ANTIPROLIFERATIVE EFFICACY OF ZERUMBONE-LOADED NANOSTRUCTURED LIPID CARRIER IN BALB/C MICE MODEL OF BREAST CANCER

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ABSTRACT

Recently, we showed that the antiproliferative effect of ZER-NLC on Jurkat cells is through the apoptotic intrinsic pathway via activation of caspase-3 and -9, release of cytochrome c (cyt-c) from mitochondria into cytosol, and subsequent cleavage of polyADP-ribose polymerase (PARP). However, there has been no available information of ZER-NLC affects murine breast cancer cells *in vivo*. Thus, In this study, *in vivo* effects of ZER-NLC on murine breast cancer 4T1 cells were investigated. Outcomes of histopathology, immunohistochemistry and TUNEL assays of BALB/c mice bearing breast cancer revealed that the number of cancer cells were significantly decreased in mammary gland tissues after four weeks of oral administration of various doses of ZER-NLC.

INTRODUCTION

Zerumbone (ZER) is derived from several plant species of the Zingiberaceae family that have been investigated and recently found to possess multiple biomedical properties, such as anti-proliferative, antioxidant, anti-inflammatory and anticancer activities. ZER poor aqueous solubility has been the hindrance into creating a sufficient bioavailable formulation. This was resolved using nanostructured lipid carrier (NLC) containing ZER, prepared by hot high pressure homogenization (HPH) technique.

MATERIALS AND METHODS

Cell culture condition

Murine mammary cancer cell line (4T1) was purchased from American Type Culture Collection (ATCC) (Maryland, USA). The cells were maintained in RPMI-1640 (ATCC, USA) medium; supplemented with 10% heat inactivated fetal calf serum (FCS) (ATCC, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin (Sigma Aldrich, USA), according to the ATCC protocol, cultured and grown in 75 cm^2 culture flasks (TPP, Switzerland) at 37˚C in an incubator with humidified atmosphere of 95% air and 5% CO_2.

Preparation of cancer cells for injection

Upon growing of 4T1 cells and reaching 90% confluence, the medium was removed and cells washed with PBS and trypsinized. The cells were immediately centrifuged, washed twice with and dispersed in PBS. Trypan blue (Sigma Aldrich, USA) staining used to exclude dead cells. Eventually, the cells were suspended in 300 µL PBS. Harvested cells were used within 1 h of preparation. All animal groups excluding the first group were anesthetized by an intraperitonal injection of a mixture of ketamine-HCl and xylazine. The remaining groups were injected intraperitonally with 4T1 cells (1×10^6 cells/animal) in 300 µL PBS using a tuberculin (TB) syringe and 26 G needle.

Experimental design and drug treatment

Group 1 comprised untreated normal healthy mice and served as the negative control (animals without cancer burden). Group 2 comprised of mice induced to develop breast cancer and served as the cancer control, while groups 3 and 4 were cancerous mice treated daily with 60 mg/kg body weight each with blank NLC (vehicle), and ZER-NLC respectively. Group 5 were treated with 4 mg/kg body weight tamoxifen (Sigma Aldrich, USA) , an anticancer chemotherapy drug, dissolved in distilled water and served as positive control. The treatments were given orally for 4 consecutive weeks to the animals through gastric intubations.

RESULTS AND DISCUSSIONS

The breast tissues of mice treated with ZER-NL displayed significant ($P<0.05$) and markedly lower numbers of neoplastic cells than those of untreated group or NLC group. Analysis using TUNEL assay showed that ZER-NLC, like suramine the anticancer drug, had anticancer activity by inducing significant ($P<0.05$) apoptosis of breast cancer cells in the mammary gland of the BALB/c mice. On the other hand, this study showed that oral ZER-NLC at doses of 60 mg/kg significantly ($P<0.05$) reduced expression of Bcl2 protein and increased expression of Bax protein using immunohistochemistry in the breast cells compared to that of cancer control group.

CONCLUSION

In conclusion, the results from these characterization studies strongly suggest that ZER-NLCs have the potential as a probable prolonged released drug-carrier system useful for parenteral application in mammary gland adenocarcinoma.

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