

Anti-cancer, antiproliferative activity of active anionic H₂O₈ oxygen solution on HCT-116 cancer cell

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Abstract

HCT116 cells are adherent epithelial cells derived from the human colorectal carcinoma cell line commonly used to study inflammatory responses in colonic epithelial cells. In this study, it was aimed to evaluate the effects of active anionic H₂O₈ oxygen solution, which is a very strong antiviral and antimicrobial agent, on HCT-116 human colorectal cancer cell line. Cell viability was determined by MTT analysis. Antiproliferative activity of the anionic H₂O₈ was investigated on HCT 116 (human colorectal carcinoma) cancer cells. Anionic H₂O₈ displayed the outstanding activities for MTT test, IC₅₀= 9.44 for 24th hour was calculated as IC₅₀= 11.73 for 48th hour on HCT 116 cell line. It is thought that it can serve as an agent with strong potential to be used in treatment.

Keywords: Active anionic oxygen; Cancer; HCT 116; Cell; MTT.

1. Introduction

Colorectal cancer is a very common type of cancer affecting the colon and rectum, which usually develops with the spread of polyps in the colon. The serious side effects of drugs used in colorectal cancer cases direct people to develop new treatments. Recently the protection of the tumor cell from the immune system constitute the basic physiopathology and inability to perform apoptosis, inactivation of tumor suppressor genes, activation of oncogenes, deterioration of DNA repair ability, reprogramming of the energy metabolism of the studied cell [1].

The basic chemical compound of anionic oxygen, whose IUPAC name is octaoxidant, contains 2 hydrogen and 8 oxygen molecules, has a molecular weight of 130.01108 g/mol, pH 7.55, density 0.9981 and chemical formula 1S/H₂O₈/c1-3-5-7-8-6-4 -2/h1- is 2H (H₂O₈) (Active Anionic, 2021). It is reported that active anionic octaoxidant liquid oxygen (H₂O₈), which is used to prevent infection, provides the production of biocidal and humoral antioxidant stimulant in the treatment of many purposes, such as eliminating diseases, stopping infectious agents, antiviral, antifungal and many purposes [2].

This study aimed to evaluate the effects of active anionic H₂O₈ oxygen solution on the growth of HCT-116 human colorectal cancer cell line.

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2. Material and methods

2.1. Chemicals

In this study, chemicals of analytical purity were used. McCoy's Medium, trypsin, penicillin, streptomycin, fetal bovine serum (FBS) from American Type Culture Collection (ATCC, Manassas, VA, USA), phosphate buffered saline (PBS) from Sigma (St. Louis, USA) were used in the cell culture step. The product named UB20 08 (Active anionic H₂O₈ oxygen solution) used in the study was supplied by BAYNOVA TECHNOLOGY Joint Stock Company. The product is a solution-shaped product containing active oxygen atoms (8 atomic oxygen molecules) in anionic structure as an active molecule. When the general content analysis information stated by the company of the product is examined, it is seen that Na⁺ 32 mmol/L, K⁺ 16.9 mmol/L, Cl⁻ 35 mmol/L and pO₂ are 158 mmHg and the ORP value (unit of oxidizing and reducing power of the solution) is 960 mV [3].

2.2. Cell culture

The human HCT116 colorectal cancer cell line was purchased from the American Type of Culture Collection (ATCC, Bethesda, MD, USA) CCL-247 LOT: 70009735 (Table 1). Cells were grown in McCoy's 5A medium containing 2mM L-glutamine, 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/ml streptomycin and incubated at 37°C in a CO₂ oven containing 5% CO₂.

