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Original Article

Pharmacological Study of the Antitumor Effect of Newcastle Oncolytic Virus in Combination with Copper Nanoparticles, Hyperthermia and Radiation on Malignant Colorectal Cancer Cell Line

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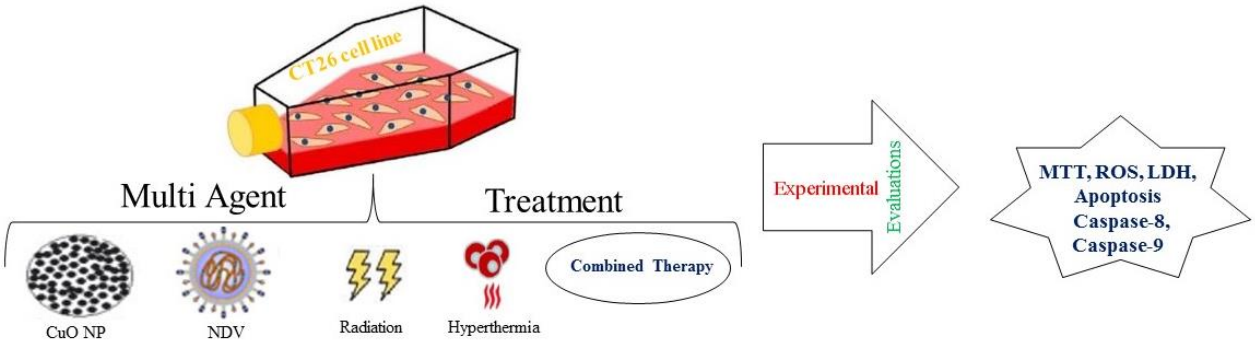
ABSTRACT

As new multifactorial method this study aimed to investigate the synergistic effects of the Newcastle oncolytic virus in combination with copper nanoparticles, hyperthermia, and radiation on the proliferation of CT26 cell line. Cultured CT26 cells were treated with the combination of CuO nanoparticles (100 µg/ml), Newcastle oncolytic virus (MOI 40), radiation (cGy200), and hyperthermia (41 °C). In order to confirm the anticancer effects of these factors, proliferation rate (MTT), percentage of cell death, generation levels of reactive oxygen species (ROS), release levels of lactate dehydrogenase (LDH), and activity levels of caspase-8 and 9 were measured. A significant decrease in cell proliferation rate (57±5.19) was observed. Also, levels of cell death (52±3.06), ROS production (27.89±0.69), and LDH release (26.54±1.27) were increased significantly in the group of Newcastle oncolytic virus combined with CuO nanoparticles, hyperthermia, and radiation in comparison with other treated and control groups. Also, the activity level of caspase-9 was significantly increased in all treated groups compared to the control group. According to the present study, it seems that the combination of multifactorial therapies such as oncovirotherapy, nanotherapy, hyperthermotherapy, and radiotherapy can inhibit the proliferation of CT26 cancer cell line.

GRAPHICAL ABSTRACT



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Introduction

There are several treatments of colorectal cancers, including surgery, chemotherapy, and immunotherapy. However, none of these methods represented successful outcomes. Besides, these approaches effectively delayed tumor growth. Chemotherapy is one of the most effective treatments for metastatic tumors. To cancer treatment, several chemotherapy regimens are used based on 5-fluorouracil (5-FU) agent [1,2] (Figure 1).

