Effects of Curcumin and Doxorubicin on the Viability of Neuroblastoma Cancer Cell Line

İlker Ünlü[1], İlhan Özdemir[2], Şamil Öztürk[3], Mehmet Cudi Tuncer[4]

1 Beykent University, Faculty of Medicine, Department of Neurosurgery, İstanbul, Türkiye
2 Private Buhara Hospital, Department of Gynecology and Obstetrics, Erzurum, Türkiye
3 Çanakkale Onsekiz Mart University, Vocational School of Health Services, Çanakkale, Türkiye
4 Dicle University, Faculty of Medicine, Department of Anatomy, Diyarbakır, Türkiye

Abstract

Aim: Neuroblastoma (NLB) has a very important place among childhood diseases, and despite all the methods used in treatment, it is very difficult to prevent neuroblastoma invasion. The number of studies showing that curcumin, the most active component of turmeric, is not toxic, is increasing day by day. In this study, the anti-cancer activities of curcumin (Cur), one of the important active compounds, were demonstrated in the human neuroblastoma cancer cell line (Na2B).

Methods: NA2B was used in the study. To determine the IC50 doses of doxorubicine (Dox) and Cur, NA2B cells line were cultivated with an automatic pipette. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) analysis was performed to analyze cell survival (viability). Inhibition levels in the cells were determined at 24 and 48 hours.

Results: While the IC50 of NA2B cell proliferation was approximately 124.5 uM at the 48th hour in Dox-treated cells, the IC50 value of Cur at the 48th hour was found to be 224.6 uM.

Conclusions: These results showed that Cur could be an alternative agent in the treatment of neuroblastoma, and its fewer side effects compared to other chemotherapeutic agents such as Dox would increase its preferability.

Keywords: *Neuroblastoma, NA2B cell line, cancer, MTT, curcumin*

1. Introduction

Neuroblastoma is the most common extracranial solid tumor in children. It constitutes 7-10% of pediatric tumors.[1] NBL, which is common from the neonatal period onwards, has a good prognosis in the early stages and in those younger than one year of age, but is a cause of mortality in older children and in the metastatic stage. It is important to recognize the epidemiological and clinical features of neuroblastic tumors, which are common and present different clinical features.

Curcumin is also known as an anti-inflammatory and an agent that reduces the effect of chemicals taken in chemotherapy.[2] Cur has many properties and shows a high anti-proliferative effect, resulting from its interaction with various molecules such as growth factors, enzymes, carrier proteins, metal ions, tumor suppressors, transcription factors, oncoproteins and nucleic acids. Thus, many studies have shown that it is beneficial against breast, cervical and melanoma cancers, such as effective induction of apoptosis.[3]

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We can also say that curcumin's low toxicity towards normal cells and tissues has attracted much attention in cancer treatment Recent studies on curcumin have revealed that this compound has poor bioavailability, hydrophobicity, poor cellular uptake and rapid metabolism.[4] To eliminate this problem, experts have conducted various experiments, many of which perform nano carriers. Additionally, curcumin affects different signaling pathways and molecular targets involved in the development of various cancers.[5] Cur has been shown to have therapeutic benefits in multiple chronic diseases.[6] In particular, inflammation, arthritis, metabolic syndrome, liver disease, obesity, neurodegenerative diseases and most importantly, cancer. There are not many alternative treatment options for cancer cases other than chemotherapy. This study aimed to reveal the effectiveness of curcumin as an alternative treatment on cancer cell invasion and migration.

Medicinal plants are very important in pharmacological research and development of drugs. The type and development of cancer is important in the fight against cancer, because many properties of different types of cells come into play and treatment with a single therapy is rarely possible.[7] At this point, combination treatments have come into play and new combination treatments are being developed every day. In combinations, targeted different pathways and preferred chemotherapy agents are reduced to lower doses and toxicity is significantly reduced.[8,9] Significant side effects occur in

patients due to the fact that the drugs are chemically synthesized. In order to overcome this problem, the discovery and development of new active substances derived from natural products has become the main focus of research.[10,11] In this study, the anticancer effects of curcumin, which can affect the neuroblastoma cell line, which is a childhood cancer, can be an alternative treatment, and has been reported in many studies to have fewer side effects, were investigated.

