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Anti-Cancer Effect of Cephalexin Loaded on Nano chitosan Against Humane Liver Cancer

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Abstract A beta-lactam antibiotic, such as cephalexin, is frequently prescribed to treat bacterial infections. In a particular investigation, cephalexin's effectiveness was assessed when it was administered ex vivo, or outside of a living organism, to the HepG2 human liver cancer cell line subsequent to its loading onto Nano chitosan. The purpose of the study was to determine how useful cephalexin would be in this situation. Positive study results showed that the drug worked well in this particular application. The ability of cephalexin to activate p53, a protein that is essential for starting the process of programmed cell death in liver cancer cells, was discovered. This shows that cephalexin, especially in combination with Nano chitosan, may be useful in the treatment of liver cancer.
Overall, the study showed that cephalexin has the ability to target liver cancer cells and activate pathways that may result in their demise. Nevertheless, more investigation and clinical testing would be required to properly evaluate the safety and effectiveness of using cephalexin in this situation.

Key Words liver cancer, cephalexin, nano chitosan

1. Introduction

Cancer is an extremely contagious disease that kills people and is typified by unrestricted cell growth. In the world, it ranks as the second cause of death [1]–[3]. Mutations caused by genetic predisposition increase the likelihood of infection, which is how cancer starts: deoxyribonucleic acid (DNA) gets exposed to harmful substances including chemicals and UV radiation [3]. Depending on the organ it invades, cancer can take many different forms. Examples include lung, brain, and blood cancer. Liver cancer, which also appears in a variety of forms, is among the most severe. Hepatocellular carcinoma is the most prevalent and deadly type. In actuality, a variety of factors, including alcohol consumption, contribute to liver carcinogenesis.

Aflatoxin exposure and obesity are the two main factors that contribute to this cancer's deadly nature. Because the condition is marked by a lack of symptoms, it was not discovered until much later, which makes treatment extremely challenging [4], [5]. Promising new technologies have recently surfaced that specifically target liver cancer cells. One such technique is nanotechnology, particularly Nanochitosan, which is made from chitin, which is found in marine crustaceans' outer shells. In addition to its bioavailability, this chemical possesses anti-cancer capabilities that enhance the therapeutic effects of medications when applied [6].

It was a unique candidate for having antibiotics loaded on it because of this. Antibiotics have been shown to be beneficial in the fight against cancer; however, the problem lies in the fact that these beneficial concentrations are lost during route to the cancer cells. In this case, chitosan's function to resolve the issue arose [7], [8]. The beta-lactam class of antibiotics, which has a lactam ring that prevents the formation of the bacterial wall, is one of those that has been shown to have anti-malignant qualities. Additionally, it was discovered that the tumor suppressor gene TP53, which is thought to have lost its function and is found on the short arm of the human seventeenth chromosome, was stimulated by chitosan and beta-lactam antibiotics. It is strongly associated with the incidence of many malignancies, and cancer cells can be eliminated by activating and targeting it [9], [10].

2. Material and Methods

A. Preparation of HepG2 Hepatocellular Carcinoma

HepG2 human liver cancer cells were melted in a 37°C water bath and then added to a specific 25 cm-diameter culture container with 96 holes (Santacruz Biotechnology

USA). The cancer cells were then cleaned using one milliliter of phosphate buffer solution. Following that, the Capricorn Company-prepared medium (RPMI-1640) was used to preserve the human liver cancer cells (HepG2). Germany), after which the cells were kept in an incubator, set to 37°C and 5% CO2. After that, an inverted microscope was used to check the HepG2 cells' viability and collect the necessary amount between (500 and 800 cells/ml) to be then the culture media was disposed of by pouring it into a glass container within the sterile culture cabinet. The cancer cells were then twice-washed for ten minutes with PBS saline solution, with the saline solution being disposed of after each wash. A quantity of trypsin enzyme was added, and the cells were treated for 30 to 60 seconds at 37°C to produce monolayer cells. However, bovine calf serum was added to block the enzyme's activity. Subsequently, the cancer cells were centrifuged at 2000 rpm for 10 minutes in order to remove the trypsin enzyme and the growth media. The cancer cells were suspended by adding culture medium supplemented with 10% bovine calf serum [10], following the removal of the filtrate.

B. Detection of Morphological Changes

Using an inverted microscope (100X), morphological alterations of HePG2 cancer cells were seen after they were cultured for 24 hours at 37°C in a plate with 24 holes at a density of (1×105 cells ml-1). Subsequently, the cells were split into two groups, one of which received control treatment and were not given the medication. Cephalexin that was placed onto Nano-chitosan was administered to the other group. It was then stained with crystal violet dye, allowed to sit for ten to fifteen minutes, and then washed under tap water to get rid of the dye. Using a digital camera mounted on a microscope, the cells were inspected and taken pictures of [11]–[13].

C. Acridine Orange/Ethidium Bromide Staining (AO/EtBr) Assay

Using a combination of two dyes (5 mg of acridine dye and 3 mg of ethidium) (Sigma-Aldrich USA) [14], HepG2 cells were cultivated in a 96-well plate and incubated for 24 hours to perform a two-stain test to determine the viability of the cells. Subsequently, one set of cells was treated as the control group while the other was exposed to a portion of the drug cephalexin loaded on chitosan for 24 hours at a concentration of half the dose (IC50 = 59.07 µg/ml). The last step was dying the cells for two minutes at 37°C using a combination of the two dyes (AO/EtBr). Under a fluorescence microscope, it was investigated [15].