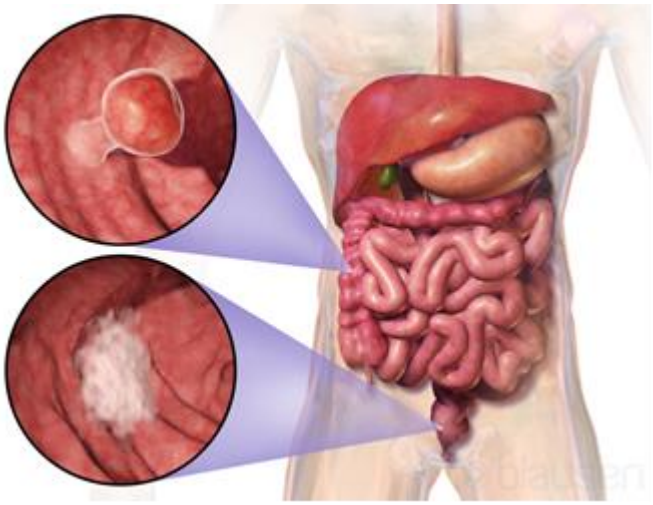

Figure 1: Colorectal cancer

Radiotherapy is one of the most critical methods of cancer treatment. Radiotherapy is performed in two forms; external and internal (brachytherapy) protocols. Radiotherapy's ideal purpose is to absorb the highest and the lowest dose of radiation by tumoral cells and surrounding healthy tissues, respectively [3-5]. Nanotherapy protocols are highly developed in various medical and therapeutic fields, including cancer treatment. Nanoparticles, based on the small molecular sizes, can be used as drug carriers to treat various diseases (cancers, cardiovascular diseases, Alzheimer's, and brain cancers) [6]. CuO is one of the most widely used nanoparticles in medicine. Several studies have demonstrated its selective cytotoxic effects on cancer cell lines through DNA damage of cancer cells. Low cost of production and availability, antibacterial (gram-positive and negative) features, selective anticancer effects, and anti-proliferative potential even in low concentrations are some of the advantages of CuO nanoparticles [7,8]. Hyperthermia is another method of cancer treatment. In this method, the tissues are exposed to a higher temperature than physiological body temperatures to damage and destroy cancer cells. Hyperthermia is primarily used in conjunction with other cancer treatments such as radiotherapy (increasing the sensitivity of cancer cells to radiation) and chemotherapy (increasing drug effectiveness) [9,10]. Cancer cell virotherapy is a method relying on the innate ability of viruses to infect, proliferate in an intracellular matrix and lysis of host cells. Thus, along with identifying a wide range of humans and animals' viruses, these viruses were analyzed for their anticancer potential, specificity to cancer types, and safety. These viruses include Newcastle virus, adenoviruses, herpes simplex virus, measles, retrovirus, lentivirus, parvovirus, vesicular stomatitis virus, mumps virus, smallpox virus, vaccinia virus, and paramyxoviruses [11]. The birds are the host of Newcastle virus; thus, this kind of virus is not pathogenic for humans. This type of virus hires the sialic acid, a highly expressed protein available on the surface of cancer cells, as a receptor for the cell entrance. Disruption of activation of cell antiviral defensive pathway (especially Type I IFN Signaling pathway), Rac1 expression, and induction of apoptosis in tumor cells are other anticancer mechanisms of this type of virus [12]. Some studies have approved the selective anticancer features of the Newcastle virus. According to the researches, the anti-proliferative effects of the Newcastle virus on cancer cells are 700-time greater than normal cells [13]. This study aimed

to investigate the synergistic effects of Newcastle virus in combination with copper nanoparticles, hyperthermia, and radiation on CT26 cell line proliferation (colorectal malignant carcinoma) as a new multifactorial method.

Materials and Methods

Cell, virus and reagents

CT26.WT (ATCC® CRL-2638™) was purchased from Pasteur Institute (Tehran, Iran). Copper oxide nanoparticles (US3070) were purchased from US Research Nanomaterials, Inc. LaSota strain of Newcastle virus was prepared from Applied Virology Research Center of Baqiyatallah University of Medical Science (Tehran, Iran). DMEM media and fetal bovine serum were prepared from GIBCO/Life Technologies Inc. Dimethyl sulfoxide (DMSO), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO) and caspase-8 and 9 kits were purchased from Abcam company.

Culture of Newcastle oncolytic virus

Following CT26 cell line (malignant colon carcinoma) preparation (C532 cell bank code, Pasteur Institute, Iran), the cells were cultured in T25 flask containing DMEM culture medium (10% FBS, and penicillin/streptomycin antibiotic). They kept in an incubator (37 °C, 5% CO_2). After 80% of cell density, they were trypsinized (0.25% trypsin-EDTA) and underwent passage. The MOI value was calculated to determine the effective concentration of the Newcastle oncolytic virus. The multiplicity of infection (MOI) is the number of viral particles divided into the number of cells at inoculation (Figure 2).

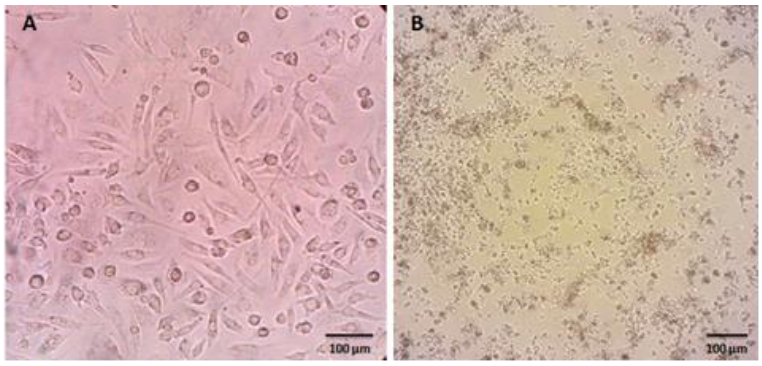

Figure 2: Phase-contrast images of CT26 cells (A) and Newcastle virus-infected cells (B)

Study design

According to Table 1**,** the CT26 cell line was divided into 13 groups containing 1 × 10^6 cells. To achieve the most effective concentrations of CuO nanoparticle and MOIs value of Newcastle virus, different concentrations and MOIs were used (no data were provided due to a large amount of information). Following IC50 obtaining, the most effective concentration of CuO nanoparticle was calculated as 100 µg/mlL for 24 h, and the most effective MOIs values were defined as 40 for 1 h. Radiation (200 cGy) and hyperthermia (41 °C) were also applied for 1 h to design multifactorial groups. 5-FU with the concentration of 10 nm was used as a positive control.

