 200 µL). The binucleated lymphocytes were harvested 28h after adding Cyt-B (Sigma, Missouri, USA); they were treated by hypotonic KCl (0.075M) to red blood cell (RBC) lysis. Then fixative solution (methanol: acetic acid =6:1) was added to the cells prior to slide preparation and staining. For slide preparation, 2-3 drops of cell suspension were thrown on a clean slide. The slides were stained with Giemsa solution (4%) for 7-10 mins. They were observed at 40× and 100× magnifications using a light microscope to estimate mitotic index (the cells with two or more nuclei per 1000 observed cells) and micronuclei frequency (the number of micronuclei in 1000 binucleated cells) are lymphocytes that were once divided by mitosis. The experiment was performed two times. Mitotic Index has a direct relation with cells' proliferative activity.

Statistical analysis

One way analysis of variance and Tukey's honestly significant differences (HSD) test were used for multiple comparisons of data. A P value less than 0.05 was considered as significant. The IC50 (half maximal inhibitory concentration) values were calculated by PRISM software using nonlinear regression. Standard deviations represent average results of double experiments. The IC_{50} values were compared using the Student's t-test measuring the effectiveness of a substance to cause cell death or inhibit cell growth. Therefore, the lower amount of IC_{50} represents a higher toxicity of a compound, which leads to death or inhibition of cell growth.

RESULTS

MTT assay

The IC_{50} of aripiprazole on MKN45 and NIH3T3 cell lines were examined using MTT assay. The IC_{50} of aripiprazole on MKN45 cell line was 21.36 µg/mL and on NIH3T3 cell line was 54.17 µg/mL. The lower IC_{50} value is representative of the higher ability of a cytotoxic compound to cause cell death or inhibit cell growth[20]. The results of the MKN45 cancer cell line (TABLE 1 and FIGURE 1) showed that aripiprazole compared to the negative control group had more cytotoxic effects in every concentration. Compared to the positive control group; cisplatin (the common anticancer drug) it had no significant difference at 100 and 200 µM, but at lower concentrations: 50, 25, 10 and 1 µM, it showed less cytotoxic effects ($P<0.05$). Furthermore, the results of the NIH3T3 cell line (TABLE 1 and FIGURE 2) showed that aripiprazole in comparison with the negative control group had more cytotoxic effects at 10, 25, 50, 100, 200 µM and no significant difference at 1 µM. Moreover, in comparison with the positive control group (cisplatin), it showed less cytotoxic effects in every concentration but 100 µM ($P<0.05$).

TABLE 1. Cell viability assay.

|	Control	1	10	25	50	100	200	Cisplatin
Mean ±Std. Deviation	100.0±2.371	89.31±1.929	73.64±1.119	62.71±3.797	45.91±6.197	24.33±3.609	21.18±0.08532	26.80±2.357
Effect of aripiprazole on NIH3T3cell viability	100.0±3.886	89.20±2.932	85.11±7.263	76.06±6.670	64.72±2.426	32.33±5.891	7.042±0.9220	29.24±1.870
Micronuclei frequency in different concentrations of aripiprazole	0.6667±0.5774	–	–	–	4.000±1.732	13.67±1.528	19.00±2.000	21.67±2.517
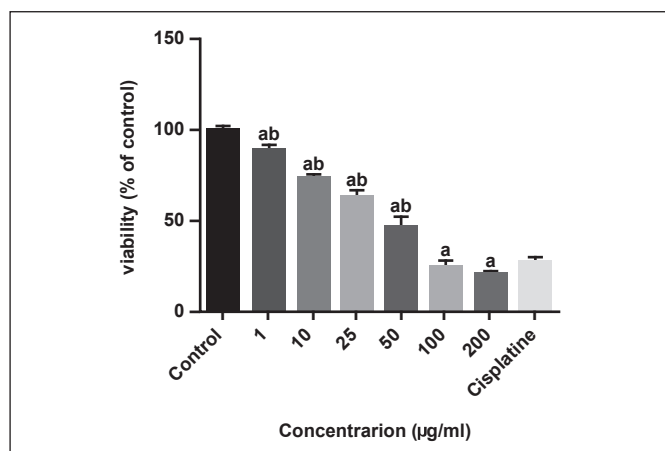
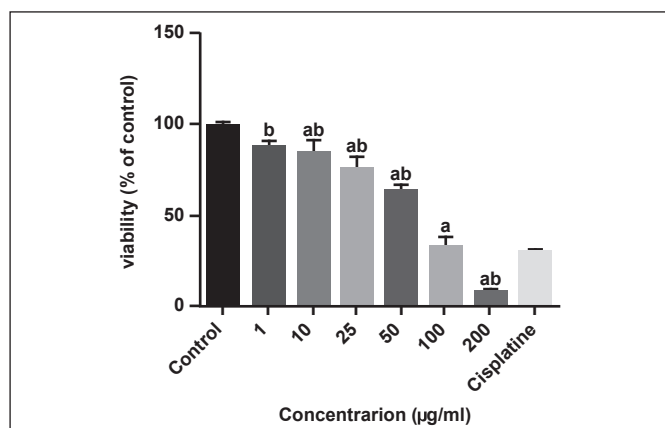**FIGURE 1.** Effect of aripiprazole on MKN45 cell viability.

