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Alteration of Alzheimer Amyloid Precursor Protein after Treatment with Carbamazepine in Colon Cancer

Ladan Akbarzadeh,[1,*] Taraneh Moini Zanjani,[1] Masoumeh Sabetkasaei,[1] and Yalda Khazaei[1]

[1]Department of Pharmacology, Shahid Baheshti University of Medical Sciences, Tehran, IR Iran

[*]*Corresponding author*: Ladan Akbarzadeh, Department of Pharmacology, Shahid Baheshti University of Medical Sciences, Tehran, IR Iran. Fax: +98-22439969, E-mail: akbarzadehladanlada@yahoo.com

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Abstract

Background: Amyloid precursor protein (APP), which is involved in cell proliferation, is observed to be over expressed in human cancer. Thus, it is crucial to investigate its role in cancer and suggest treatment strategies to alter its effects on cell growth.
Objectives: The main purpose of this study was to determine APP concentrations in colon cancer SW480 cell line after treatment with histone deacetylase inhibitors (HDACIs), namely valproic acid (VPA) and carbamazepine (CBZ).
Methods: In the present experimental study, carried out during the period 2014 - 2016 in Iran, 1000000 cells were seeded and incubated in a six-well plate for each sample preparation. The cells were then treated with drugs and APP was evaluated. The concentration of this protein was detected using the enzyme-linked immunosorbent assay (ELISA) method.
Results: We found that treatment with CBZ ($P < 0.001$) and VPA ($P < 0.001$) reduced the APP levels significantly compared to controls. The APP level reduction by CBZ was 41.6% more than that by VPA.
Conclusions: The results of this study suggest that APP reduction by HDACIs can apparently play an important role in the treatment of colon cancer.

Keywords: Histone Deacetylase Inhibitor, Colon Cancer, Amyloid Precursor Protein

1. Background

Colon cancer is the third most common male and the second most common female cancer in the world. This disease is the second cause of cancer mortality and more than half of the patients with this illness do not survive (1). Although there are many treatment protocols at the present time, most of patients die from metastasis due to the disease progression. A large number of studies are carried out to understand the mechanisms underlying this disease; however, the exact mechanism of action remains unknown. Reported studies indicate amyloid precursor protein (APP) over expression in cancer, yet its levels are not measured prior to and after drug treatment (2, 3). Here for the first-time, the levels of APP were measured in human colon cancer cells after treatment with carbamazepine (CBZ), one of the least toxic histone deacetylase inhibitors (HDACIs). To date, no study has been conducted on APP levels in colon cancer using this drug, which has far less side effects than the presently used chemotherapy drugs. Hence, the results presented here may be very valuable.

2. Objectives

The main purpose of this study was to determine the APP levels in colon cancer cells after treatment with HDACIs, namely valproic acid (VPA) and CBZ that have fewer side effects than chemotherapeutic agents. Thus, it can be considered as a new treatment protocol in colon cancer.

3. Methods

3.1. Materials

APP enzyme-linked immunosorbent assay (ELISA) was purchased from R&D Systems (USA). All other chemicals used in this study were obtained from Sigma (USA). The human colon adenocarcinoma cell lines SW480 were obtained from Avicenna Institute (Iran).

3.2. General Information

The present experimental study was performed during the period 2014 - 2016 in Tehran, Iran. The study was approved by the ethics committee of Shahid Baheshti University of Medical Sciences in Tehran, Iran (code: IR.SBMU.SM.REC.1394.4). Date of approval is 2015/8/18.

3.3. Cell Culture

The human colon cancer SW480 cell line was maintained in culture medium containing RPMI 1640. The medium was supplemented with 10% fetal calf serum and incubated at 37°C in an atmosphere of 95% air and 5% CO_2.

3.4. Sampling

In this study, SW480 colon cancer cells were used to evaluate the APP concentrations. The number of cells used was according to instructions recommended by ELISA kit producer. The colon cancer cells were seeded into a six-well plate at a density of 1000000 cells well for each sample preparation and incubated overnight.

3.5. Treatment Protocols

The treatment was performed through determining the inhibitory concentration of 50% (IC_{50}) levels which were obtained from our previous study. IC_{50} represents the drug concentration required for 50% cell growth inhibition (4). Here, IC_{50} was defined as a drug concentration which is required to kill 50% of cancer cells. The IC_{50} values considered here for VPA and CBZ were 2.5 mM and 5 μM, respectively. The optical density (OD) values were obtained by using ELISA reader. The MTT assay was used to measure cell viability defined as: (OD treated cells / OD untreated control cells) *100. The colon cancer cells were treated with different concentrations of VPA, 2.5 and 5 mM, for 72 hours and CBZ, 5 and 10 μM, for 48 hours. Cells in the control group were treated with vehicle. We removed particulates by centrifugation. The cell culture supernatants were used to determine APP levels. In this experiment, mean level of each drug concentration was the average of 6 samples.