Table 1: Single-factor and multi-factor study groups

Groups	Group abbreviation	Group	Group abbreviation
Negative Control	**NC**	Radiation	**R**
Hyperthermia	**H**	Radiation + Hyperthermia	**R + H**
5-Fluorouracil	**5-FU**	CuO nanoparticle	**CuO NP**
Newcastle oncolytic virus	**NOV**	CuO nanoparticle + Radiation	**CuO NP + R**
CuO nanoparticle + Hyperthermia	**CuO NP + H**	CuO nanoparticle + Newcastle oncolytic virus	**CuO NP + NOV**
Newcastle oncolytic virus + Radiation	**NOV + R**	Newcastle oncolytic virus + Hyperthermia	**NOV + H**
Newcastle oncolytic virus + CuO nanoparticle + Radiation + Hyperthermia	**NOV + CuO NP + R + H**		

Determination of amount of CuO nanoparticles uptake

CT26 cells with concentrations of 0,5, 10, 20, 40, 80 and 100 µg/mL were incubated (37 °C, 5% CO_2) for 24 h in DMEM culture medium. 24 h later, the cells were washed with phosphate buffer saline (PBS) to remove non-uptake nanoparticles. To destroy CT26 cells treated with different concentrations of CuO nanoparticles, respectively 0.3 mL of 3% HCL concentrated with HNO_3 (overnight at room temperature), 5 µL of 5 ppm iodine, and 5ml of 2% HCL solution, and 2% HNO_3 were used. The content of nanoparticles released from cell degradation was measured by spectrophotometer (light absorption of 280 nm). Levels of CuO nanoparticles uptake were calculated using a standard curve [14,15].

Radiotherapy and hyperthermia therapy

One-hour treatment with radiation (200 cGy) and hyperthermia (41 ^{o}C) was applied to evaluate the effects of multifactorial groups of CT26 cells in both forms of alone and in combination with CuO and NOV nanoparticles. Following 70% of cell density, the cells were incubated at 41 °C. After cell treatment with CuO and NOV nanoparticles in combination therapy groups, the cells underwent hypnotherapy and radiotherapy according to the mentioned stages [16].

Determination of cell proliferation

To assess the proliferation of CT26 cells, the MTT assay was used according to the study of Jabbari *et al*. (2018). Briefly, 10^6 cells were transferred into the wells of the 96-well plate. Then, the cells were treated with NOV, CuO NP, R, and H agents (alone or in combination with the abovementioned agents). Finally, 20 µL of MTT solution (5 mg/mL PBS) was added to all 96 wells of the well-plate. Following incubation (4 h, 37 °C, 5% CO2), 100 µl of DMSO solution was added to all 96 well plates. Immediately the light absorption of the wells was recorded at 492 nm through Elisa reader. The following formula was used to evaluate the proliferation rate [16]: *cell viability (%): optical density value of test / Optical density value of control × 100.*

Determining the rate of cell death

Acridine orange/propidium iodide staining was used to evaluate the rate of CT26 cell death induced by multifactorial treatments. Briefly, cultured CT26 cells were incubated (15 min, 37 °C) with 10 µL/mL acridine orange. Then, the cells were washed twice with PBS solution. 10 µL/mL propidium iodide was added to the culture medium and incubated for 5 min at 37 °C. Finally, the cells were rewashed, and the number of dead cells was assessed using a fluorescent microscope [17].

Measurement of ROS generation

Dichlorodihydrofluorescein (DCF-DA, 2.5 µM, Invitrogen, St Louis, MO, USA) was used to calculate the amount of produced ROS. After passive intracellular transportation of DCFH-DA, the DCF (a fluorescent agent with a high affinity to ROS) was formed. Following CT26 cell staining with DCFH-DA (20µM), they were incubated (30 min, 37 °C, 5% CO_2). Then, the cells were washed with PBS. The fluorescence of the cells was compared with the control group using a fluorescence microscope, and the amount of ROS production was calculated [15].

LDH Measurement

Lactate dehydrogenase (LDH) is an enzyme available in the cytosol to change the lactate to pyruvate. The increased LDH level in culture medium indicates cell membrane damage. According to the instruction of the related kit (Abcam, ab65393), the supernatant available in wells was aspirated 48 hours after CT26 cell culture. Measurement of LDH enzyme was applied using ELISA reader at 492 nm [15].

Measurement of Activity of Caspase-8 and 9 (MCA)

According to the study of Jabbari *et al*. (2018), the concentrations of caspase-8 and 9 in CT26 cells were measured using commercial kits (Sigma-Aldrich, St. Louis, MO). CT26 cells were suspended in lysis buffer (50 mM of Tris-HCL [pH: 7.4], 0.5% NP-40, 250 mM NaCl, 5 mM EDTA, and 50 mM NaF) and they were followed by incubation on ice for 10 min. Lysates (derived from the effect of lysis buffer on CT26 cells) were vortexed every 15 min. Following centrifugation (1,1000 rpm, 20 min), 20 µL of supernatant was

added to the specific p-nitroaniline (pNA)-conjugated buffer to measure the concentration of caspase-8 and 9. This solution was incubated for 1 hour at 37 °C. Then, the light absorption was measured at 492 nm using ELISA reader [15].

Statistical Analysis

The obtained data were analyzed using one-way ANOVA statistical method, and the final results were presented as mean ± standard deviation (SD). Tukey's multiple post-hoc tests was used to compare the significance of results among different groups. Also, $p < 0.05$ was considered as the significant level. Due to the presence of various study groups, the lowercase letters were used to represent the significance value among groups. Therefore, dissimilar and similar small letters indicated significant and non-significant differences, respectively, at the level of $P < 0.05$.

Results and Discussions

Rate of CuO nanoparticles uptake

According to the standard diagram (Figure 3), the absorption rate of CuO nanoparticles by CT26 cells at concentrations of 5, 10, 20, 40, 80, and 100 µg/mL were calculated as 2.980, 5.570, 13.600, 31.730, 63.589 and 80.295 µg/mL. In this study, a concentration of 100 µg/mL was used.

