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STUDYING THE APOPTOTIC AND NECROTIC CELLS OUTCOMES UPON TARGETING ORAL SQUAMOUS CELL CARCINOMA WITH TOLL LIKE RECEPTOR 7 AGONIST (IMIQUIMOD): IN- VITRO AND IN- VIVO STUDY

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INTRODUCTION

Oral cancer is the 11th prevalent type of malignancy worldwide (1). Oral squamous cell carcinoma (OSCC) represents about 90% of oral cancer. Among the various treatment options of cancer, immunotherapy has become the most effective therapy (2). Toll like receptors (TLRs) are specific pattern recognition receptors that are normally expressed by the immune cells and the epithelial cells. They play critical roles in both the innate and adaptive immune responses. Studies have demonstrated that tumor cells express TLRs, opening a wide platform for targeted cancer therapy by manipulating the immune response using TLR agonists. Imiquimod is a TLR7 agonist that was approved in 2014 for the treatment of superficial basal cell carcinoma (3). The role of TLRs inducing apoptosis (programmed cell death) is not clearly understood. There are contradicting reports stating that targeting TLRs can have anti and/or pro apoptotic effects (4). Therefore, more studies are needed to investigate the apoptotic and necrotic inducing activities of this TLR agonist on OSCC in an off-label pattern.

METHODOLOGY

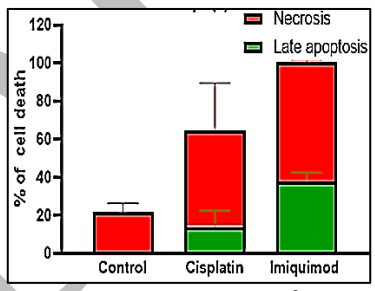
The In-vitro study: Human oral squamous cell carcinoma (SCC-4) cell line was seeded with a density of 3x 10^5 cells per well in one 6- wells plate. After 24 hours, duplicates of wells were treated for 6 hours: control (2 mL culture media), Cisplatin (7uM), and Imiquimod (80 ug/ml). Cells were stained with Annexin V-FITC/PI Apoptosis Detection Kit, then assessed by FACScan flow cytometer. Necrosis and late apoptosis were calculated among the cell population exhibiting the cell death profile.

The In-vivo study: Fifteen Syrian golden male hamsters underwent cancer induction in their left buccal pouches. After developing cancerous lesions, animals were randomized into 3 groups (5 per group) and treated for 4 weeks: control (0.2 mL saline); Cisplatin (7mg/kg body wt. intraperitoneal, once per week), and imiquimod (topical 50 mg Aldara 5% cream, 3 times per week). The tumor volumes were measured before treatment, then they were recorded on a weekly basis till the 4th week after treatment or until death. Biopsies were extracted and analyzed for histopathology and apoptosis (Annexin V-FITC/PI Apoptosis Detection Kit.) which was assessed by the immunofluorescent microscope.

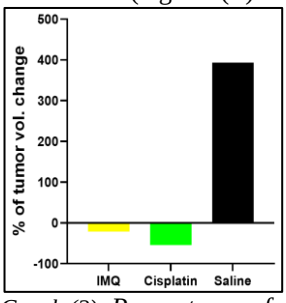
RESULTS AND DISCUSSION

The in-vitro study: Imiquimod treated SCC-4 cells demonstrated significantly increased percentages of apoptosis (37.63%) and necrosis (62.95%) compared to Cisplatin (14.18% apoptosis and 50.35% necrosis) and control (0.5 % apoptosis and 21.03 % necrosis). Two- way ANOVA test was used (Graph 1).

The in-vivo study: After 4 months of cancer induction, SCC tumors were developed at the corner of mouth and left cheeks. The tumors were presented as exophytic masses, with evident areas of necrosis (Figure 1(a). The soft tissue sections from the extracted biopsies revealed a dysplastic hyperplastic epithelium with evident points of invasion of the malignant cells into the underlying lamina propria. (Figure 1(b). Tumor volume assessment showed an increase by 393% in the control group. A significant decrease in the mean tumor volume was recorded in groups treated by Cisplatin and Imiquimod by -54.37% and – 21.16% respectively (Graph 2). Specimens from tumors treated with Imiquimod and Cisplatin showed evident Annexin V stain and scarce PI stain located on cell membranes and nuclei respectively, in comparison to the negative control. (Figure 1(C).



Graph (1): Percentages of apoptotic and necrotic cells in scc-4 cell line.



Graph (2): Percentages of tumor volume change in induced OSCC.

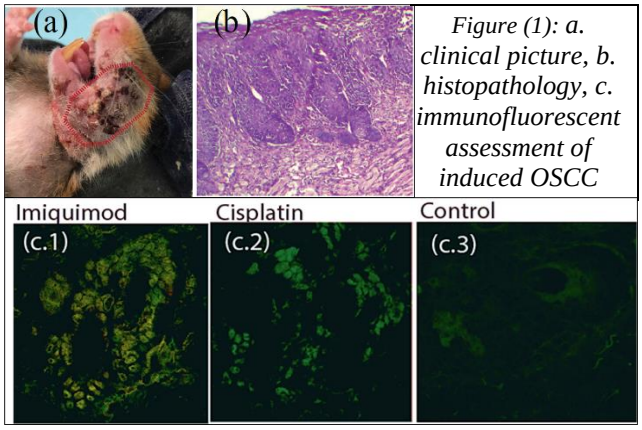


Figure (1): a. clinical picture, b. histopathology, c. immunofluorescent assessment of induced OSCC

CONCLUSION

Imiquimod induces apoptosis and necrosis in OSCC, both in-vitro and in-vivo. This suggests a strong immunotherapeutic effect of this drug on OSCC in an off-label pattern.

REFERENCES

1. Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. Oral Oncology. 2017; 70:23-8.
2. Esfahani K, Roudaia L. A review of cancer immunotherapy: from the past, to the present, to the future. 2020;27(s2):87-97.
3. Hanna E, Abadi R, Abbas O. Imiquimod in dermatology: an overview. International journal of dermatology. 2016; 55:831-44.
4. Salaun B, Romero P, Lebecque SJEjoi. Toll-like receptors' two-edged sword: when immunity meets apoptosis. 2007;37(12):3311-8.

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