 DOI: 10.21608/adjalexu.2022.273516

Therapy-Induced Senescence: A Promising Treatment Modality Using Imiquimod in Oral Squamous Cell Carcinoma (An Invitro Study)

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a malignant tumor that accounts for 90% of all oral cancers with less than 50% survival rate. Traditional therapy methods such as chemotherapy rely on cytotoxic strategies that cause severe side effects (1). Therefore, cytostasis or cell arrest has been introduced as an alternative modality with fewer and less severe side effects (2). Cellular senescence is an irreversible cell cycle arrest characterized by distinct numerous cellular phenotypic changes. A canonical criterion for senescence is the increased activity of the senescence associated β-galactosidase enzyme (SA-β-gal). Immortal cancer cells can undergo senescence in response to several stresses induced by a wide variety of cancer therapeutics. This variant of senescence is termed, Therapy-Induced Senescence (TIS) (3). Imiquimod is an FDA-approved immunotherapeutic drug that acts through the activation of toll like receptor-7 (4). Upon triggering, signaling not only leads to programmed cell death, but it also leads to the induction of senescence against carcinogenesis (5). It is suggested that TIS may be valued as a novel alternative cytostatic strategy in the battle against cancer (3).

METHODOLOGY

1- Cytotoxicity assay: In this study, the purpose is to reach a sub- IC50 dose instead of the IC50 to allow senescence induction. To establish the optimum working dose, various concentrations of Imiquimod were selected. In a 96 well plate, wells were seeded with Human OSCC cells (SCC-4) purchased from ATCC (American Type Culture Collection) and incubated in 5% CO2, 37 °C incubator. Twenty-four hours after plating, the calculated volumes of the drug were added to the wells. After 48 hours of the drug application, CCK-8 reagent (Dojindo, CA, USA) was applied as 10% of the total volume of media to all the wells and incubated for 4 hours. Finally, the color absorbance was read, and the data was processed with the reader at wavelength 450nm.

2- Senescence detection using flow cytometer: The sub- IC50 dose selected from the previous cytotoxicity test was used in this assay. Six well plates were seeded with density $3X10^5$ cell/ well. Twenty-four hours after plating, the cells were grouped in replicas and treated with either Cisplatin 2 ug/ml as a golden standard senescence inducer chemotherapy (6), the selected Imiquimod drug concentration, or received no treatment (negative control). After adding the drugs, cells were incubated for another hour and stained with the canonical senescence marker, beta galactosidase antibody (Dojindo for SG03: Cellular Senescence Detection Kit - SPiDER-βGal) following the manufacturer's instructions.

Finally, stained cells were collected and analyzed using a FACS caliber flow cytometer with 488 nm excitation laser. Data was processed with the Flow Jo software.

RESULTS AND DISCUSSION

1- Cytotoxicity assay results: As demonstrated (fig 1a), all the 5 doses (100%) showed cell viability. The percentage ranged from 62% to 80%. Negative control showed 100% cell viability. All the doses showed more than 50% cell viability. However, Imiquimod 80 ug/ml was the highest (red arrow). Thus, this dose was used in the flow cytometry assay as it represented the least lethal sub- IC50 dose.

2- Flow cytometry results: As shown (fig 1b), all groups; negative control, Imiquimod 80ug/ml, and Cisplatin 2ug/ml expressed positive beta gal-stained cells. However, Imiquimod showed the highest percentage of positively stained senescent cells. The differences were statistically significant between the Imiquimod and both the negative control and Cisplatin groups (red Asterix). Furthermore, a statistical significance was found between all the 3 groups

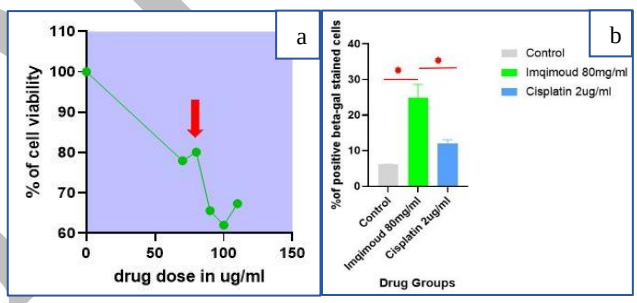

Figure (1): (a)line graph showing the viable cell percentage of imiquimod different doses, dose 80mg/ml (red arrow) represents the least lethal dose. (b)bar graph represents the percentage of positively stained senescent cells in the 3 groups. Imiquimod was higher and statistically significant than the other 2 groups (red Asterix)

CONCLUSION

The therapy induced senescence of Imiquimod outperforms the effect of the golden standard Cisplatin. As immunotherapeutics provide less cytotoxic side effects, this could introduce it as a favorable possible alternative to the traditional cytotoxic chemotherapy.

ACKNOWLEDGMENT

This study was funded by The Science, Technology, and Development Funding Authority (STDF) (30036)

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