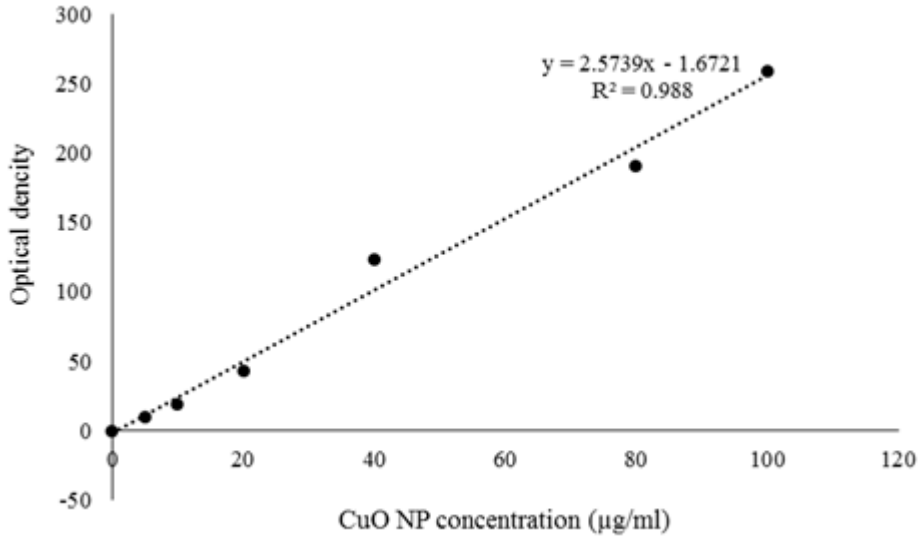

Figure 3: Standard curve for cellular uptake of copper oxide nanoparticles (CuONPs) into the CT26 cells in concentrations of 5, 10, 20, 40, 80 and 100 µg/ml

Rates of cell proliferation, cell death and LDH

According to Table 2, the MTT, apoptosis, and LDH tests were hired to evaluate the cytotoxicity of Newcastle oncolytic virus on CT26 cells in combination with nanoparticles, hyperthermia, and radiation. Results of the MTT assay revealed that CuO, NOV, R, and H groups had the highest significant proliferative inhibitory potential (57±5.19), and the R group represented the lowest significant inhibitory potential (85±8.66) in comparison with the NC group. Apoptosis, or programmed cell death, is characterized by morphological features and energy-dependent biochemical mechanisms. Acridine orange (AO) and propidium iodide (PI) was used to evaluate the apoptosis rate. By binding Acridine Orange to the structure of DNA, the highest and lowest excitation and emission were 502 and 525 nm, respectively. Acridine Orange binds to the DNA of living cells. Therefore, the green emission indicates the living cells. PI dye also binds to DNA and RNA (this binding has no association to its sequence). PI binds to the DNA of necrotic or apoptotic cells with no interaction with living cells. The results of the apoptotic assay showed that the CT26 cells in CuO, NOV, R, H, and R groups had the highest (52±3.60) and lowest (18±3.46) cell apoptosis, respectively in comparison with the NC group. Lactate dehydrogenase is one of the most stable cytoplasmic enzymes found in most cells. This enzyme leaks into the extracellular matrix following cell membrane damage, which can be measured using an LDH assay kit. Parallel to the

results of MTT and apoptosis assays, the rate of LDH release (which indicates damage to the cytoplasmic membrane) showed that the CuO, NOV, R, and H groups had the most significant damage (26.54±1.27) and the R group (7.26±1.22) represented the least significant damage compared to the NC group.

Table 2: Mean ± standard deviations (SD) of investigated parameters in the study groups

Groups	MTT assay (%)	LDH assay (%)	Cell death (%)
NC	100^{a}	2.32 ± 1.12^{a}	$\mathbf{6\pm1.73^{a}}$
R	85 ± 8.66^{b}	7.26 ± 1.22^{b}	$\mathbf{18\pm3.46^{b}}$
H	93 ± 6.92^{abc}	4.72 ± 0.88^{a}	$\mathbf{9\pm4.58^{a}}$
R+H	80 ± 6.07^{bcf}	8.88 ± 1.42^{b}	$\mathbf{21\pm4.35^{b}}$
5-FU	38 ± 4.03^{d}	27.98 ± 1.66^{c}	$\mathbf{61\pm8.54^{c}}$
CuO NP	66 ± 6.08^{egh}	15.54 ± 2.02^{df}	$\mathbf{33\pm4.35^{de}}$
NOV	75 ± 5.27^{ef}	13.42 ± 1.62^{dh}	$\mathbf{26\pm3.60^{df}}$
CuO NP+R	65 ± 4.35^{gi}	19.35 ± 1.14^{e}	$\mathbf{36\pm2.64^{de}}$
CuO NP+H	66 ± 5.56^{hi}	16.72 ± 1.00^{f}	$\mathbf{33\pm6.08^{deg}}$
CuO NP+NOV	63 ± 7.54^{ghj}	24.11 ± 1.74^{g}	$\mathbf{40\pm7.81^{e}}$
NOV+R	73 ± 7.01^{begh}	13.19 ± 1.24^{h}	$\mathbf{27\pm7.93^{bfg}}$
NOV+H	74 ± 5.29^{begh}	12.43 ± 1.51^{h}	$\mathbf{23\pm4.35^{bf}}$
CuO NP+NOV+R+H	57 ± 5.19^{j}	26.54 ± 1.27^{g}	$\mathbf{52\pm3.60^{h}}$
p-Values	$p<0.05$	$p<0.05$	$\boldsymbol{p<0.05}$

The abbreviations are provided in experiment design of section "Materials and methods".5-FU was considered as positive control; CuO: Copper oxide nanoparticles; R: radiation; H: hyperthermia. Significant statistical differences between groups in each index are indicated by the different superscript letter ($p < 0.05$).

