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Study of the neutralization of cell multiplication, in vitro, with Botulinum Neurotoxin Type A

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Abstract— *Non-melanoma skin cancer is the most frequently diagnosed cancer in humans. The process of cutaneous carcinogenesis is not fully understood. However, several studies have been carried out to better explain the mechanisms that lead to malignancy. Methods: We tested in vitro the neutralization of non-melamoma carcinoma cell replication with botulinum toxin. Results: There was no significance in cell inhibition. Conclusion: Although our results have not been promising, further research with botulinum toxin should be encouraged to provide additional data on its effects on skin lesions, as with current evidence it is possible to correctly plan clinical trials aimed at testing its action on the skin. non-melanoma skin cancer*

I. INTRODUCTION

Despite the many advances in the diagnosis and treatment of cancer, tumor diseases are one of the main reasons for death worldwide. The side effects of chemotherapy and drug resistance of some types of cancer belong to the current major therapeutic problems. Therefore, the search for new anticancer substances and drugs is very important. (Henkel; Baldwin; Barbieri 2010)

Currently, ten major characteristics of cancer have been universally recognized, including unlimited multiplication, evasion from growth suppressors, promoting invasion and metastasis, resisting apoptosis, stimulating angiogenesis, maintaining proliferative signaling, elimination of cell energy limitation, evading immune destruction, genome instability and mutation, and tumor enhanced inflammation. (Hanahan; Weinberg, 2011)

There are key features in cancer formation that have been universally recognized, including unlimited multiplication, evasion of growth suppressants, promotion of invasion and metastasis, resistance to apoptosis, stimulation of angiogenesis, maintenance of proliferative signaling, elimination of cell energy limitation, evasion of immune destruction, genome instability, and tumor-enhanced mutation and inflammation (Hanahan; Weinberg, 2011).

Although there is already an understanding of most of the characteristics of cancer, the cellular and non-cellular components of the tumor niche help tumors acquire these characteristics. (Chen, et al. 2015)

Skin cancer can be classified into major subtypes: melanoma, basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and rare tumors. (Amaral ;Garbe , 2017).

Non-melanoma skin cancer (NMSC) represents one of the most common malignancies in humans, with basal cell carcinomas derived from keratinocytes (BCCs) and squamous cell carcinomas (SCCs) accounting for approximately 80% and 20% of NMSC cases, respectively. (Didona, et al 2018)

Although a high recurrence rate is observed, these cancers rarely metastasize and the results are promising with targeted therapies, however the development of resistance has been described. (NCCN,2018)

Botulinum neurotoxin Type A (BoNT/A) is one of the most potent toxins known (Pirazzini et al., 2017). It blocks neurotransmission via the specific cleavage of the synaptic protein SNAP-25 (synaptosomal-associated protein of 25 kDa). Atualmente, existeminúmerasindicações para o uso das neurotoxinasbotulínicas (BoNT) tipos A e B naclínicamédica. Their specific inhibitory action on

cholinergic synapses makes them desirable for the treatment of various hyperkinetic movement disorders as well as those caused by glandular hyperactivity. (Jankovic, 2017)

However, the literature is limited on the addition of BoNT/A to the culture of cancer cell lines. Some articles report that there is a delay in cell growth and mitotic activity of certain cancer cells and promote cell apoptosis (Matak.; Lacković, 2015)

Promising studies have been developed, such as those by Karsenty et al., (2019), which reported the inhibition of the growth of LNCaP and PC3 cells in vitro and in vivo (prostate cancer xenografts in mice) after the application of abobotulinum toxin, a in this regard they observed that TXB significantly reduced LNCaP cell proliferation as well as a dose-dependent increase in apoptosis, but did not affect PC-3. (Piamo; Ferrer 2020). Therefore, the search for TXB action in skin cancer cell lines is necessary, so that we can have a view of the in vitro activity on them.

I.1 Objectives

The present study aims to investigate experimentally the neutralization of skin cancer cell growth using botulinum toxin. If this hypothesis is confirmed, thus establishing a model with the next objective of studying possible mechanisms involved in this neutralization.

