



# **Inhibitory Potential of Combination of Macrolide Antibiotic with Conventional Chemotherapeutic Agent Sorafenib on Growth Rate of Cancer Cell Population**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Over the past few years great progress has been achieved in anticancer therapy, but development of resistance and unavoidable side effects have incapacitated these fulfillments. Keeping in view this demanding condition, numerous drugs with unique antitumor mechanisms are under investigations including antimicrobials which have been shown to possess anti-inflammatory, immunomodulatory and cytotoxic effects. In this regard, both conventional and novel antimicrobials are being studied to explore their anticancer potential along with underlying mechanisms which may render them as effective anticancer drugs in near future. Moreover, the new approach of drug repurposing is also being encouraged especially in cancers in order to reduce cost and limit

adverse effects. In recent times a cumulative number of studies have laid stress upon the antitumor properties of antimicrobials. Consequently, this study has been conducted to see comparative inhibitory effect of Sorafenib and its combination with a macrolide antibiotic Azithromycin on growth rate HepG2 cell line.

*Keywords: Macrolide; anticancer; inhibitory potential.*

## 1. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the familiar cancers around the globe responsible for the highest rate of incidence in the region of East Asia and Africa [1]. It stands as sixth most common malignancy worldwide [2] but due to poor prognosis it is third leading cause of cancer related mortality around the world [3] with adenocarcinoma of liver being the commonest type [4]. Presently few drugs are available for managing HCC beside surgical or radiological interventions which improve median survival rate for few months, among which Sorafenib is the only FDA approved drug [5]. Uptill now it is the only approved drug which progresses the overall median survival in liver cancer patients [4]. As cited by Intaraprasong et al in 2016, the median survival rate was enhanced from 7.9% to 10.7% in 602 patients suffering from HCC who received Sorafenib in comparison with placebo group [6] In this context researches are not only being conducted on natural herbs but also on well-known drugs which are previously approved for other illnesses, a phenomenon defined as drug repositioning which is searching for new uses of existing drugs [7]. Several drugs like metformin and paracetamol revealed remarkable antiproliferative potential in certain in vitro studies [8]. This strategy with a cost-effective way offers a rare opportunity for the treatment of human neoplastic disease, facilitating rapid clinical translation. With an increased understanding of the hallmarks of cancer and the development of various data-driven approaches, drug repurposing further promotes the holistic productivity of drug discovery [9]. In this study, we are aiming to demonstrate the comparative potential of anticancer drug Sorafenib and its combination with a macrolide antibiotic, Azithromycin as a cell growth inhibitor of cancer cells in hepatoma cell line.

## 2. MATERIALS AND METHODS

### 2.1 Drug Preparation and Optimization

Sorafenib and Azithromycin were purchased from Med Chem Express (USA). Both drugs

were dissolved in 100% DMSO in order to make their stock concentrations followed by their storage at -20°C. Then the working concentrations were freshly prepared from the respective stock solutions by dissolving them in Dulbecco modified Eagle medium (DMEM).

An optimized MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed for cytotoxicity analysis and the IC<sub>50</sub> concentrations of Sorafenib and combination of Sorafenib with Azithromycin were obtained.

### 2.2 Cell Cultures of HepG2

Human hepatocellular cell line (HepG2) cells were cultured in T75 flask in DMEM (Sigma Chemicals) supplemented with 1% penicillin and streptomycin 1%, 1% L-glutamine and 10% FBS in humidified atmosphere at 37°C containing 5% CO<sub>2</sub>. When cells achieved 80% confluency, they were detached using 0.05% trypsin. 6-well plates were used for the main experiment, cells were seeded into them in a concentration of  $1.2 \times 10^6$  cells/well in triplicate after which morphological analysis and cell counting were performed for Sorafenib and its combination with Azithromycin at their IC<sub>50</sub> concentrations at different time intervals. The IC<sub>50</sub> concentrations obtained after MTT came out to be 1.5 µg/ml for Sorafenib while for its combination with Azithromycin is 1.01 µg/ml.

### 2.3 Cell Morphology

Cells were observed for morphological changes under the effect of Sorafenib and combination treatment under inverted phase microscope after 24 and 48 hours after treatment at their IC<sub>50</sub> concentrations.

