Effect of Green (520 nm)Light in the Photodynamic Therapy of HCT Colon Cancer Cells

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Abstract

Background
Colon cancer comes under first most common cancer in the world. It is a very deadly disease. Various chemotherapy drugs are there to treat the cancer. But the drawbacks of chemotherapy are well known and discussed. To overcome the side effects and cost of the treatment, the attention is now given to many other areas of treatment modalities.

Objective
To study effect of green light in photodynamic therapy of HCT-116 colon cancer and compare it with standard chemotherapeutic drug cisplatin.

Methods
Dose response study (MTT assay) of cisplatin in HCT-116 cell line was carried out and effect of that dose on normal cell line HEK-293 was checked. The dose response of green light (520 nm) was carried out in HCT-116 and effect of the same was checked on normal cell line.

Results
The IC50 of cisplatin after 48 hours of treatment was 19µM. At this concentration the viability of HEK-293 was 34%. The MTT of green light showed IC50 of 15 min treatment for HCT-116 and HEK-293 was found to be safe at this time interval.

Conclusion

Cisplatin is very toxic for normal cells as we all know. Our treatment modality of green light PDT stands out in future of treatment for colon cancer. Further work can be done using green light along with phytochemicals to make treatment better for metastasis state of the cancer.

Keywords: *Green Light, PDT, Colon cancer, Cisplatin, MTT assay.*

1. Introduction

Colon cancer is the most common cancer in men (3rd commonly) and women (2nd commonly). Worldwide colon cancer accounts for 10% of all other cancers. In different countries the incidence rates tend to differ and is mainly 25% found to be higher in males. Annually 6,00,000 deaths have been estimated which makes colon cancer the 4th most common death causing cancer globally.[1,2]Lifestyle of westernization, obesity, alcohol consumption, smoking and physical inactivity is the growing cause of increasing incidence case of colon cancer.[3] Some data also shows effect due to imbalance in microbial flora of the gut.[4-6]

Many new tests have been developed like genomic profile of tumor and tumor DNA test [7-11] but staging of cancer can only help to diagnose the cancer as well as indicates the recurrence of the cancer.[12] Stage 1 disease includes surgery and no use of adjuvant chemotherapy. Stage 2 and Stage 3 includes surgery and combination of adjuvant chemotherapy.[13-15] Cisplatin is the like a platinum treatment for many solid tumors, but the side effects of it makes it less pursuable. Many alternate therapies are under development for colon cancer. One of it is the light therapy or phototherapy which we propose to use in this present study.

Photodynamic therapy (PDT) was proposed in 1970. In comparison to radiation and chemotherapy, PDT is considered to be latest therapy. After the introduction of PDT, it has been well occupied in treating various cancers like breast, lung, colon and melanoma with fewer invasive effects.[16] When compared to conventional therapies used for treating cancer, like chemotherapy, radiation therapy and surgery, PDT is more particularly selective and is less toxic with fewer or no effect on normal cells.[17] PDT includes three factors namely, light source, oxygen and photosensitizers (chemical agent sensitive to light). The light source can be laser light or halogen light. The photosensitizers used are sometimes more costly and shows fewer disadvantages such as phototoxicity, less sensitivity towards light and restricted to few tumors.[18-20] This leads to explore cheaper and easily available sources. Green light (520nm) has been used in cosmetic application to photosensitize IAA to treat acne.[21,22] So, referring to the disadvantages of costly photosensitizers, we decided to use green light only to check its effect on colon cancer without any commercial photosensitizers and check its effect against normal cells too. The aim of our study was to increase the effect of light-based cancer therapy against colon cancer using green light as PDT.

2. Materials and Method

2.1 Chemicals and reagents

Cisplatin was obtained from CelonLabs, India (CelonLabs, CSI2167BC), Dulbecco's Modified Eagle Medium (DMEM), (Thermo, 10569010), Anti-Anti (Penicilin/ Streptomycin/ Amphotericin B), (Thermo, 15240096), Gentamycin (Thermo, 15710064), Fetal Bovin Serum (FBS), (Thermo, 10270106), 0.25% Trypsin EDTA (Thermo, 25200056), Phosphate Buffer Saline (PBS), (HIMEDIA, TS-1006) and green light (7 color LED mask. WBC, Haryana, ASIN: B09NYP24F9) were used in the study.