2. Materials and methods

2.1. Cell culture

NA2B neuroblastoma cells were purchased from American Type Culture Collection (ATCC, Manuassas, VA). NA2B cell line; It was incubated in appropriate medium (DMEM; Dulbecco's modified Eagle's medium) containing 10% fetal bovine serum (FBS) and 1% antibiotics (Penicillin, Streptomycin) and in an environment of 37°C and 5% CO_2. The culture medium was changed every 24 hours until the cells reached the required majority.[12]

2.2. Cell Viability Test (MTT assay)

To determine the IC_{50} doses of Dox and Cur, Na2B cells were seeded in 96-well plates at 3000-5000/well/cell. At the end of one night, 10-1000 nM doses of Dox and 10-1000 µM doses of Cur were applied in 9 different concentrations with serial dilution and incubated for 24 and 48 hours. The MTT test is a method that determines cell proliferation and viability by reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to the formazan product. While performing the MTT test, all procedures were performed in as little light as possible. On the first day, Na2B cells were incubated for 24 hours, with 16,000 cells added to each well, making a total volume of 300 µL. The same experiment was repeated after inducing toxicity with Dox for 24 h and adding negative control concentrations. On the second day, cells were incubated in proliferation medium. Cur containing medium was then removed and 10 µL of MTT stock solution was added, and the cells were incubated at 37°C for 4 h. After pipetting the MTT solution, DMSO was added and left at room temperature for 10 minutes. A microplate reader spectrophotometer with a wavelength selection system was used to determine the absorbance at 570-690 nm. The absorbances measured compared to the control group after three replicates were used to determine the protective effect of Cur.[13]

2.3. Statistical analysis

Statistical analyzes were performed with SPSS 20.0 software (IBM, USA) and are presented with standard error bars in the graphs. Analysis results were made with one-way ANOVA and Tukey's multiple comparative test.

3. Results

The antiproliferation effects of Cur and Dox against the NA2B cell cell line are shown in Table 1 and Table 2.

At the end of 24 hours of treatment of 250 nM Dox concentration, cytotoxic activity and 86.6% cell viability were obtained in the NA2B cell line. However, the highest cytotoxic activity of the same 24-hour Dox application was obtained with a cell viability of 57.4 at a concentration of 1000 nM. Additionally, IC_{50} could not be found in 24-hour Dox application. After 48 hours of Dox application, IC_{50} was determined as 124.5 nM. The highest 48-hour Dox toxicity was obtained at 750 nM and 1000 nM concentrations, respectively, with 33.4% and 8.2% cell viability (Figure 1, Table 1). 250 µM Cur concentration for 24 hours, the highest cytotoxic activity and cell viability of 70.4% were obtained in the NA2B cell line. IC_{50} could not be determined in 24-hour Cur application. After 48 hours of Cur application, IC_{50} was determined as 224.6 µM. The highest 48-hour Cur toxicity was 16.8% and 18.4% cell viability at 750 nM and 1000 nM concentrations, respectively (Figure 2, Table 2).

Table 1

Cell viability obtained from 24 and 48 hours of Dox application in NA2B cell line at 9 different concentrations obtained as a result of serial dilution in the concentration range of 0.5-50 µM.

NA2B-Dox		N	Cell viability (%)	Std.Dev.	Std. Err.
S24h	,00	6	100,00	5,40	1,70
	10,00	6	96,21	2,92	1,70
	25,00	6	98,56	5,79	2,27
	50,00	6	94,51	4,05	1,45
	75,00	6	96,33	3,22	1,33
	100,00	6	94,26	2,84	1,12
	250,00	6	86,65	4,23	1,73
	500,00	6	68,47	1,67	0,62
	750,00	6	62,34	2,75	1,43
	1000,00	6	54,75	1,34	0,21
S48h	,00	6	100,00	5,47	2,34
	10,00	6	78,45	4,29	1,47
	25,00	6	76,76	2,52	0,89
	50,00	6	76,20	2,53	1,13
	75,00	6	78,13	3,73	2,24
	100,00	6	53,95	1,20	0,29
	250,00	6	19,09	1,14	0,46
	500,00	6	10,78	0,64	0,34
	750,00	6	11,21	0,37	0,11
	1000,00	6	8,51	0,77	0,26

Figure 1

% Cell viability compared to the vehicle control group after 24 and 48 hours of Dox application.