ROS production

This value was significantly increased in CuO, NOV, R, and H groups (27.89±0.697) than in the NC control group (3.71±0.647) and represented the highest ROS level among others treatment groups. Group of H showed the lowest level of ROS production (5.92±0.833) compared to other treatment groups (Figure 4).

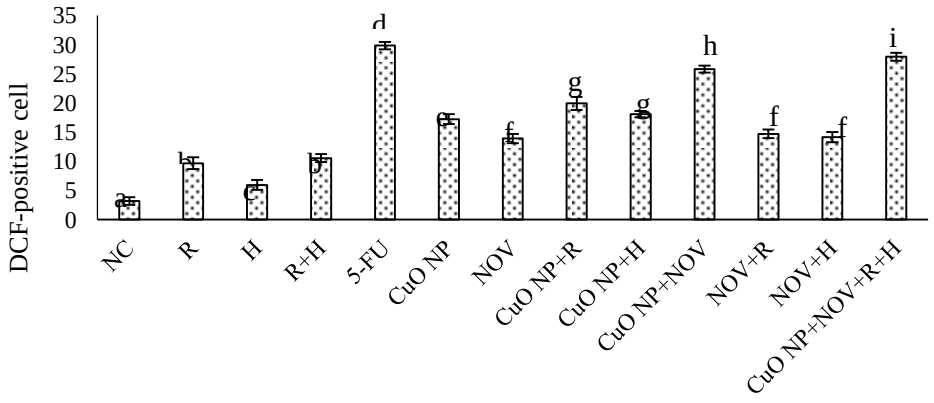

Figure 4: Effects of radiation (R), Hyperthermia (H), CuO nanoparticle (CuO NP) and 5-FU, as positive control (PC), on ROS production of CT26 cells in the studied groups. The abbreviations are provided in section "Materials and methods" experiment design. The values were normalized to those of the negative control group. The different superscript letters indicate significant statistical differences between groups in each index ($p < 0.05$)

Caspase measurement

The level of caspase-8 activity in all treatment groups found a non-significant increase. However, the activity level of caspase-9 in CuO, NOV, R, and H groups revealed the highest significant value (0.354±0.026), and in the R group represented the lowest significant value (0.124±0.022) compared to the NC group (Table 3).

Table 3: Descriptive statistics (mean ± standard deviations (SD)) of assessment of caspase-8, and -9 activities in studied groups

Groups	Caspase-8 activity (absorbance)	Caspase-9 activity (absorbance)
NC	0.313±0.036[a]	0.060±0.013[a]
R	0.334±0.038[a]	0.124±0.022[b]
H	0.332±0.046[a]	0.062±0.014[a]
R+H	0.313±0.034[a]	0.148±0.028[b]
5-FU	0.360±0.042[a]	0.361±0.040[c]
CuO NP	0.330±0.03[a]	0.227±0.024[df]
NOV	0.332±0.043[a]	0.175±0.019[eg]
CuO NP+R	0.338±0.042[a]	0.253±0.026[df]
CuO NP+H	0.311±0.049[a]	0.198±0.036[deg]
CuO NP+NOV	0.338±0.061[a]	0.281±0.024[f]

While radiotherapy is one of the most effective methods for cancer treatment, this procedure also has different side effects such as nausea and vomiting, damage to the epithelial layer, mouth, throat, and stomach sores, intestinal discomfort, swelling, infertility, fibrosis, epilation, dryness, lymphedema, cardiovascular diseases, cognitive decline, radiation enteropathy, radiation-induced polyneuropathy, radiation necrosis, cumulative side-effects, effects on reproduction, effects on pituitary system, and radiation therapy accidents [19]. Therefore, it is necessary to use adjuvant therapy to reduce the dose and side-effects of radiation.

In recent years, rapid growth in nanotechnology to develop nanoscale pharmaceutical agents provides great promise to improve therapeutic approaches against various cancers. Today, nano-based drug therapies offer a fantastic opportunity to achieve sophisticated and multi-purpose targeted strategies in cancer treatment. These nano-based drug therapies include synthetic nanoparticles with therapeutic properties such as CuO nanoparticles or nano-sized anticancer agents like oncolytic viruses. Various studies have demonstrated the selective cytotoxic potential of CuO nanoparticles against various types of cancers, including hepatocarcinoma, lung carcinoma, nasopharynx cancer, breast cancer, cervical carcinoma, and pancreatic cancer [20]. Today, CuO nanoparticles are essential in nanotechnology as an absorption candidate to deliver many small drug molecules or large biomolecules (DNA, RNA, and proteins). For a long time, the copper ion was known for its antibiotic properties. Although the antibacterial mechanism of copper has not been fully understood, recently, this ion has been widely used due to its usage in nanoparticle fabrication. Many studies have identified the anticancer properties of CuO nanoparticles [21]. CuO nanoparticles disrupt the cellular communication network by perturbation of cell signaling pathways. Also, CuO nanoparticles play an essential role in DNA damage and induction of increased caspase-3 expression influential in cell death. CuO can lead to mediation and amplification of death signaling proteins. This concept is crucial in cancer treatment [22].

