RGC Ref. No.:
UGC/FDS11/M07/19
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Anti-emetic potential of cocaine- and amphetamine-regulated transcript (CART) system in *Suncus murinus*

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. CHAN Sze-wa / Associate Professor	School of Health Sciences / Caritas Institute of Higher Education
Co-Investigator(s)	Prof. RUDD John Anthony / Professor and Director	School of Biomedical Sciences and the Laboratory Animal Services Centre / The Chinese University of Hong Kong
	Prof. SAKAI Takafumi / Professor and Dean	Graduate School of Science and Engineering / Saitama University, JAPAN
	Prof. SAKATA Ichiro / Associate Professor	Graduate School of Science and Engineering / Saitama University, JAPAN
	Prof CHAN Dominic Tak-wah / Professor	Department of Chemistry / The Chinese University of Hong Kong
Others	N/A	N/A

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2020	N/A	N/A
Project Completion Date	31/12/2022	30/6/2023	Institution Approval granted on 12/10/2022
Duration *(in month)*	36	42	Institution Approval granted on 12/10/2022
Deadline for Submission of Completion Report	31/12/2023	30/6/2024	N/A

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To identify the anatomical distribution of CART peptide and CART mRNA in S. murinus.
2. To investigate the mechanism of action of the CART system to modulate emesis, nausea biomarkers and physiological changes indicative of nausea (PCIN; blood pressure changes, heart rate variability, temperature, gastric myoelectric activity, and respiratory function) induced by cisplatin.
3. To establish if CART system activation provides a board inhibition of emesis by modulating activation of brainstem and forebrain areas that may be involved in nausea and emesis.

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change:

1.

2.

3. ..

5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Cocaine- and amphetamine-regulated transcript (CART) peptide is a neuropeptide that appears to contribute to the regulation of energy homeostasis and appetite, which may be altered during cancer treatment. CART mRNA and peptide are expressed in brain regions involved in stress, feeding, and emesis. The aim of this project was to elucidate the potential involvement of the CART system in emesis control in *Suncus murinus*.

Objective 1: The anatomical distribution of CART mRNA and peptide in the brain and gastrointestinal tissues was identified using reverse transcription PCR, Western blot analysis, and immunohistochemistry. CART mRNA was widely expressed in the brain as well as in the gastrointestinal tissue. The expression of CART peptide was confirmed by Western blot analysis. CART immunoreactive cells were detected in several hypothalamic nuclei, the Edinger–Westphal nucleus, as well as the brainstem. In the periphery, CART immunoreactive cells were detected mainly in the mucosal layer, gastric glands and external muscle layer along the intestine and in the stomach.

Objective 2: Acute cocaine or amphetamine administration has been shown to upregulate CART mRNA in rat striatum. We repeated earlier studies using *d*-amphetamine and demonstrated that it induces emesis, inhibits food intake and increases locomotor activity in a dose-dependent manner over a period of 2 h. Interestingly, a low dose of *d*-amphetamine enhanced water intake. In a subsequent study, animals were pre-treated with *d*-amphetamine or saline prior to cisplatin. We found that pre-treatment with *d*-amphetamine produced a non-significant reduction in cisplatin-induced emesis. It also inhibited food intake and increased locomotor activity compared with saline/cisplatin-treated animals. We then assessed the dose-response effect of central administration of CART (55-102) on food and water intake. Our data showed that CART (55-102) failed to modulate food and water intake. Studies were then set up to investigate the effect CART (55-102) on cisplatin-induced emesis. We found that CART (55-102) did not induce emesis during the pre-treatment period, but subsequently reduced the number of retches induced by cisplatin. To investigate the neurochemical changes in the brain, we measured the levels of neurotransmitters associated with feeding and emesis, including acetylcholine, norepinephrine, dopamine, tyrosine, 5-HT, tryptophan, 5-HIAA, GABA, and glutamate, in the brain homogenates using LC-MS. We found that CART (55-102) and cisplatin had no significant effect on the concentrations of neurotransmitters in the hypothalamus and brainstem. In a separate set of studies, cisplatin inhibited food and water intake and induced emesis, but had no significant effect on the gastric myoelectric activity, body temperature, cardiovascular or respiratory functions over a period of 24 h. Pre-treatment with CART (55-102) did not antagonize the effects induced by cisplatin.

Objective 3: To investigate if CART peptides have board inhibitory effect on emesis by modulating activation of brainstem and forebrain areas, we examined the c-Fos expression in the hypothalamus and brainstem using immunohistochemistry. Our data showed that both CART (55-102) and cisplatin induced c-Fos expression in the nucleus tractus solitarius, lateral hypothalamus, paraventricular hypothalamus, and bed nucleus of the stria terminalis (BNST), compared to saline-treated animals. Cisplatin also induced c-Fos expression in the area postrema (AP), arcuate nucleus, and central nucleus of the hypothalamus. Notably, pre-treatment with CART (55-102) significantly attenuated cisplatin-induced c-Fos expression in the BNST and AP. Nicotine induces emesis via a central mechanism, whereas copper sulphate pentahydrate is a gastric irritant. We found that that CART (55-102) was ineffective in antagonizing nicotine- or copper sulphate pentahydrate-induced emesis. Furthermore, palonosetron antagonized cisplatin-induced emesis, however, the anti-emetic effect was not enhanced when used in combination with CART (55-102).