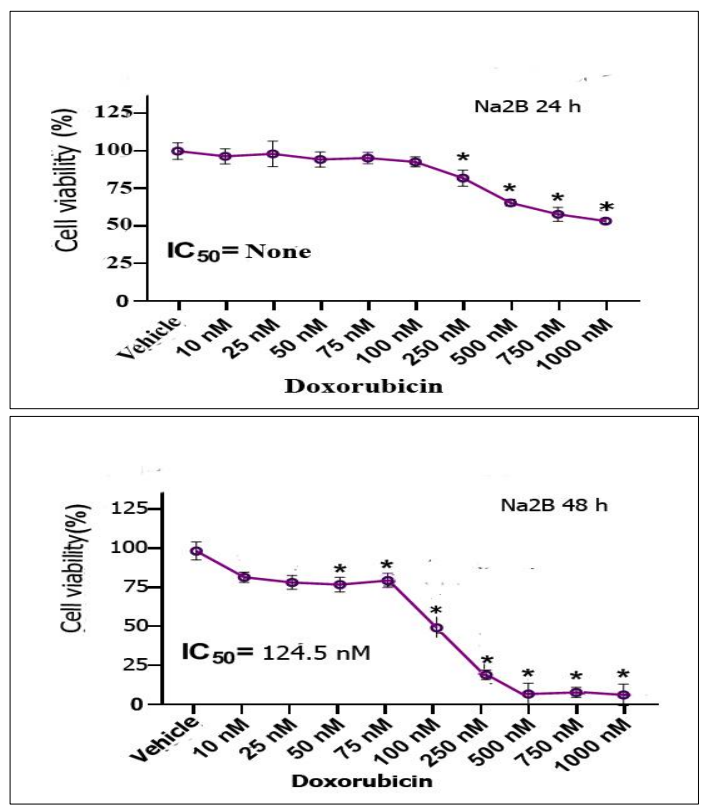


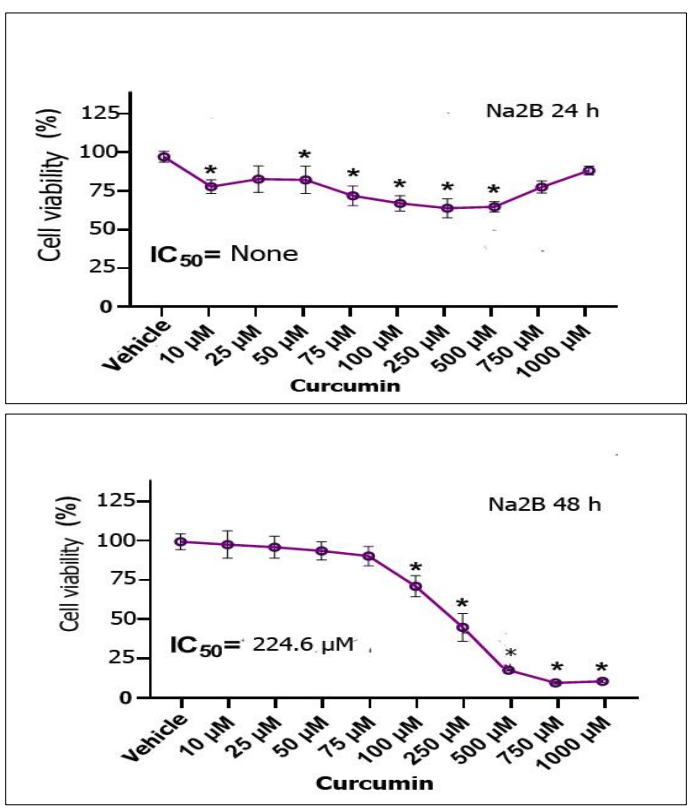
Table 2

Cell viability obtained from 24 and 48 hours of Cur application in 9 different concentrations obtained as a result of serial dilution in the NA2B cell line in the concentration range of 0.5-50 µM.