Oncolytic cancer therapy involves a viral application for cancer cell lysis. This method relies on the innate ability of viruses to infect, intracellular replication, and ultimately lysis of host cells. According to trials performed on Newcastle oncolytic virus, this agent was found as one of the most potent oncolytic viruses with inhibitory effects on breast, brain, and cervical cancers in both conditions of in vitro and in vivo [23].

In addition to inhibition of cancer cell proliferation, mild hyperthermia (39–41 °C for 1 hour) could increase the effectiveness of radiation, chemotherapy, and other adjuvant therapies in both conditions of in-vitro and in-vivo [24].

Based on the results of this study, the synergistic effects of combination therapy can be concluded in colorectal cancer treatment. The present study results showed that the use of multifactorial therapy of Newcastle oncolytic virus,

nanoparticles of copper, hyperthermia, and radiation in addition to reducing the proliferation and survival rates of CT26 cells could also increase the production of LDH and ROS. These conditions can restrict cancer cells' growth, proliferation, and migration. Guo *et al*. (2020) (21), Joel *et al*. (2020) [25], Esmaeili Gouvarchin Ghaleh *et al*. (2019) [15], and Shafagh et al. (2015) [26], reported that CuO nanoparticles in a concentration-dependent activity could decrease proliferation (MTT) and viability (increased apoptosis) of cancer cells. In this manner, the growth of cancer cells in the culture medium can be inhibited.

One of the known anticancer mechanisms of CuO nanoparticles is that these released ions can inhibit cancer cell cycle and growth through DNA damaging and histone deacetylase inhibition. Keshavarz *et al*. (2019) [13], Meng *et al*. (2020) [27], and Najmuddin *et al*. (2020) [28] reported that Newcastle oncolytic virus could significantly reduce the survival rate and increase apoptosis of cancer cells.

There are various anticancer mechanisms of the Newcastle oncolytic virus, including induction of apoptosis through intrinsic stimulation (mitochondrial) and extrinsic pathway receptors, stimulation of cascading activities of caspases, and the use of hemagglutinin-neuraminidase protein. In the present study, the obtained results of both evaluations of cell proliferation rate (MTT) and concomitant survival rate (apoptosis) showed that combination treatment of Newcastle oncolytic virus with nanoparticles, hyperthermia, and radiation could synergistically reduce the growth and viability of CT26 cells. Al-Zharani *et al*. (2020) [29], and Esmaeili Gouvarchin Ghaleh *et al*. (2019) [15] found that the increased level of LDH in cancer cell culture medium following treatment with CuO nanoparticles. After cell membrane rapture, the LDH is released. Thus, LDH release and cell membrane damage are characteristics of necrotic cells. Measurement of LDH leakage is based on the intracytoplasmic release of this enzyme following cell membrane damage, while the MTT is measured mainly based on intra-mitochondrial enzymatic changes. Researchers have approved that in cell culture conditions and MOI-dependent manner, the Newcastle oncolytic virus can increase the status of LDH release in TC1 cancer cells (cervical cancer). Also, it has been reported that the 40 MOI is the best concentration of Newcastle virus to inhibit TC1 cells growth [13]. In our study, the results of the LDH assessment showed that the CT26 cells treated with multifactorial combination therapy had a significant increase in LDH release compared to the negative control group. In addition, the obtained results of LDH assessment were in line with MTT and apoptosis outcomes in this study. Liu *et al*. (2020) [30], Mariadoss *et al*. (2020) [31], Fahmy *et al*. (2020) [32], and Ali *et al*. (2020) [33] reported that CuO nanoparticles could increase ROS generation in cancer cells.

The mechanism of action in most compounds, drugs, and cancer treatment procedures is based on ROS generation. Among these mechanisms, the nanotherapy with CuO nanoparticles has a unique position. By induction of oxidative stress and lipid peroxidation, Nanoparticles play a crucial role in DNA damage, membrane destruction, and cell death. Many studies indicated that increased ROS levels in cancer cells are directly associated with increased apoptosis rate. Also, based on the nanotherapy reviews, the increased level of ROS and cell death related to DNA damage are considered characteristics of nanoparticles [34]. ROS is one of the essential molecules regulating the physiological and pathological pathways involved in cancer and viral diseases. Oncolytic viruses increase ROS production through mitochondrial damages. Researchers also reveale that treating TC1 (cervical cancer) cells with the Newcastle virus could potentially increase ROS-dependent autophagy. Scientists have approved that the HVJ-E oncolytic virus (sendai virus, hemagglutinating virus of Japan envelope) can elevate the rate of ROS-dependent autophagy in cancer cells through various pathways, including JNK, p38, and PI3K / beclin-1 [13,35].

Conclusions

The results of ROS assessment in the present study also revealed that treatment of CT26 cells with the combination of multifactorial therapies

can increase the ROS level and consequently increase the apoptosis rate.