2.2.1. Cell passage

The cryo vial containing cells from ATCC was thawed by shaking in a 37°C water bath, transferred to a 15 mL falcon and centrifuged at 1200 rpm for 4 minutes by adding 5 mL of medium. Cell precipitate was obtained at the bottom of the falcon. HCT 116 colorectal cancer cells at the bottom of the falcon were transferred to T-25 cm cell culture flasks, examined under an invert microscope, and incubated at 37°C in a CO₂ oven containing 5% CO₂. When the bottom of the flask was 80%, the medium in the flask was removed. PBS was used to purify cells from serum. After removing the PBS, 2 mL of 0.25% trypsin EDTA was added and left in the incubator for 5 minutes. At the end of this period, it was checked with an invert microscope whether the cells adhered to the flask were separated from the surface or not, and the cells were completely separated from the base by lightly tapping the flask. Adding 5 mL of medium, it was taken into a 15 mL falcon tube and centrifuged at 2500 rpm for 5 minutes, and the supernatant was poured away. 1 mL of medium was added and lightly pipetted. By adding 4 mL of medium to the new flask, the cell medium in the falcon was transferred to the flask. Cells were followed under an inverted microscope and incubated for 24 hours in a CO₂ oven.

Table 1 Details of ATCC HCT 116 cell line

ATCC HCT 116 Cell Line Description	
Organism	Homo sapiens
Tissue	Large intestine; Colon
Age	Adult
Gender	Male
Morphology	Epithelial
Growth properties	Adherent
Disease	Carcinoma; Colorectal

2.3. Determination of cell count with Trypan Blue

While dead cells are stained with trypan blue staining method, living cells are not stained with dye because the cell membranes of living cells are intact, but under invert microscope appears transparent. After the cells covered the flask base by 80%, the medium of the cells was discarded by pulling with the help of a pipette. Afterwards, the flasks were washed with PBS without Ca²⁺ and Mg²⁺, and the remaining proteins in the flask were removed. The cells remaining in the flasks were incubated with 0.25% trypsin EDTA for 5 minutes in an incubator, 4 mL of medium was added, and the supernatant was removed by centrifugation at 1200 rpm at +4 °C for 5 minutes. 1 mL of medium was added and pipetted.

50 μl of cell suspension from eppendorf was taken into a separate 1.5 ml eppendorph, the same volume (50 μl should be in equal volume) of trypan blue (0.4%) was added and pipetted so that it became homogeneous. 10 μl of the cell/trypan blue mixture was pipetted and dropped into the middle of the Thoma slide. The Thoma slide was covered with a coverslip so that no air bubbles remained. The count lines of the Thoma slide placed on the microscope were counted with a 40x objective microscope. Only viable cells that were not stained blue in the 16 squares in the 1 mm² area where the lines intersect in the center were counted (Dead cells are all blue). This process was performed 5 times and the average was taken. The number of viable cells in 1 mL was calculated according to the formula below.

Number of viable cells (cells/mL) = Average number of cells $\times 2 \times 10^5$

Average cell number: It is obtained by dividing the total number of cells $\times 2 \times 10^5$

Average cell number: It is obtained by dividing the total number of cells by 5 after counting 16 squares in 1 mm² area 5 times.

10⁵: Multiply by 10⁵ to find the number of cells per mL, since each 1 mm² area equals 0.1 mm³ in volume, which equals 10⁵ mL.

2: Since an equal amount of trypan blue is added to the sample volume taken, it will be diluted 2 times, so it is multiplied by 2 (If the sample taken for counting is diluted, the result found is multiplied by the final dilution factor).

2.4. Cell viability with MTT

The proliferative or cytotoxic effects of any therapeutic agent on the cell are determined with MTT, which is a method in which the amount of cell proliferation is determined based on the colorimetric measurement of enzymatic activity or formazone dyes depending on the decrease in MTT. The chemical basis of the experiment is based on the reduction of MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide]. The distinctive yellow color of MTT produces a blue formazan color as a result of chemical reduction.

The cytotoxic effect of anionic H2O8 on the HCT116 cell line was performed according to the instructions for use specified in the commercially available MTT kit (Abcam ab211091). The method is based on the principle of colorimetric determination of color change in cells incubated with MTT agent. The resulting discoloration occurs as a result of the reduction of tetrazolium salt in the active cell mitochondria of formazone salts colored with yellow. The absorbance value of these compounds is proportional to the determination of their metabolic activities. Method;

One day before the application of the MTT method, 100 μl of medium was prepared with 1×10^4 cells counted in a 96-well plate and inoculated into the wells. The cells were allowed to adhere to the surface by keeping the microplate in an incubator set at 37°C and 5% CO₂ for 24 hours. The reason for this is that the damage caused by the trypsin enzyme on cell membrane proteins and growth factor receptors can be eliminated, a 24-hour period is required for these proteins and growth factors to be resynthesized, and in this way, the cells gain metabolic activity before the therapeutic agents (anionic H2O8) in the medium are treated with the cell.