I.2 Method

Cell growth assay (MTT)

SCC-25(oral squamous cell carcinoma cell line) or HUVEC (noncancerous control cell line) (2.104) cells were seeded in 96-well plates and cultured in 10% FBS medium for 48 h, in the presence of botulinum toxin. Cells were harvested, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml in normal media) was added, and the cells were incubated for 4 h; then, 100 µl DMSO (Dimethyl Sulfoxide) were added, and the optical density of 570λ value was detected. round bracket (i.e., (3) reads as "equation 3").

II. RESULTS

The results revealed that was not lethal to the cell lines.

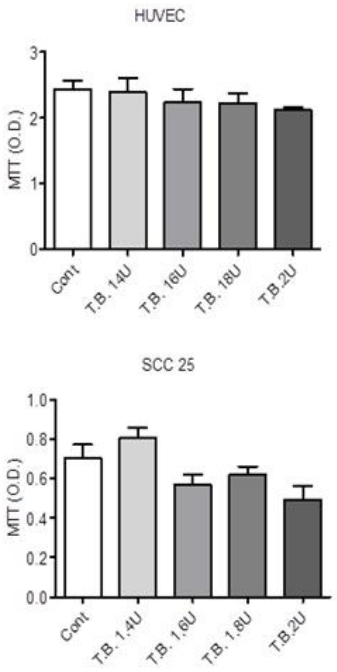

Fig. 1: Test results

III. CONCLUSION

Although our results were not encouraging, the positive effect of BoNTs on different cancer cell lines and their direct effects on certain cancer tumors is encouraging. More studies are needed to verify these results and if verified to develop a methodology by which BoNT injections can be safely used for the treatment of certain human cancers.

Currently, there are vast indications for the use of botulinum neurotoxins BoNT/A in clinical medicine. Their specific inhibitory action on cholinergic synapses makes them desirable for the treatment of several hyperkinetic movement disorders as well as symptoms caused by glandular hyperactivity and bladder dysfunction (Jankovic, 2017)

The rationale for the use of BoNT/A is that, under conditions of increased muscle tone, the administration of the toxin alleviates pathological symptoms by blocking neuromuscular transmission. However, experimental evidence indicates that not all effects of BoNT/A can be explained by blocking the neuromuscular junction alone (Marchand-Pauvert et al., 2013).

There is no consensus on how other actions arise. Experimental studies of skin effects with botulinum neurotoxins, in vivo and in vitro, have identified a number of direct effects of BoNT/A on non-neuronal cells in the skin. In experimental use of BoNT / A demonstrates ability to protect skin flaps, reducing cutaneous lymphocyte

infiltration and improving acanthosis in KC-Tie2 ard NL, (Kavlick et al. 2012) and decrease mast cell activity.(Park, 2013)

Anticancer properties of BoNT/A have been identified in three types of cancer cell lines, prostate, breast and colon. BoNT/A inhibits the growth of LNCaP human prostate cancer cells in vitro and in vivo, (Karsenty, et al. 2009) in addition to increasing the phosphorylated form of phospholipase A2. This would be the likely mechanism that explains how the toxin reduces cell growth and proliferation (Proietti, et al2012), rats, the intraprostatic injection of BoNT / A altered cell dynamics inducing apoptosis, inhibiting proliferation and down-regulation a1-adrenergic receptors, which were associated to apoptosis and atrophic alteration. (Nishiyama 2009). In a comparative study, rats treated with BoNT/A showed reduced epithelial staining of Bcl-xL and consistently increased staining of Bax and caspase-3 when compared to saline-treated animals. (Scott ,et al2021).

How breast and colon cell lines have been shown to respond to BoNT/A

through changes in gene expression in RNA and protein levels, (Dreyfus,et al,2021). Therefore, other types of cancer may become a potential target for BoNT 's anti - cancer activity.Relevant evidence that BoNTs exhibit biological effects in many types of human cells, with a much broader effect based on individual cellular responses to the cholinergic impacts of BoNT/A. (Grando;Zachary, 2018).

Although our results have not been promising, further research with botulinum toxin should be encouraged to provide additional data on its effects on skin lesions, as with current evidence it is possible to correctly plan clinical trials designed with the aim of trying out its action in non-melanoma skin cancer.

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