Apoptosis often occurs through two classical intrinsic and extrinsic pathways. The extrinsic pathway is associated with binding essential ligands such as TNF-α and Fas to death-inducing membrane receptors. Intrinsic apoptotic pathway is also related to permeability changes in outer mitochondrial membrane and release of apoptotic agents. The initiation of both apoptotic pathways is mediated by caspases during the cascade activation process. Extrinsic apoptotic pathway hires caspases 8 and 10 as initiating caspases.

In contrast, caspase-9 as an initiating caspase is used in the intrinsic apoptotic pathway. Caspases 3, 7, and 6 are involved in both intrinsic and extrinsic apoptosis pathways, leading to cell destruction [36]. Most studies revealed the involvement of both intrinsic and extrinsic pathways for apoptosis induction by the Newcastle virus in cancer cells. In this manner, the main pathway directly depends on the type of cancer. For instance, the extrinsic (TNF-dependent) and intrinsic signalling in Hela and Vero cells were seen as the main apoptotic pathways, respectively [13]. Several studies have also shown that CuO nanoparticles and Newcastle oncolytic virus can induce apoptosis by increasing the activity of caspase-9 in the intrinsic mitochondrial pathway [13, 15].

According to the results of our study, CT26 cells treatment with multifactorial combination therapy could induce ROS-dependent cell death through an intrinsic mitochondrial pathway.

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Conflict of Interest

The authors report no conflicts of interest.

Informed consent

This manuscript reports the results of experimental investigations conducted with the mouse model (Ethics: IR.BMSU.REC.1398.332).

Abbreviations

NOV: Newcastle oncolytic virus
LDH: lactate dehydrogenase
ROS: Reactive Oxygen Species
CuO NP: copper nanoparticles
H: Hyperthermia
R: Radiation
NC: Negative Control
5-FU: 5-Fluorouracil

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References

[1]. Sur D., Havasi A., Cainap C., Samasca G., Burz C., Balacescu O., Lupan I., Deleanu D., Irimie A., *J. Clin. Med.*, 2020, **9**:182 [Crossref], [Google Scholar], [Publisher]

[2]. Lichtenstern C.R., Ngu R.K., Shalapour S., Karin M., *Cells*., 2020, **9**:618 [Crossref], [Google Scholar], [Publisher]

[3]. Gozzi G.J., Gonzalez D., Boudesco C., Dias A.M., Gotthard G., Uyanik B., Dondaine L., Marcion G., Hermetet F., Denis C., Hardy L., *Cell. Death. Differ.*, 2020, **27**:117 [Crossref], [Google Scholar], [Publisher]

[4]. Ito K., Yamaguchi T., Ogawa H., Nakajima Y., Karasawa K., *Jpn. J. Clin.*, 2020, **50**:1442 [Crossref], [Google Scholar], [Publisher]

[5]. Lu C.C., Wang F.N., Lin H.H., Hsu C.H., Lin J.P., Lai L.H., *Radiat. Phys. Chem.*, .2020, **172**:108792 [Crossref], [Google Scholar], [Publisher]

[6]. Tiwari A., Saraf S., Jain A., Panda P.K., Verma A., Jain S.K., *Drug. Deliv. Transl. Res.*, 2020, **10**:319 [Crossref], [Google Scholar], [Publisher]