3.6. Sample Collection

In this study, the cancer cells were divided into four different groups: 1-CBZ treated, 2-VPA treated, 3- control vehicle treated (DMSO) for CBZ and 4- control vehicle treated (culture media) for VPA. A total number of 24 and 12 samples were used for the two drugs treated and vehicle control treated groups, respectively.

3.7. Measurements

Cells were harvested after overnight incubation for protein analysis. The total protein concentrations were determined using BCA protein tests. The amount of APP concentrations in the supernatant of cell cultures was assessed using ELISA kit as described by the manufacturer (R&D systems, USA). The samples were added in duplicates to appropriate pre-coated plates. After the plates were washed, horseradish peroxidase-conjugated detection antibody was added. The substrate used for color development was tetramethylbenzidine. The optical density was measured at 450 nm with an ELISA reader (Bio-Tek E1800, USA). All tests were repeated three times and the samples for protein analysis were measured in duplicates (kappa coefficient > 96%).

3.8. Statistical Analysis

Data were analyzed using SPSS version 16 (Chicago, USA) and graph pad prism version 5(USA). One-way ANOVA was used to compare differences between the groups. P-values below 0.05 were considered statistically significant.

4. Results

The APP levels in SW480 cells treated for 72 hours with 2.5 and 5 mM VPA decreased by 37.07% and 75.13%, respectively, (Figure 1A) and those treated for 48h with 5 and 10 μM CBZ decreased by 78.68% and 92.46 %, respectively, compared to the levels in the control group (Figure 1B). Based on one way ANOVA, the reductions were significant ($P < 0.001$) (Table 1). The APP level reductions with CBZ compared to VPA were 41.6% and 17.3% higher in IC_{50} and $2IC_{50}$ concentrations, respectively.

5. Discussion

Evidence indicates an important role for APP in cell-growth and proliferation. APP is a transmembrane protein expressed in different tissues that contains a large N-terminal and a short C-terminal domain (3). Three secretases α, β, and γ are involved in APP breakdown through two main pathways: the amyloidogenic and the nonamyloidogenic. In the amyloidogenic pathway, APP is cleaved by β and γ secretases resulting in the creation of amyloid β (Aβ) protein. The Aβ protein has been found pathophysiologically to be an important factor in progressing Alzheimer disease (AD). In the nonamyloidogenic pathway, APP is cleaved by α and γ secretases resulting in the creation of P3 protein (5). Increased risk of cancer seems to be associated with decreased chance of AD and there is a complex mechanism relating to these two diseases. These findings suggest the possibility that cancer can be linked to some neurodegenerative diseases (6). At the present time, the biological action of APP is not well explained in cancer cells. Several studies indicate that APP supports adhesion, neurite outgrowth, protein-protein interaction, cell migration, synapse remodeling, and cell signaling activities (7). Takayama et al. found that APP is a principal androgen target gene that raises prostate cancer growth (2).

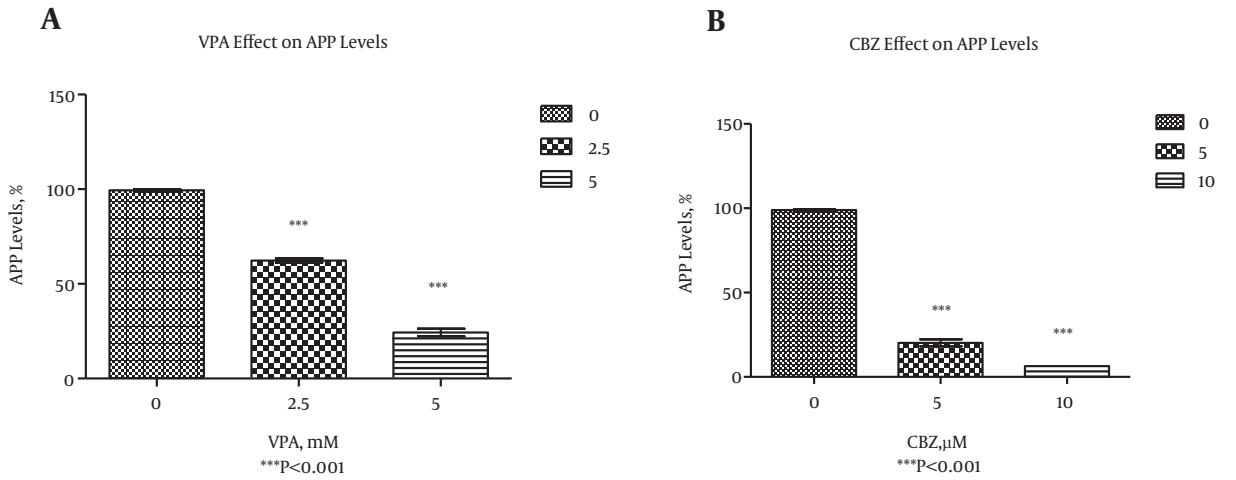

Figure 1. Amyloid Precursor Protein (APP) Levels in SW480 Cell Line (A) after Treatment with Valproic Acid (VPA) and (B) after Treatment with Carbamazepine (CBZ) which Significantly Differ from Control Levels ($P < 0.001$).