2.2Cell culture

HCT-116 (Human Colon Cancer Cell line) and HEK-293 (Human embryo kidney cell line) were obtained from National Centre for Cell Science (NCCS), Pune. HCT-116 and HEK-293 cell lines were maintained and cultured in DMEM complemented with 10% FBS, 0.2% Anti-Anti (10,000 unit of Penicillin, 10,000 µg/ml Streptomycin and 25 µg/ml Amphotericin B) and 0.1% gentamycin and were incubated at 37ºC in 5% CO_2. When the cells in the culture flask were 80% confluent, the spent media were discarded, cells were washed with PBS and detached with 0.25% trypsin EDTA.

2.4 Dose response analysis of Cisplatin

The cells were seeded in 96-well plate and were left to adhere overnight. The next day a working solution of 0.3 mM of cisplatin was prepared in DMEM media. For dose-dependent analysis, the working solution was administered to the cells with different concentration of Cisplatin (7.5 –100 µM) for 48 hours. After 48 hours MTT was carried out to find the inhibitory concentration (IC50) of the cisplatin.

2.5 Effect of cisplatin IC50 on normal cells

The same range of concentration of cisplatin was used for HEK-293 cells to check the toxic effect of cisplatin. The cells were treated for 48 hours and then MTT was carried out.

2.5 Dose response analysis of green light (520nm)

The light source used in the experiment was seven color LED face mask (World Beauty Care, Haryana) with a green light (500-520 nm).[22] The cells were seeded in 96-well plates and were left to adhere overnight. The next day cells were treated with 5 ,1 10, 15 and 20 minutes of green light in separate plates. Further the cells were incubated for 24 hours and MTT was carried out the next day to check the cytotoxicity of the cells. Normal cell line HEK-293 was also used to check the cytotoxicity of PDT.

2.6 Statistical analysis

All the experiments were carried out in triplicates and statistically analyzed using one way ANOVA test in SPSS software. The values were represented as mean+ standard error with significance value less than 0.05.

3. Results

3.1 Dose response study of cisplatin

HCT-116 cells and HEK-23 were treated with different doses of cisplatin for 48 hours. The morphological changes were photographed using inverted microscope after treatment. Figure 1 and 2 shows morphological changes in HCT-116 and HEK -293 cells after 48 hours of treatment with cisplatin.

The IC50 for HCT-116 cells treated for 48 hours was 19 µM. The viability of normal cells HEK-293 was 34% only (Figure 3 and Figure 4). All the experiments were carried out in triplicates.