3. Results and Discussion

A. Detection of Morphological Changes

Crystal violet dye is one tool used to find phenotypic alterations in HepG2 cancer cells. The group of cells that were not exposed to the medication is displayed in Figure 1, which displays the results that were achieved. The clear dye in Figure 2 indicates that the cells were treated with Nano-chitosan.

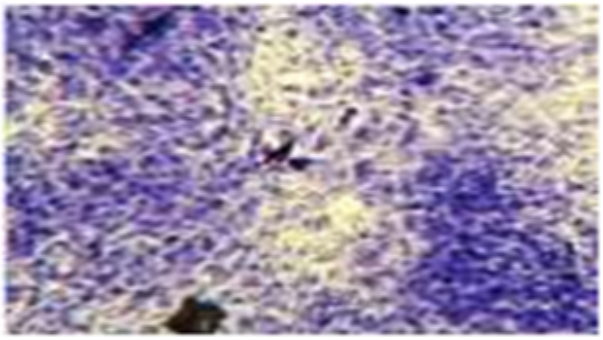

Figure 1: HepG2 Un treated With the Drug

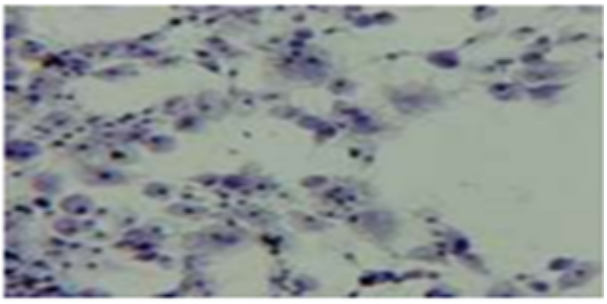

Figure 2: Morphological changes in HepG2 cells after been treated with Chitosan-Cephalexin

The ability to distinguish the borders of cancer cells suggests that they are in good condition and have not undergone any observable morphological alterations. On the other hand, cells that have initiated the programmed death process exhibit diffused and faded dye, making it difficult to differentiate cell boundaries, a sign of membrane damage. Additionally, we see gaps between cells as a result of cytoplasmic shrinkage [16]. This is in line with our findings, which demonstrate how well the antibiotics placed onto the Nano -chitosan worked.

B. Acridine Orange/Ethidium Bromide Staining (AO/EtBr) Assay

Changes in the nuclei of HepG2 cancer cells treated with Nano-cephalexin were observed using a combination of ethidium bromide and acridine orange dyes. Figures 3 and 4 display the outcomes we were able to acquire for the control group and liver cancer cells treated with Nano-chitosan, respectively. The integrity of the mitochondrial membrane is essential to the test's premise. Dead cells are stained orange, but the membranes of healthy cells selectively absorb the green hue of the dye. The cells are in a more advanced stage of programmed death when the color is darker [17]. This is in line with the outcomes that we saw. Numerous experts discovered that cancer cells are more susceptible to newly discovered classes of antibiotics than their treated counterparts. This means that the antibiotics are more effective in inhibiting cancer, especially in liver cancer cells, even when they are targeted. Antibiotics suppress it even in the

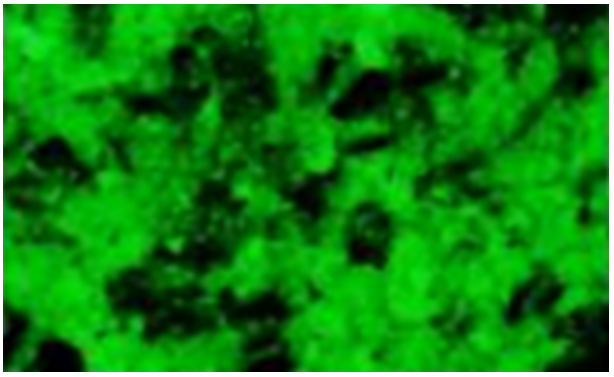

Figure 3: HepG2 hepatocellular carcinoma cells not treated with the drug

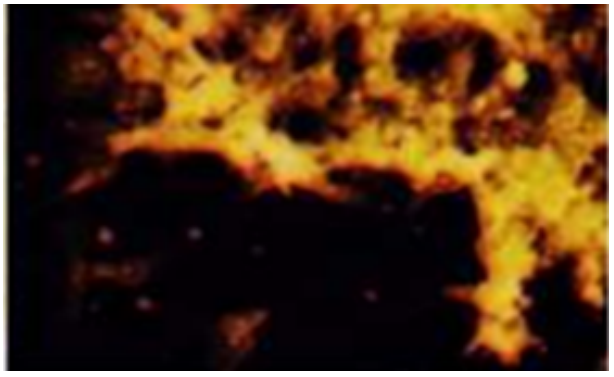

Figure 4: HepG2 hepatocellular carcinoma cells treated with cephalexin loaded on Nano chitosan

G0 dormant phase, when it enters and continues to spread. This is achieved by increasing the tumor protein P53, which influences P21 and activates cyclin B, which in turn inhibits Cde2 [18].

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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