

MASTER'S THESIS

Biosynthetic studies of tetrodotoxin and its anticancer activities assessment in vitro

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Abstract

In this study, the synthesis of TTX by three species of TTX-producing bacteria (*Vibrio alginolyticus*, *Microbacterium arabinogalactanolyticum* and *Serratia marcescens*) was conducted in a 10-L fermentor under the same controlled fermentation conditions for each of a period of 60 hours. The bacterial growth curves were monitored and the TTX synthesized in the culture medium was determined by HPLC. The TTX biosynthesis was found limited at the microgram level per L of culture medium with toxicities 14.7 MU (mouse unit) and 13.0 MU per mL in the partially purified culture medium of *V. alginolyticus* and *M. arabinogalactanolyticum* respectively by mouse bioassay. In the studies on SW480 and SW620 colorectal carcinoma cell lines, the expression, distribution, invasion and proliferation of voltage-gated sodium channels (VGSCs) were investigated by MTT assay (24-48 hours) and wound healing assay (0-120 hours). The different subtypes of VGSCs were expressed by semiquantitative RT-PCR and the locations of Nav1.5 and Nav 1.7 were detected by immunofluorescence microscopy. In the MTT assay, 40 μ mol/L of TTX showed significant inhibitory effect on both cell lines, with maximum inhibition rate, 33% and 40%, in SW480 and SW620 respectively. In the wound-healing assay, the inhibitory rate of 80 μ mol/L of TTX on SW480 reached 22% after 120 hours, compared with 30% in the control group. Moreover, VGSCs were highly expressed in both SW480 and SW620, with the main subtypes of Nav1.5 and Nav1.7 located on the cell surface, which might increase the metastatic rate of the cell lines.

Keywords: Tetrodotoxin (TTX), Bacterial synthesis, Anticancer, VGSCs

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