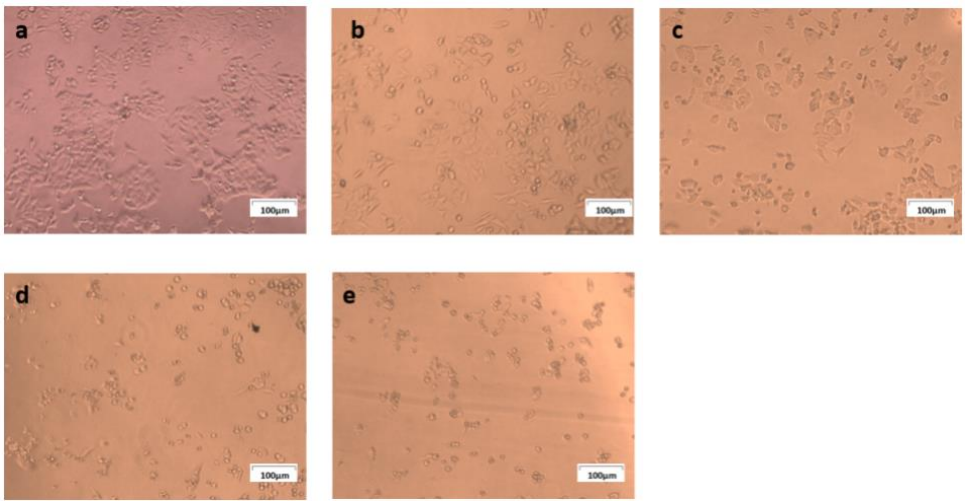

Figure 1: Morphological changes of HCT-116 by cisplatin treatment in different doses. Where, (a) are untreated cells; (b-e) are cells treated with 12.5, 25, 50 and 100 µM of cisplatin, respectively. (20X magnification).

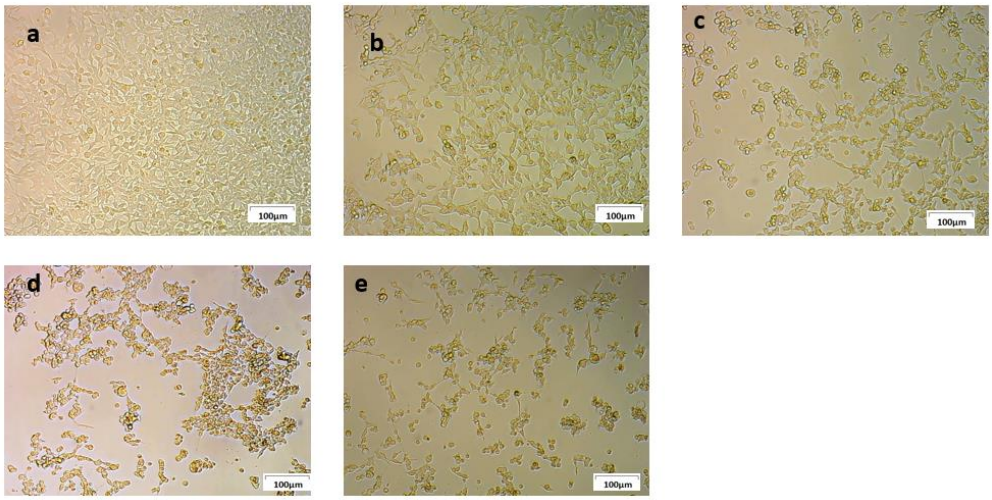

Figure 2: Morphological changes of HEK-293 by cisplatin treatment in different doses. Where, (a) are untreated cells; (b-e) are cells treated with 12.5, 25, 50 and 100 µM of cisplatin, respectively (20X magnification).

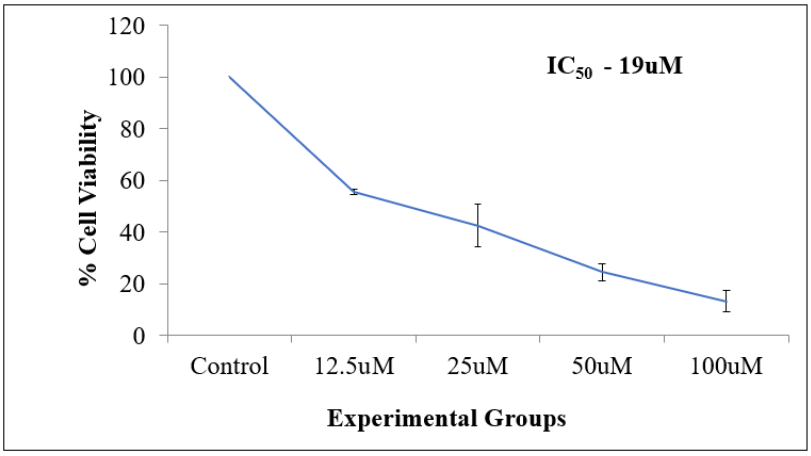

Figure 3: Effect of different doses of cisplatin on the percentage viability of HCT-116 colon cancer Cells after 48 hours of treatment. Values were presented as mean±SD from independent triplicate experiments.