FIGURE 2. Effect of aripiprazole on NIH3T3 cell viability.

Micronucleus assay

The genotoxic effects of aripiprazole were studied based on the number of MN produced in peripheral blood lymphocytes following treatment with different concentrations of aripiprazole (TABLE 1 and FIGURE 3). Results showed that the MN number was relatively increased based on the increase in the aripiprazole concentration. Fifty-µM concentration of aripiprazole did not produce any significant difference in MN number relative to the control group. While, 100 and 200 µM concentrations of aripiprazole significantly increased the number of MN. Comparison of different concentrations of aripiprazole and cisplatin showed that treatment of lymphocytes with 50 and 100 µM of aripiprazole significantly increased the number of MN. Two hundred-µM concentration of aripiprazole did not produce any significant difference in MN number relative to the cisplatin ($P<0.05$).

DISCUSSION

In this study, the cytotoxic effect of Duloxetine on MKN45 gastric cancer cell line was investigated and compared to NIH3T3 normal cell line by MTT assay. Moreover, the genetic damage caused by this drug was also evaluated using MN assay. The IC_{50} of aripiprazole on MKN45 cell line and NIH3T3 cell line was calculated 21.36 µg/mL and 54.17 µg/mL respectively. However, the IC_{50} of cisplatin on MKN45 was 12.49 µg/mL and on NIH3T3
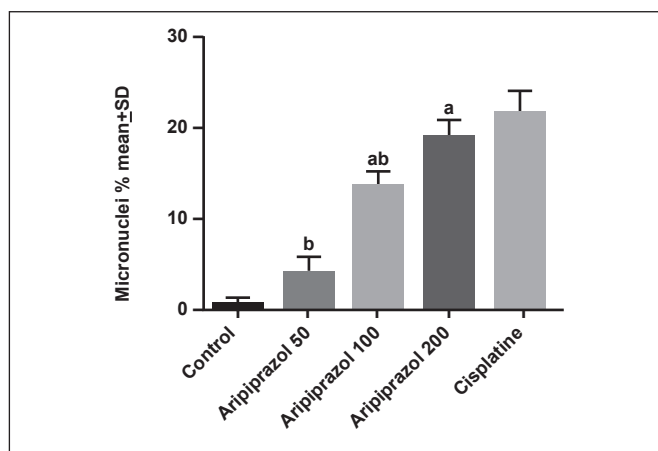

FIGURE 3. Micronuclei frequency in different concentrations of aripiprazole {a: significant difference compared to the negative control group b: significant difference compared to the positive control group ($P<0.05$)}.

cell line was 24.9 µg/mL. The lower IC_{50} value is representative of the higher ability of a cytotoxic compound to cause cell death or inhibit cell growth. Taken together, it seems that this drug can be mentioned as a good cytotoxic compound. As reported here, the cytotoxicity of aripiprazole on MKN45 cancer cell line is consistent with some other researches[5,21]. Anti-cancer effects of aripiprazole on various malignant tumor cells and its molecular mechanism were examined by using cell proliferation assay, xenograft mouse model, immunoblotting analysis, migration assay, luciferase reporter gene assay, kinase assay, and overexpression strategy. Treatment with aripiprazole induced cytotoxicity in U251 glioma cells, MKN-1 gastric adenosquamous carcinoma cells, and CT26 colon carcinoma cells[5]. Moreover, the effects of aripiprazole alone and in combination with chemotherapeutic agents in order to test growth ability, the ability to form stem cells/differentiation/chemical resistance, the markers of cancer stem cells, cancer cells and normalized cells was investigated. According to their study, the growth of cancer cells and cancer stem cells was inhibited by aripiprazole in non-toxic concentrations of normal cells. Generally, it could be concluded that aripiprazole could be a good candidate as an anticancer stem cell[21].