Table 1. Comparing Experimental Results of Amyloid Precursor Protein (APP) Levels after Treatment with Valproic Acid (VPA) and Carbamazepine (CBZ)[a]

	Drug			
Variable	**VPA**	**P Value**	**CBZ**	**P Value**
APP				
Control	99.43	< 0.001	98.92	< 0.001
IC_{50}	62.36	< 0.001	20.24	< 0.001
$2IC_{50}$	24.3	< 0.001	6.46	< 0.001

[a]Values are expressed as %.

Hansel et al. suggested that APP is promoted in pancreatic tumors playing an important role in carcinogenesis (3). Krause et al. found higher expression of APP in cold thyroid nodules compared to normal thyroid tissues (8). This protein seems to have a critical role as a growth factor, which is explained by considering the structure of APP large N-terminal domain with cysteine- rich and heparin-binding sections. Moreover, ligands that act on the growth factor receptors can increase APP secretion (9). Hebert et al. suggested that APP plays a key role in gene transcription and signaling in cancer (10). Other studies described that cancer patients with enhanced APP levels die earlier; hence it is valuable to measure its concentration in clinical trials for cancer prognosis (2, 11). VPA and CBZ are antiepileptic drugs and, similar to other anticonvulsants, they prevent sodium and potassium channel activities and may alter neurotransmitters action (12). VPA also shows a range of activities like anti-cancer effects, causing suppression of tumor growth and metastasis in vitro and in vivo models (13). Xia et al. reported that VPA inhibits prostate cancer due to its HDACI effects (14). Recently, Beutler et al. reported that CBZ has also HDACI effects (15). Regarding APP production, previous studies have shown that glucose-regulated protein 78 (GRP78) can decrease the production of APP (16). Interestingly, other studies indicate that HDACIs can increase GRP78 expression (17). In this study for the first time, APP concentration in human colon cancer cells before and after CBZ treatment, as a HDACI compound, was measured using the sensitive ELISA technique. VPA has been used in cancer treatment because of its HDACI effects. Today, VPA is used in various cancer protocols in clinical trials. In this study to compare CBZ anticancer effects, we used VPA as a positive control (18). To date, no study has been reported regarding the effects of CBZ on APP which can be considered as a new potential treatment protocol for cancer disease. We found that both drugs can lower APP concentrations after drug treatment. This confirms the effectiveness of HDACIs in decreasing APP levels and supports the results of previous studies in this field (16, 17). In this regard, our data are consistent with those of previous studies on cyto-

toxicity effects of HDACIs in cancer cell growth (14). However, we observed that CBZ lowers APP level considerably more efficient than VPA. This suggests the possibility of activating some unknown mechanisms by CBZ, other than those affecting GRP78, in lowering APP concentration compared to VPA. Earlier studies have recognized a role for APP as a growth factor in different cells (9). Here, we demonstrated the involvement of APP in the colon cancer development and showed that HDACIs drugs, and in particular CBZ, can inhibit colon cancer tumors growth through effectively lowering the levels of APP, as an important growth factor in this type of cancer. According to the results of our study, it seems that small doses of CBZ can be effective in reducing cell proliferation with lower incidence of side effects. Moreover, APP as a biomarker in cell growth can allow one to evaluate cancer development at different stages. These findings not only improve our understanding of molecular mechanisms involved in colon cancer, but also they facilitate early colon cancer detection. Moreover, earlier reported results indicate that HDACIs, when used with chemotherapeutic drugs, increase the sensitivity of metastatic cells that can prevent drug resistance, which is the main cause of chemotherapy failure (19). In view of these findings, we determined that targeting APP can probably be a valuable new treatment protocol for colon cancer treatment, and it seems there is an essential need for drugs that can alter this protein. We suggest that drugs affecting APP, as an important factor involved in cell growth, can possibly be effective when used with or without chemotherapeutic agents. Hence, the results of this study can lead to a more effective treatment protocol for this disease which can also allow its earlier detection with far less side effects. The key remaining task for future studies is to evaluate the effects of CBZ in clinical trials on colon cancer patients as a novel treatment strategy.

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Footnotes

Authors' Contribution: All Authors contributed to design of the study, analysis, and interpretation of the data, and drafting of the manuscript.

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