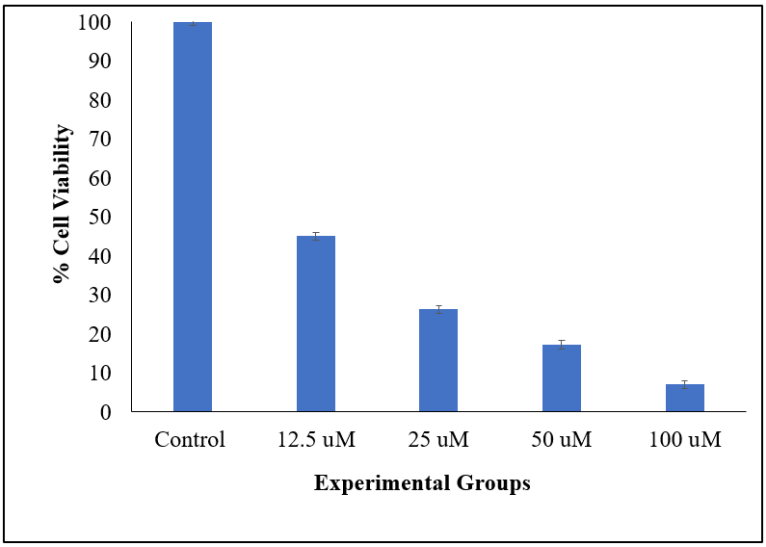

Figure 4: Effect of different doses of cisplatin on the percentage viability of HEK-293 human embryo kidney Cells after 48 hours of treatment. Values were presented as mean±SD from independent triplicate experiments.

3.2 Dose response study of green light, 520nm

HCT-116 cells and HEK-23 were treated with different green light for 5, 10, 15, and 20 minutes. Figure 5 shows morphological changes in HCT-116 after 24 hours of incubation. HEK-293 showed no visible morphological changes.The IC50 for HCT -116 cells was 15 minutes. The normal cells HEK-293 was least effected at this time interval. (Figure 6 and Figure 7). All the experiments were carried out in triplicates.

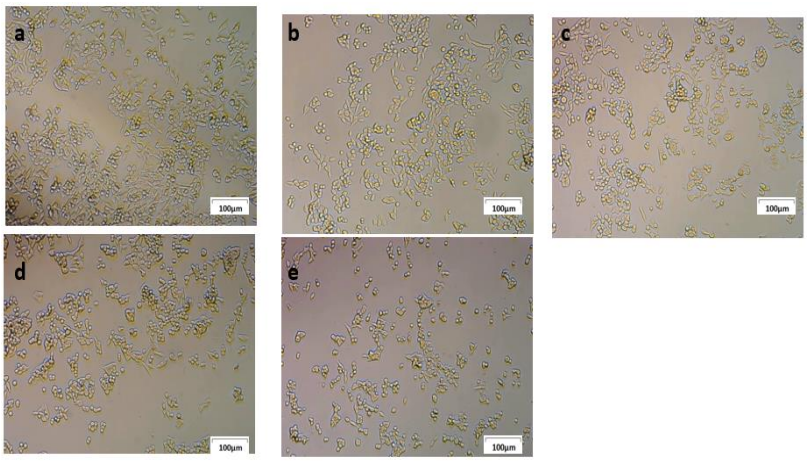

Figure 5: Morphological changes of HCT-116 by green light treatment for different time intervals. Where, (a) are untreated cells; (b-e) are cells treated for 5, 10, 15 and 20 minutes of green light, respectively (20X magnification).