Additionally, we used lymphocytes in our in vitro studies to assess the potential genotoxic effect of aripiprazole. Results from the micronucleus assay confirmed the ability of aripiprazole to induce the formation of micronuclei. The induction of micronuclei is commonly used to evaluate the chromosomal damage. The cellular and tissue toxicity was observed in the increased therapeutic concentrations of aripiprazole. Aripiprazole and its metabolites can bind DNA, causing damage that can result in chromosome breaks, micronucleus formation, and cell death. As evident, the frequency of micronuclei was increased with aripiprazole in comparison with the negative control group but in comparison with the positive control group, it caused less DNA damage at 100 and 50 µM concentrations. The genetic damage caused by aripiprazole at concentrations below 200 µM was much less than cisplatin, indicating that it could be studied more widely due to its low genotoxicity. The results of our findings in this regard coincided with the work of other researchers[22,23]. The mutagenic and antimutagenic effects of some quinoline- and isoquinoline-sulfonamide analogs of aripiprazole were evaluated and their

results showed that newly synthesized azinesulfonamide analogs of aripiprazole might be considered as genotoxically safe as they do not display mutagenic activity on the tester strains[22]. In addition, genetic toxicity of 545 medications including aripiprazole were studied by using information from 1999 to 2008. The data also contained anti-cancer and antiviral drugs, nucleosides (all with known mechanistic genotoxicity), steroids with class-specific genotoxicity and biologicals or peptide-based drugs. Their results discussed the link between positive genetic toxicity findings, rodent carcinogenesis, and silica predictions and found that there was supporting evidence for the idea that just the presence of an N-dialkyl group or piperidine aryl ketone may somehow be associated with genotoxicity[23].

CONCLUSION

Aripiprazole can be a great cytotoxic compound and beneficial candidate for further studies of cancer therapy. This study in comparison to other studies showed that aripiprazole can be considered as one of the candidates for the synthesis of a safe anticancer drug. Further studies are needed.

Authors' contribution
Shokrzadeh M: study design and management. Mohammadpour A: micronucleus assay. Modanloo M: methyl tetrazolium assay. Hassani M: micronucleus assay. Barghi NG: writing article. Niroomand P: statistical analyses. All authors read and approve final manuscript.

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Shokrzadeh M, Mohammadpour A, Modanloo M, Hassani M, Barghi NG, Niroomand P. Efeitos citotóxicos do aripiprazol nas linhagens celulares MKN45 e NIH3T3 e efeitos genotóxicos nos linfócitos sanguíneos periféricos humanos. Arq Gastroenterol. 2019;56(2):155-9.

RESUMO – **Contexto** – O câncer gástrico é conhecido como o quarto câncer mais comum. Os tratamentos atuais para o câncer danificaram os tecidos sensíveis do corpo saudável e, em muitos casos, o cancro será recorrente. Portanto, a necessidade de tratamentos que são mais eficazes é desejada. Recentemente, os pesquisadores mudaram sua atenção para os antagonistas antipsicóticos da dopamina para tratar o câncer. As atividades anticâncer de aripiprazol permanecem desconhecidas. **Objetivo** – Este estudo objetivou avaliar a eficácia e a segurança do aripiprazol no câncer gástrico e nas linhagens celulares normais. **Métodos** – A este respeito, a citotoxicidade e a genotoxicidade do aripiprazol foram investigadas em linhas celulares MKN45 e NIH3T3 por ensaio de metil tetrazólio e em linfócitos periféricos de sangue por ensaio de micronúcleos. Para este efeito, as células foram cultivadas em 96 placas. As soluções de estoque de aripiprazol e cisplatina foram preparadas. Após incubação celular com diferentes concentrações de aripiprazol (1, 10, 25, 50, 100 e 200 μL), a solução de metil tetrazólio foi adicionada. Para o ensaio do micronúcleo o sangue fresco foi adicionado ao meio de cultura RPMI 1640 suplementado, e as concentrações diferentes de aripiprazole (50, 100 e 200 μL) foram adicionadas. **Resultados** – O presente estudo mostrou que o IC50 de aripiprazol na linhagem celular cancerosa (21,36 μg/mL) foi menor do que na linha celular normal (54,17 μg/mL). Além disso, o ensaio de micronúcleos demonstrou que a frequência de micronúcleos de aripiprazol em concentrações inferiores a 200 μM foi muito inferior à cisplatina. **Conclusão** – O aripiprazol pode ser um bom composto citotóxico e bom candidato para estudos adicionais da terapia do câncer.

DESCRITORES – Neoplasias gástricas. Antagonistas de dopamina. Aripiprazol, uso terapêutico. Sais de tetrazólio. Testes para micronúcleos. Testes de mutagenicidade.

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