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Cucurbit[7]uril: *In vivo* Toxicity Assays and Reversal of General Anesthesia on Zebrafish Models

by

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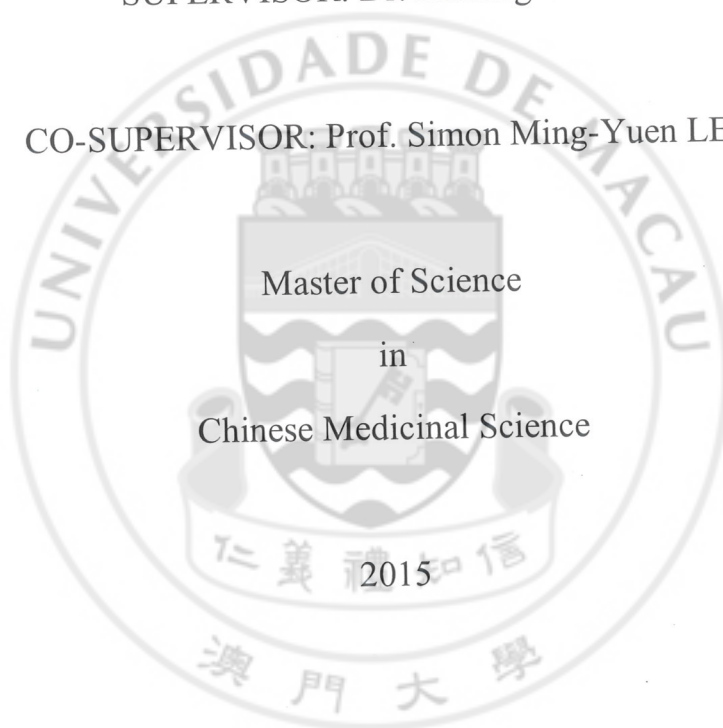
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Abstract

The macrocyclic molecular container cucurbit[7]uril (CB[7]) has attracted the most attention in the cucurbit[*n*]uril family (*n* = 5-8, 10, 14) during the past few years for pharmaceutical and biomedical studies, owing to its superior water-solubility and its optimal cavity-size that may accommodate many pharmaceutically- and biologically-relevant molecules. To advance the use of CB[7] in biomedical research further, the *in vivo* toxicity of CB[7] and its reversal effect on general anesthesia induced by tricaine on zebrafish models have been examined in detail.

In this study, firstly CB[7] was evaluated for its *in vivo* toxicity profile, including its developmental toxicity such as its effect on hatching, growth and survival, as well as its potential organ-specific toxicities such as cardiotoxicity, hepatotoxicity, and locomotion and behavioral toxicity, using zebrafish models. The results revealed that CB[7] has measureable cardiotoxicity and locomotion and behavioral toxicity at concentrations of ~500 μM or higher, and negligible developmental and hepatotoxicity at concentrations up to 750 μM , although extended exposure to CB[7] at the 500-750 μM concentration range induced the mortality of tested fish. These results demonstrate for the first time with live *in vivo* animal models that CB[7] has relatively low developmental and organ specific toxicity, and support further exploration of the use of CB[7] in biomedical research at sub-toxic concentrations.

Additionally, the reversal effect of CB[7] to a general anesthetic agent was explored. A general anesthetic is a drug that brings about a reversible loss of consciousness, during a surgical or therapeutic procedure to render the patient free of pain and anxiety. However, the effect of anesthetics may linger far beyond the necessary time required and induce adverse effects. In addition, many surgical patients need to recover to a conscious state that allows them to make important decisions soon after their surgery. Unfortunately, there are currently no clinically-available anti-dotes to reverse the effects of anesthetics. In this thesis, the *in vitro* supramolecular host-guest complexations between CB[7] and a commonly used general anesthetic in fish, tricaine mesylate (TM), was investigated; and for the first time the *in vivo* reversal effect of CB[7] to general anesthesia induced by TM was demonstrated with zebrafish models. These findings will likely lead to a new approach that may allow patients to regain lucidity much faster than their natural recovery from general anesthesia, and may also be potentially used to reverse

potentially life-threatening toxic effects encountered by some patients in response to general anesthesia.



摘要

大環分子瓜環[7]是瓜環[n]家族 ($n=5-8, 10, 14$) 中近年來在藥學和生物醫學研究中最受關注的一員, 這得益於其極好的水溶性, 以及可以容納眾多藥學或生物學相關分子的理想孔隙大小。為了推進瓜環[7]在生物醫學研究的進一步應用, 本論文詳細考察了瓜環[7]在斑馬魚模型上的體內毒性以及其對三卡因甲磺酸(TM)誘導的全身麻醉的逆轉作用。

本文首先通過研究和評價瓜環[7]在斑馬魚上的發育毒性如其對孵化、生長和存活的影响, 以及潛在的器官特異性毒性如心臟毒性、肝毒性和運動與行為毒性, 得到其體內毒性資料。結果顯示, 瓜環[7]在 $\sim 500 \mu\text{M}$ 或更高濃度具有可量化的心臟毒性和運動與行為毒性, 在高達 $750 \mu\text{M}$ 表現出不可忽視的發育毒性和肝毒性, 而在 $500-750 \mu\text{M}$ 的濃度範圍給藥, 瓜環[7]可誘導斑馬魚的死亡。這是首次使用在體的動物模型闡明瓜環[7]具有相對較低的發育及器官特異性毒性, 為進一步探索亞毒性濃度的瓜環[7]在生物醫學研究中的應用提供了依據。

另外, 論文探討了瓜環[7]對一種全身麻醉藥的逆轉作用。全身麻醉藥是指在手術或治療過程中引起可逆轉的意識喪失的藥物, 以使患者免受痛苦和焦慮。但是, 麻醉藥的作用往往比實際需要持續更長的時間, 並導致不良反應。另外, 很多外科手術患者需要在手術不久後恢復至清醒狀態以讓其做一些重要決定。遺憾的是, 如今未有一種臨床可用的解藥以逆轉麻醉藥的作用。本文研究了瓜環[7]和一種常見的魚用麻醉藥三卡因甲磺酸(TM)的體外超分子主客體結合作用, 並且首次論證了瓜環[7]在斑馬魚模型上對TM誘導全身麻醉的逆轉作用。這些發現可能將轉化成一種使全身麻醉患者比自然蘇醒更快恢復清醒新方法, 並可能用於逆轉某些病人在全身麻醉過程中產生的威脅生命的潛在毒性效應。

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