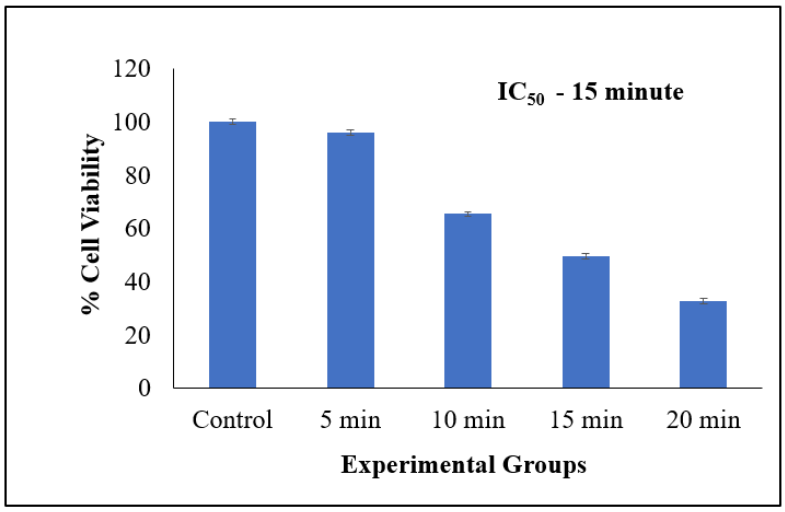

Figure 6: Effect of different doses of cisplatin on the percentage viability of HCT 116cancer cells after green light treatment for different time intervals. There was significant difference between control and treated group. Values were presented as mean±SD from independent triplicate experiments.

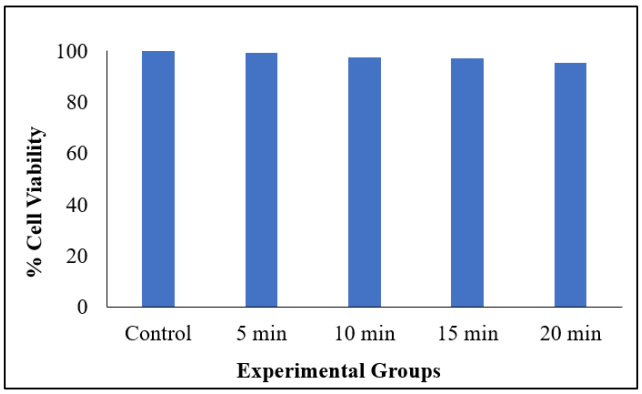

Figure 7: Effect of different doses of cisplatin on the percentage viability of HEK-293 cells after green light treatment for different time intervals.

4. Discussion

Cancer pathological process is very complex and includes numerous mechanisms.[23] There have been many advancements against cancer in surgery, radiation and chemotherapy area.[24] However, the side effects of this conventional therapies and the cost of treatment has never been reduced. Therefore, there has been a need to develop, characterize and study new anticancer formulations with low price and toxicity for patient and high efficiency towards cancer.[25-27]

Advances in colon cancer chemotherapy are limited mainly to the accepted or FDA approved drugs. These drugs have been used since long time for treating colon cancer.[15] Many other treatment modalities have been worked on but the cost and side effects are difficult to decrease. So, our try was to find something cheaper and safer treatment for treating colon cancer.

In our study we used cisplatin as a reference drug and studied its dose repose in HCT-116. Though the dose response study was carried out earlier we still did it so we can check the reproducibility of the cited data in our lab environment and it also helped us to decide the dose range for normal cells. The IC50 of cisplatin was 19 µM matched the cited data.[15]The viability of normal cell at this particular dosage was only 34% which indicates that while 50% of the cancer cells will be dead, the normal cells will be approximately 70% dead. This shows that cisplatin is highly toxic to the normal cells.

We then carried out green light PDT dose response. The colon cancer cells showed 50% death at 15 minutes exposure. The normal cells showed least or almost no effect at this time interval. The advantage of the study is that the light source used was the cheapest and is also used for cosmetic purposes. The simple light if can show this much of advanced effect then by developing a pure green light standard source will help even more to strengthen the effect of the treatment.

5. Conclusion

Our aim of the study was to find an alternative replacement of conventional therapies like chemotherapy and radiation therapy using PDT for the treatment of colon cancer was achieved successfully. Green light shows a promising mode of treatment for colon cancer. All the components used in the treatment are cheaper as well as easily available. Moreover, if green light is used in conjugate with the phytochemicals conjugated to hybrid nanoparticle, then there will be even more significant help in addition of drug which furtherhelp in designing more efficient and effective treatment for colon cancer patient.

6. References

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