Stock solutions of the H2O8 was prepared in medium at different concentrations (2, 5, 10, and 15 $\mu\text{l}/\text{mL}$) for MTT assay. After 24 hours of incubation, H2O8 was added at 2-15 $\mu\text{l}/\text{mL}$ for varying time intervals (3, 24, and 48 h) MTT analysis was performed at the end of the determined periods by applying the determined concentrations.

2.4.1. MTT analysis

The medium in the 96-well plate is carefully emptied and 50 μl of serum free medium and 50 μl of MTT reagent are added to each well. It was incubated in an incubator at 37 °C for varying time intervals (3, 24, and 48 h). After three hours of incubation, 150 μl of MTT solvent was added to each well. It was mixed with foil and mixed in an orbital shaker for 15 minutes. At the end of the incubation, the absorbance value of 590 nm was measured with a microplate reader spectrophotometer (μ -Quant, Epoch BioTek Instruments, Winooski, Vermont, USA). The applied dose and % cell viability curve were determined with the help of Microsoft Excel program, and the 50% inhibitory concentration (IC₅₀) value was calculated with a logarithmic slope graph (Fig. 2).

$$\% \text{ Cytotoxicity: } 100 \times (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}) / \text{Control}$$

2.5. Statistical analysis

Data were analyzed using SPSS software package, version 22.00. Statistical analysis was done by one-way analysis of variance (ANOVA). Post-hoc Tukey's test was used to compare the biochemical parameters between the groups. P values <0.05 were considered as significant. The results are expressed as mean±standard error (SEM) for each group.

2.6. Abbreviations

HCT 116 (human colorectal carcinoma), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), IC 50 (The half-maximal inhibitory concentration)

3. Results and discussion

In order to determine anionic H2O8 sensitivity HCT116 cells were exposed to 2-15 $\mu\text{L}/\text{mL}$ for 3, 24, and 48 h using MTT analysis (Fig. 1). While the viability of HCT 116 cells were 100% at 3, 24 and 48 hours, it was 84.53%, 69.67%, 60.77%, and 50.00% at 24 hours in the groups administered 2, 5, 10 and 15 $\mu\text{L}/\text{mL}$ H2O8, while it was found to be 71.48, 59.63, 46.40 and 40.28 at 48 hours. Respectively. After 24- and 48-h treatment, cell viability decreased significantly in response to H2O8.

The statistics of the data of MTT viability test obtained as a result of the study were made and presented in Figure 1