### 2.4 Cell Counting

For performing cell count, cells were trypsinized and cell count formula was used to count cells at 48 hours post treatment. Cell counting was performed in Neubauer counting chamber where the mixture of 10 µl of trypan blue dye and 10 µl of re-suspended cells were loaded. Finally, cell

counting was done by observing the cells under inverted microscope. The number of cells counted is the sum of all cells counted across squares in one chamber. And the final count is derived with the help of following formula:

$$\text{Cell count} = 2 * \text{Number of cells in a chamber} * 10^4$$

## 2.5 Statistical Analysis

Data was analyzed via SPSS version 20. All numerical values were presented as mean  $\pm$  S.E of mean (SEM). The mean and SEM of the groups was generated by ANOVA (Analysis of variance). Tukey's post hoc tests was applied to find comparison among the groups. The significant difference between and within the treatment groups was considered significant at set P-value < 0.05

## 3. RESULTS

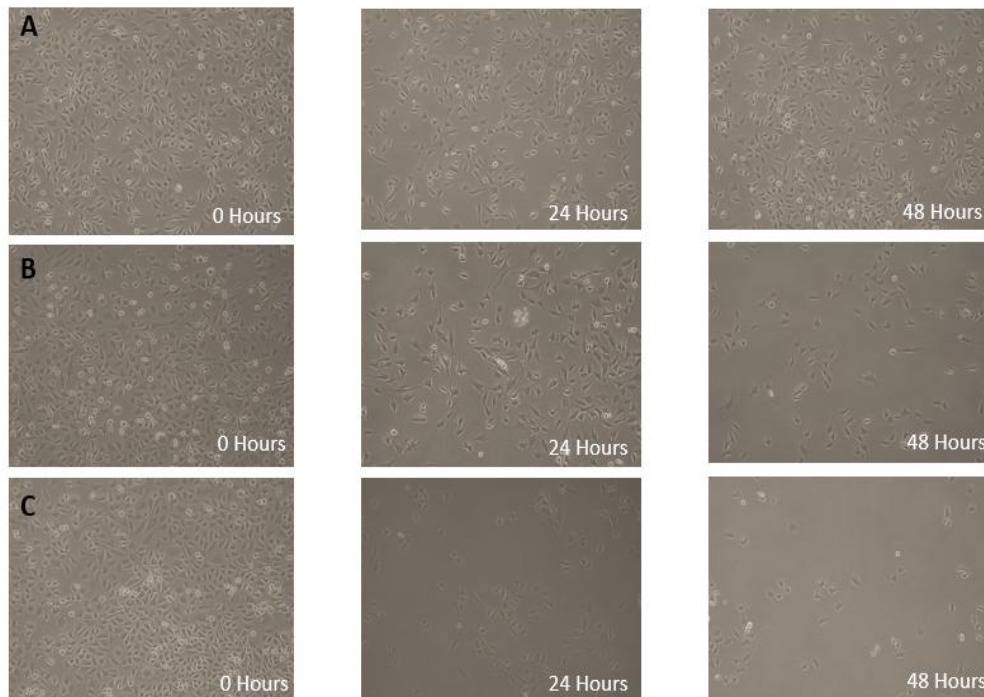
### 3.1 Effect of Sorafenib and Combination Treatment on Morphology

The effect of Sorafenib and combination treatment on morphology of HepG2 cells was

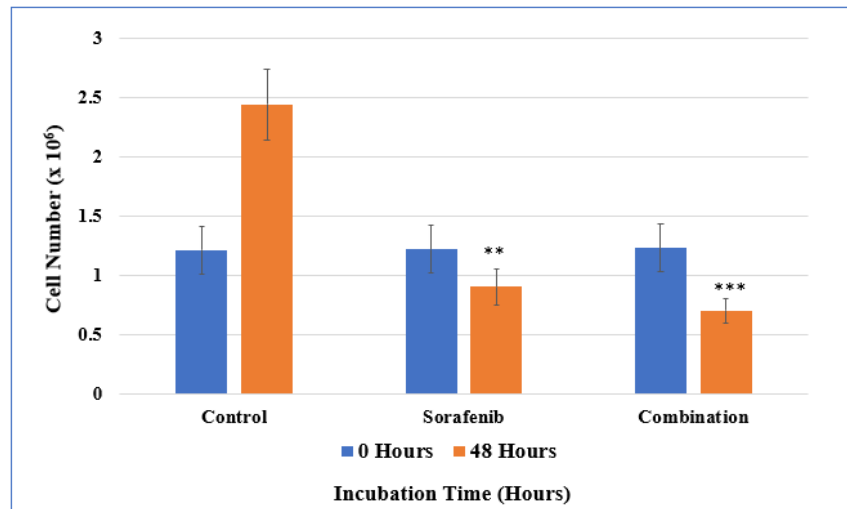
studied at their respective IC<sub>50</sub> concentrations using inverted phase microscope at 0, 24 and 48 hours of treatment. The control (untreated) group showed increased confluency after 48 hours of incubation. The Sorafenib (B) and combination (C) treated group showed significant morphological changes with decreasing cell count compare to normal untreated control group (A) when tested on different time intervals.