NA2B-Cur		N	Cell viability (%)	Std.Dev.	Std. Err.
S24h	,00	6	100,00	4,40	1,70
	10,00	6	76,41	3,92	1,68
	25,00	6	78,65	3,79	2,12
	50,00	6	79,43	2,05	1,23
	75,00	6	75,33	2,22	1,33
	100,00	6	74,26	2,64	1,21
	250,00	6	73,65	5,23	1,63
	500,00	6	74,47	1,87	0,42
	750,00	6	77,34	2,25	1,37
	1000,00	6	80,73	1,64	0,22
S48h	,00	6	100,00	4,47	1,34
	10,00	6	94,24	2,29	1,57
	25,00	6	89,66	3,52	0,24
	50,00	6	86,46	3,53	1,13
	75,00	6	84,41	3,43	2,25
	100,00	6	73,85	2,20	0,24
	250,00	6	44,19	1,24	0,32
	500,00	6	16,75	0,35	0,33
	750,00	6	8,43	0,33	0,13
	1000,00	6	11,41	0,57	0,24

Figure 1

% Cell viability compared to the vehicle control group after 24 and 48 hours of Cur application.



4. Discussion

In this study, we showed that curcumin and doxorubicin prevent the proliferation of neuroblastoma NA2B cells and exert this effect by suppressing cell viability. With these results, we found that curcumin, a natural phenolic compound, plays protective roles in highly metastatic cancer types such as neuroblastoma and its antioxidant potential can be benefited by using it in combination with chemotherapeutics used in treatment. Neuroblastoma is a complex disease that affects the sympathetic nervous system.[14] Most of the mortalities associated with neuroblastoma occur due to metastasis to lymph nodes and bones.[15] Therefore, preventing cancer cell invasion and migration is an important step to prevent metastasis. Neuroblastoma cell invasiveness and metastasis depend on the ability of tumor cells to break down the extracellular matrix to detach from the primary tumor and enter the bloodstream or lymphatic system, then travel to distant sites and reattach.[16] Phenolic compounds have been shown to suppress MMP2 and MMP9 levels in vascular endothelial cells, thus It has been reported that it has a protective effect against degenerative diseases.[17]

The anti-oxidant effects of natural phenolic compounds have been determined by many studies. With their radical scavenging effects, phenolic compounds also reduce the risk of cancer formation. In an in vitro study, the antioxidant activity of curcumin was determined.[18] In addition to the primary antioxidant effects of these compounds, it was also determined that they had a broad-spectrum biological effect on carcinogenesis.[19] Studies on this subject show that phenolic compounds, especially polyphenols, prevent the formation of cancer.[20] Studies have shown that compounds are effective on tumor cell growth and proliferation.[21,22] It has been determined that natural compounds with phenolic structures have an anti-proliferative effect on human tumor cell lines. It has been stated that the anti-proliferative activities and therefore the potential anti-cancer effects of phenolic compounds originate from their aromatic rings and hydroxylic groups.[23] In one of the studies conducted with curcumin, the anticancer properties of nanocurcumin and its anticancer effects with nanocarriers in different types of cancer were investigated.[24] It has been shown that curcumin nanoformulation can affect cellular pathways effective in differentiation and cell proliferation.[25] Particularly from in vivo studies, they reported that in the tumor model with BALB/c mice, the existing tumor size was reduced more effectively and efficiently when nano-curcumin was applied to the mice.[26] In an in vitro study conducted on neuroblastoma cells, it was determined that curcumin had significant effects on cell viability. In our study, it was determined that curcumin had an anti-proliferative effect on the neuroblastoma cell line. In this study, we found that curcumin reduced the proliferation of Na2B cells, similar to the effects of previous phenolic compounds. Although plant phenolic compounds have been shown to be effective on tumor growth and cell proliferation, more experimental studies are needed on this subject due to the mortality of cancer disease and deficiencies in treatment.

5. Conclusion

The current study shows that Cur may be a promising treatment option for the treatment of neuroblastoma cancer and an alternative to commonly used chemotherapy treatment. More and comprehensive studies involving cell culture and animal models are needed regarding the effectiveness of Cur in treatment.

Statement of ethics

Ethical approval is not required because commercially available cell lines are used as an in vitro study.

Source of Finance

The authors declare that they have received no financial support for this study

Conflict of interest statement

The authors declare that they have no conflict of interest.

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