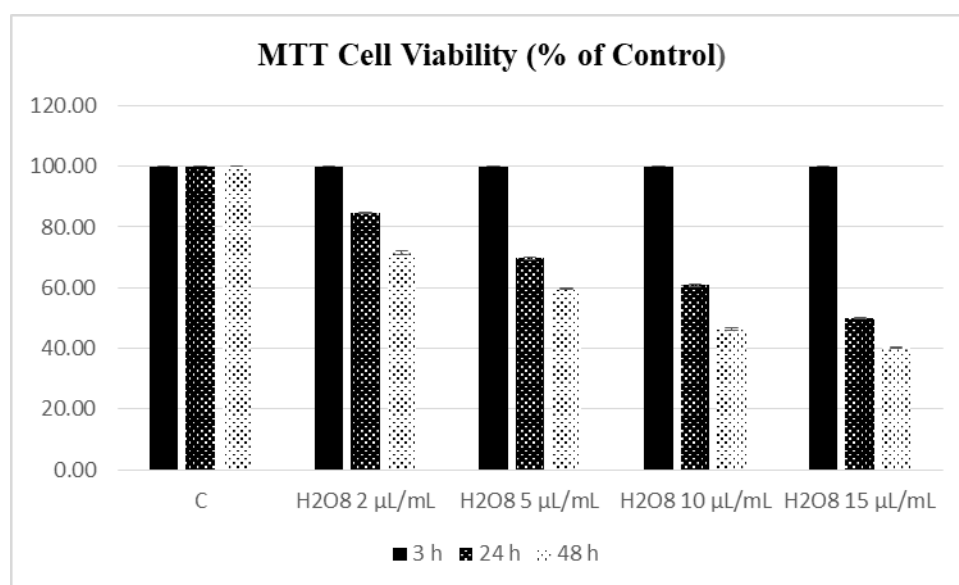


Figure 1 Percentage of cell viability on different concentration of H2O8 against HCT 116 cells using MTT Assay. C: Control (HCT 116 cell), H2O8: Groups treated with anionic H2O8 to HCT 116 cancer cells. P <0.001 were considered as significant

For MTT test, IC₅₀= 9.44 for 24th hour was calculated as IC₅₀= 11.73 for 48th hour (Fig. 2). H2O8 inhibited HCT116 cell growth in a dose and time dependent manner (Fig. 3)

UB20 08; It is an anionic H2O8 oxygen solution with anti-inflammatory, antiviral, antibacterial, antiseptic, antipruritic and antihistamine effects in all living things. By helping to transport oxygen in the circulation, it maximizes the oxygen level in the blood and does not allow infected viruses and bacteria to multiply. It also activates the enzymes superoxide dismutase, dehydrogenase, catalase, and glutathione peroxidase, which protect the organism from free oxygen radicals. It increases protein synthesis in the cell by increasing the amount of ribosomes and mitochondria [3].

Wang et al., 2019 found that Rab27a expression increased in hypoxia, resulting in exosome secretion from G-MDSCs, decreased the number of colon cancer cells by stopping GM-Exo production in respiratory hyperoxia condition, and increased plasma MDSC exosomal S100A9 level in human colorectal cancer patients [4].