### 3.2 Effect of Sorafenib and Combination Treatment on Proliferation Rate

The effect of treatment groups, Sorafenib and Combination at their respective IC<sub>50</sub> concentrations was tested on proliferation rate of HepG2, at 0 and 48 hrs. In the control (untreated) group it is seen that the cell population reaches double after 48 hours of incubation while cell population has decreased under the effect of treatment. Both Sorafenib and combination groups showed highly significant reduction in cell number with p-value (<0.01) for Sorafenib and p-value (< 0.001) for combination group as compare to untreated (control) group after 48 hours of treatment.



**Fig. 1. Morphological examination of HepG2 cell line Control group (A), Sorafenib treated (B) and Combination treated (C) groups observed under inverted microscope. Images were taken at 0, 24 and 48 hours post treatment at 40X magnification**



**Fig. 2. Comparative effects of Sorafenib and combination treatments on proliferation rate of HepG2 cells at 0 and 48 hours of treatment**

(\* = *p*-value <0.05, \*\* = *p*-value <0.01, \*\*\* = *p*-value <0.001)

(\* = significant, \*\* = highly significant, \*\*\* = very highly significant)

(Experiments were run in triplicates, data represented as mean ± SEM)

#### 4. DISCUSSION

In our study, a macrolide antibiotic Azithromycin has been carefully chosen over other macrolides owing to its distinct pharmacokinetic profile and pronounced anti-inflammatory [10], anti-proliferative as well as immunomodulatory role more than other members of macrolide group [11].

In order to see the comparative reducing effect of our treatment groups on cancer cell growing population, cells were seeded into 6-well plate in a concentration of  $1.2 \times 10^6$  cells/well in triplicate for each of the untreated (control) and treated groups (Sorafenib and combination). Our study showed that the count became double in number i.e., ( $2.44 \times 10^6$ ) at 48 hours (Figure 2) after seeding  $1.2 \times 10^6$  number of cells in 6-well plate which is also found to be consistent with other studies [12]. This number had the highest viability and live cells, but the lowest dead cells which means that this number of cells is enough to communicate each other and access the medium. Indeed if the number of cells is a lot, they will be destroyed [13]. On the other hand the treated groups showed significant decrease in cell count for both treated groups such as Sorafenib ( $0.9 \times 10^6$ ) with *p*-value (<0.01) and combination ( $0.75 \times 10^6$ ) with *p*-value (<0.001) after 48 hours of treatment (Figure 2). We also observe morphology of the untreated (control) and treated (Sorafenib and combination) groups

which showed morphological alterations along with decrease in cell number in both the treated groups when compared to untreated control (Fig. 1).

Recently combination therapy of chemotherapeutic agents with novel drugs are being considered for targeting cancer inducing and sustaining pathways, based on the phenomenon of drug repositioning which is an efficient approach, suggesting the use of FDA approved drugs for the treatment of cancer having known pharmacokinetic and pharmacodynamic profiles for other diseases. Recently Acetazolamide [15], Metformin [16], Macrolides [17], have been tailored for treating certain cancers not merely as solo agent but as adjuvant too.

Macrolides are natural/synthetic antimicrobials that retard bacterial growth by inhibiting protein synthesis after binding to 50S ribosomal subunit [18]. They have been evaluated for their anti-inflammatory [19], immunomodulatory [20] and anticancer properties [21]. For later effects macrolides are believed to inhibit the over expression of matrix metalloproteinase-9 which is considered to play role in tumorigenesis of hepatoma via downregulation of apoptotic proteins. Macrolides such as Azithromycin, Clarithromycin, Erythromycin have been evaluated for their inhibitory effect on some HepG2 cell line and chemically induced

hepatocarcinogenesis model in rats. Among macrolides, Clarithromycin demonstrated substantial decline in anti-apoptotic proteins and marked reduction in serum TNF-alpha after 17 weeks of treatment with either Clarithromycin or Azithromycin when compare to control group. Furthermore, cytotoxic analysis revealed that HepG2 treated with clarithromycin showed cytotoxicity of 24%, 23%, 28% and 29% at concentrations of 5, 12.5, 25 and 50 µg/ml respectively, while azithromycin at the concentration of 50 µg/ml showed 29% [22].

## 5. CONCLUSION

HCC is known for its aggressiveness and treatment resistance, therefore the recognition of antimicrobials with anticancer properties is a novel approach that may offer better prospect for the management of this cancer. Combining Sorafenib with Azithromycin has reduced the cytotoxic dose of Sorafenib, thus exhibited enhanced inhibitory potential of this combination on cancer cell population.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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