[7]. Kannan K., Radhika D., Vijayalakshmi S., Sadasivuni K.K., A. Ojiaku A., Verma U., *Int. J. Environ Anal. Chem.*, 2020, **27**:1 [Crossref], [Google Scholar], [Publisher]
[8]. Chandrasekaran R., Yadav S.A., Sivaperumal S., *J. Clust. Sci.*, 2020, **31**:221 [Crossref], [Google Scholar], [Publisher]
[9]. Jose J., Kumar R., Harilal S., Mathew G.E., Prabhu A., Uddin M.S., Aleya L., Kim H., Mathew B., *Environ Sci. Pollut. Res.*, 2020, **27**:19214 [Crossref], [Google Scholar], [Publisher]
[10]. Oltolina F., Peigneux A., Colangelo D., Clemente N., D'Urso A., Valente G., Iglesias G.R., Jiménez-Lopez C., Prat M., *Cancers*, 2020, **12**:2564 [Crossref], [Google Scholar], [Publisher]
[11]. Macedo N., Miller D.M., Haq R., Kaufman H.L., *J. Immunother. Cancer*, 2020, **8** [Crossref], [Google Scholar], [Publisher]
[12]. Ma J., Ramachandran M., Jin C., Quijano-Rubio C., Martikainen M., Yu D., Essand M., *Cell. Death. Discov.*, 2020, 22; **11**:1 [Crossref], [Google Scholar], [Publisher]
[13]. Keshavarz M., Nejad A.S., Esghaei M., Bokharaei-Salim F., Dianat-Moghadam H., Keyvani H., Ghaemi A., *Saudi J. Biol. Sci., 2020*, **27**:47 [Crossref], [Google Scholar], [Publisher]
[14]. Jabbari N., Zarei L., Esmaeili Govarchin Galeh H., Mansori Motlagh B., *Artif. Cells Nanomed Biotechnol.*, 2018, **46**:364 [Crossref], [Google Scholar], [Publisher]
[15]. Esmaeili Govarchin Ghaleh H., Zarei L., Mansori Motlagh B., Jabbari N., *Artif. Cells Nanomed. Biotechnol.*, 2019, **47**:1396 [Crossref], [Google Scholar], [Publisher]
[16]. Azemati F., Jalali Kondori B., Esmaeili Gouvarchin Ghaleh H., *Iran. J. Pharm. Sci.*, 2020, **19**:271 [Crossref], [Google Scholar], [Publisher]
[17]. Galeh H.E.G., Delirezh N., Froushani S.M.A., Ahangaran N.A., *Turk. J. Biol.*, 2014, 6; **38**:365 [Crossref], [Google Scholar], [Publisher]
[18]. Afrin S., Giampieri F., Gasparrini M., Forbes-Hernández T.Y., Cianciosi D., Reboredo-Rodriguez P., Zhang J., Manna P.P., Daglia M., Atanasov A.G., Battino M., *Biotechnol. Adv.*, 2020, **38**:107322 [Crossref], [Google Scholar], [Publisher]
[19]. Dulong J., Kouakou C., Mesloub Y., Rorteau J., Moratille S., Chevalier F.P., Vinasco-Sandoval T., Martin M.T., Lamartine J., *Front. Oncol.*, 2020, **10**:2800 [Crossref], [Google Scholar], [Publisher]
[20]. Mousa A.M., Aziz O.A., Al-Hagar O.E., Gizawy M.A., Allan K.F., Attallah M.F., *Appl. Radiat. Isot.*, 2020, **166**:109389 [Crossref], [Google Scholar], [Publisher]
[21]. Guo L.M., Xu X.M., Zhao D., Cai X.G., Zhou B., *AMB Express*, 2020, **10**:1 [Crossref], [Google Scholar], [Publisher]
[22]. Ali K., Saquib Q., Ahmed B., Siddiqui M.A., Ahmad J., Al-Shaeri M., Al-Khedhairy A.A., Musarrat J., *Process. Biochem.*, 2020, **91**:387 [Crossref], [Google Scholar], [Publisher]
[23]. Kennedy B.E., Sadek M., Gujar S.A., *Mol. Ther.*, 2020, **28**:1417 [Crossref], [Google Scholar], [Publisher]
[24]. Hedayatnasab Z., Dabbagh A., Abnisa F., Daud W.M., *Eur. Polym. J.*, 2020, **20**:109789 [Crossref], [Google Scholar], [Publisher]
[25]. Joel C., Jebakumar D.S., Bennie R.B., Stuart J.G., Raj A., Paul N., David S.T., Redox Mediated Synthesis of Ag-CuO Hybrid Nanoparticles-DNA/BSA Binding Studies and in vitro Evaluation of Anti-cancer Activity on MCF-7 Cancer Cell Line. *Appl. Organomet. Chem.*, 2020, **34**:e5509 [Crossref], [Google Scholar], [Publisher].
[26]. Shafagh M., Rahmani F., Delirezh N., *Iran. J. Basic Med. Sci.*, 2015, **18**:993 [Google Scholar], [Publisher]
[27]. Meng G., Li B., Chen A., Zheng M., Xu T., Zhang H., Dong J., Wu J., Yu D., Wei J., *Br. J. Cancer*, 2020, **122**:111 [Crossref], [Google Scholar], [Publisher]
[28]. Najmuddin S.U.F.S., Amin Z.M., Tan S.W., Yeap S.K., Kalyanasundram J., Veerakumarasivam A., Chan S.C., Chia S.L., Yusoff K., Alitheen N.B., *Peer. J.*, 2020, **8**:e9761 [Crossref], [Google Scholar], [Publisher]
[29]. Al-Zharani M., Qurtam A.A., Daoush W.M., Eisa M.H., Aljarba N.H., Alkahtani S., Nasr F.A., *Environ Sci. Pollut. Res.*, 2021, **28**:1587 [Crossref], [Google Scholar], [Publisher]
[30]. Liu Y., Zeng Z., Jiang O., Li Y., Xu Q., Jiang L., Yu J., Xu D., *Mater. Res. Express.*, 2021, **8**:015011 [Crossref], [Google Scholar], [Publisher]
[31]. Mariadoss A.V.A., Saravanakumar K., Sathiyaseelan A., Venkatachalam K., Wang M.H.,

Int. J. Biol. Macromol., 2020, **164**:2073 [Crossref], [Google Scholar], [Publisher]
[32]. Fahmy H.M., Ebrahim N.M., Gaber M.H. *J. Trace Elem. Med. Biol.*, 2020, **60**:126481 [Crossref], [Google Scholar], [Publisher]
[33]. Ali K., Saquib Q., Ahmed B., Siddiqui M.A., Ahmad J., Al-Shaeri M., Al-Khedhairy A.A., Musarrat J., *Process Biochem.*, 2020, **91**:387 [Crossref], [Google Scholar], [Publisher]
[34]. Lu K.Y., Jheng P.R., Lu L.S., Rethi L., Mi F.L., Chuang E.Y., *Int. J. Biol. Macromol.*, 2020, **166**:98 [Crossref], [Google Scholar], [Publisher]
[35]. Nejad A.S.M., Fotouhi F., Mehrbod P., Keshavarz M., Alikhani M.Y., Ghaemi A., *Microb. Pathog.*, 2020, **147**:104438 [Crossref], [Google Scholar], [Publisher]
[36]. Dwivedi R., Pandey R., Chandra S., Mehrotra D., *Oncol. Rev.*, 2020, **14** [Crossref], [Google Scholar], [Publisher]

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