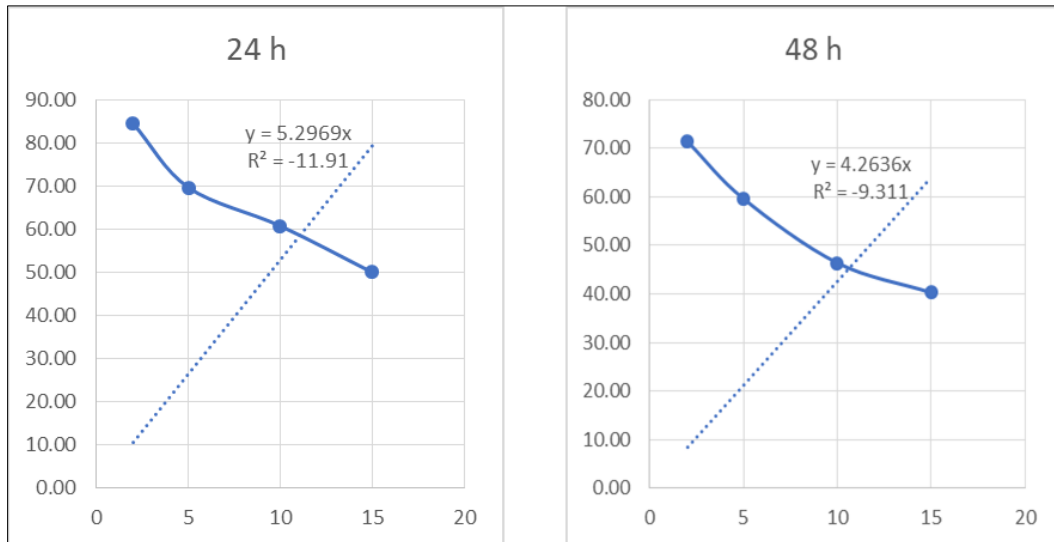


Figure 2 IC50 of H2O8 measured 24 and 48 h after incubation with HCT 116 cell

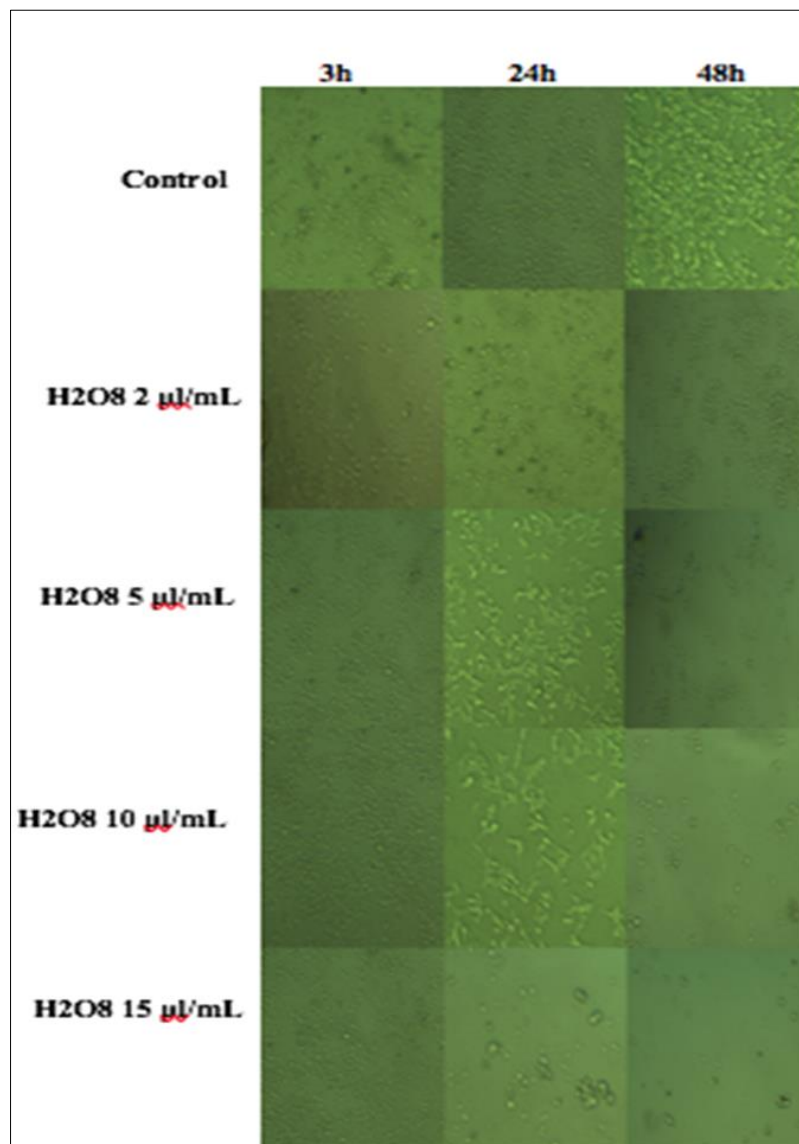


Figure 3 H2O8 inhibited HCT116 cell growth in a dose and time dependent manner

4. Conclusion

As a result, in this study, it was concluded that the anionic H₂O₈ oxygen solution has an antiproliferative effect on HCT 116 colorectal cancer cells, it acts as an agent with a strong potential to be used in the treatment in vitro, and it should be supported by in vivo studies to determine the pathways affected by molecular studies and to determine their effects. Conclusion was reached.

Compliance with ethical standards

Acknowledgments

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References

- [1] Kitagawa Y, Daniel T. Dempsey. Stomach In: Schwartz SI, ed. Principles of Surgery. Seventh ed. New York: McGraw-Hill International Inc. 2015; 1035- 98.
- [2] Ugur Bursali, Hakan Kusbaygi, Erdal Can Alkoclar, A Biocidal Active Anionic H₂O₈ Oxygen Solution with Bronchodilative & Oxygenizing Features for the Treatment of Coronaviral Infection Related Hypoxia. Journal of Design and Science. 2020.
- [3] Active Anionic Octaoxidant Liquefied Oxygen Solution with Ultra Biocide Enzyme. 2021.
- [4] Wang Y, Yin K, Tian, J, Xia X, Ma J, Tang X, Wang S. Granulocytic Myeloid-Derived suppressor cells promote the stemness of colorectal cancer cells through exosomal S100A9. Advanced Science. 2019; 6(18): 1901278.