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Short Communication

Citotoxicity of Ultradiluted *Viscum Album* (1x10-400) in a Lineage of Human Osteosarcoma

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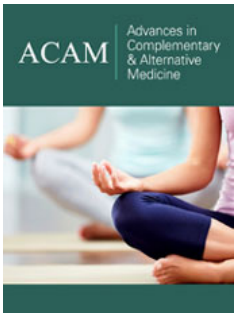

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Introduction

Homeopathy is a complementary therapy which aims to help the organism to be cured through the administration of oral or injected ultradiluted medications in sick patients [1]. It has been widely used by patients with chronic diseases, mainly aiming at a better quality of life, as well as mitigating the side effects caused by the continuous administration of conventional medicines or even in cases where chemotherapy is necessary [2].

Homeopathic medicines are derived from the animal, vegetal and mineral sources. These are produced according to the descriptions of the German and French pharmacopoeias, which describe the process according to the writings of the german physician Samuel Hahnemann, creator of Homeopathy. Hahnemann, in his studies, proposed that drugs should be diluted and streamlined. Thus, the raw material used should be diluted in water or alcohol [3], until a certain concentration (potency), which could not cause toxic effects to the patients.

One effective medicine that is being widely disseminated is obtained from the extract of the plant *Viscum album* (VA). This medication is gaining emphasis in oncological treatments due to its cytotoxic action performed by viscotoxins and lectins, some of its pharmacologically active compounds [4]. However, VA homeopathic medicine has not already been studied, so the mechanism of action of this ultradiluted remedy has not yet been elucidated. In this context, the aim of the present study was to evaluate the action of the ultra-diluted VA medicine at the potency of 200CH ($VA1x10^{-400}$), in cell culture of a human osteosarcoma lineage (U2-OS), as this is a type of cancer with poor prognosis [5] and the possibility of including it as new support therapy can be interpreted in a positive way.

Material and Methods

U2-OS cells were plated in 96 well plates at a concentration of 2.5x104 cells/ml. After 24 hours, the medium was replaced by medium added with $VA1x10^{-400}$ at 5 concentrations ranging from 10 to 100µl/ml. With the same methodology, at the same time, the control was performed with the addition of the same water used in the dynamizations in the same 5 concentrations, from 10 to 100µl of water per ml of medium (DC) and control wells were used only with medium without the addition of any other product (NC).

After 48 hours of culture, the MTT colorimetric assay was performed, with absorbance reading at 570nm and the mean inhibitory concentration (IC50) of $VA1x10^{-400}$ was calculated. This is a concentration that inhibits the growth of 50% of the cells in culture, meaning that it makes possible to evaluate the action of the medicine in the cell culture. Subsequently, a new experiment was carried out with the same methodology, however, with VA in concentrations ranging from 1 to 10µl/ml in cell culture medium, and the IC50 was also calculated.

Results

No difference in MTT assay was observed between the control groups DC and NC analyzes in either experiment, which means that these products did not interfere in cell growth. This

finding was not observed in cells that were in contact with the concentrations of VA1x10^{-400} ranging from 10 to 100µL/mL. In these groups, cells were not viable, what means that the medicine caused cell death and the IC50 could not be calculated, since even the lowest concentration (10µL/mL) caused an intense decrease in cell viability. For this reason, lower concentrations ranging from 1 to 10µL/mL were tested, so that the IC50 could be calculated, and the value of 9.09µL/mL was determined, what means that this concentration of VA1x10^{-400} in culture medium caused the death of 50% of the osteosarcoma cells.

Discussion

Osteossarcoma is an invasive and aggressive type of tumor and, if not soon diagnosed, provides poor prognosis [5]. There are several types of chemotherapeutic medicines, but most of them causes adverse effects, and a new support medicine would be extremely important to treat the cancer and mitigate these side effects. *Viscum album* extracts has been widely used with this purpose, as its mode of action includes cytotoxicity and immunomodulation [6], but it still can cause some undesired effects, so its ultradiluted formulation is a promissing possibility of treatment. So, the ultra-dilution has being commonly used for decades as an alternative to allopathy with therapeutic function and no side effects [7].

The results of the present study demonstrate both the effectiveness of the homeopathic medicine acting on tumor cell and its remarkable potency in inhibiting the growth of these cells. Some studies have already been performed in order to evaluate the antitumor effects of *viscum album* extract, both *in vitro* and *in vivo* (clinics patients), providing evidence that there may be a combination of medicine-mediated pharmacological aspects [8,9] evaluated that there was cytotoxic potential of VA extract in 5 breast tumor cell lines [10]. Analyzing the action of VA extract in breast cancer cells, found an IC50 of 172mg/L, demonstrating a cytotoxic effect, but requiring a higher concentration to promote cell growth inhibition, when compared to our study. In the 15 *in vitro* studies performed with VA extract and analyzed in a retrospective review, the results showed induction of apoptosis, cytotoxic activity, decreased cell viability, tumor cell toxicity [8] These evaluations are in accordance with what is reported in our study, however, data on ultradiluted VA cytotoxicity assessments as well as evaluations of VA extract in osteosarcoma cells have not been found.

Conclusion

The ultradiluted VA1x10^{-400} medicine showed high cytotoxicity action in human osteosarcoma lineage at initial evaluation, performed at concentrations ranging from 10 to 100µL/mL, meaning that it was not possible to evaluate the IC50 with these results. It also showed an intense action of VA1x10^{-400} in cell culture. So, a new experiment was performed, and it was possible to calculate the IC50 at lower concentrations ranging from 1 to 10µL/mL. These results are promising, as they demonstrate that VA1x10^{-400} promoted changes in cellular mechanisms, culminating in its cytotoxicity, proving that it acts directly in osteossarcoma cell function, causing cell death. This medicine should be evaluated in more detailed researches including the analysis of the molecular mechanisms involved in this cell death. Thus, VA1x10^{-400} may present, in the future, an important therapeutic source for cases of human osteosarcoma.

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