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To identify the anatomical distribution of CART peptide and CART mRNA in *S. murinus*.	✓	100 %
2. To investigate the mechanism of action of the CART system to modulate emesis, nausea biomarkers and physiological changes indicative of nausea (PCIN; blood pressure changes, heart rate variability, temperature, gastric myoelectric activity, and respiratory function) induced by cisplatin.	✓	100 %
3. To establish if CART system activation provides a board inhibition of emesis by modulating activation of brainstem and forebrain areas that may be involved in nausea and emesis.	✓	100 %

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

We confirmed that cocaine- and amphetamine-regulated transcript (CART) mRNA and peptide were widely expressed throughout the entire brain and in the intestine and stomach. In the brain, CART immunoreactivity was detected in regions that regulate emotion, energy homeostasis, pupillary control, as well as nausea and emesis. Along the gastrointestinal tract, CART immunoreactivity was mainly detected within the myenteric plexuses, mucosal layer, gastric glands and external muscle layer.

In conscious, freely moving animals, *d*-amphetamine induced emesis, inhibited food and water intake and enhanced locomotor activity. Pre-treatment with *d*-amphetamine failed to modulate cisplatin-induced emesis significantly but increased the locomotor activity and inhibited food intake compared to saline/cisplatin-treated animals. These data suggest that *d*-amphetamine may have differential effects in emesis, feeding, and locomotor activity in *Suncus murinus*. The effect of a low dose of *d*-amphetamine to enhance water intake requires further investigation.

Central administration of CART (55-102) has been shown to inhibit food intake in rodents. In our studies, central administration of CART (55-102) did not modulate food and water intake, suggesting potential species-specific differences in responsiveness to this peptide. Furthermore, CART (55-102) alone did not induce emesis, but pre-treatment with CART (55-102) significantly antagonized cisplatin-induced retches over an observation period of 2 h. LC-MS analysis of neurotransmitters revealed that CART (55-102) and cisplatin did not affect the concentrations of monoamines and amino acid neurotransmitters in the hypothalamus and brainstem. The failure of cisplatin to modulate the gastric myoelectric activity, body temperature, cardiovascular and respiratory functions was unexpected. The failure of CART (55-102) to antagonize cisplatin-induced emesis over a 24-h period may be due to the rapid metabolism of the peptide in the animals and/or clearance from the central nervous system and ventricular system.

CART (55-102) and cisplatin induced c-Fos expression in both the hypothalamus and brainstem, whereas pre-treatment with CART (55-102) significantly attenuated cisplatin-induced c-Fos expression in the area postrema and bed nucleus of the stria terminalis, suggesting that the action of CART (55-102) to attenuate cisplatin-induced emesis may involve both the forebrain limbic system and the brainstem. In a separate set of studies, pre-treatment with CART (55-102) failed to antagonize copper sulphate- or nicotine-induced emesis, indicating that the CART system appears to be differentially involved in the control of drug-induced emesis. Furthermore, CART (55-102) did not enhance the anti-emetic effect of palonosetron, indicating that using CART (55-102) in combination with a 5-HT_3 antagonist may not produce synergistic anti-emetic effect.

6.2 Potential for further development of the research and the proposed course of action
(Maximum half a page)

The hypothalamus is a key brain region for autonomic control, including feeding and emesis, further studies may involve administering CART (55-102) into the hypothalamus to investigate whether the anti-emetic action of CART (55-102) to antagonize chemotherapy-induced emesis involves the hypothalamus. As CART peptide is expressed along the gastrointestinal tract, future studies may be extended to examine the effect of peripherally administered CART (55-102) on feeding and emesis. Recent evidence suggests that some of the actions of CART peptide may be mediated via a GPR160 in the brainstem. If GPR160 exist in *S. murinus*, further studies may modulate GPR160 to dissect the mechanism of action of the CART system in emesis control.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Cocaine- and amphetamine-regulated transcript (CART) peptide plays an important role in regulating energy balance and appetite. CART mRNA and peptide are found in brain regions considered important in stress, feeding, and vomiting. In this project, we studied the role of CART system in feeding and emesis control in *Suncus murinus*, a small animal capable of vomiting. We found that CART mRNA and peptide were expressed in the gastrointestinal tract and brain areas that control emotion, feeding, pupillary control and vomiting. In conscious, freely moving animals, *d*-amphetamine modulated feeding and locomotor activity and induced vomiting. CART peptide, on the other hand, didn't affect feeding and drinking or physiological functions, but reduced cisplatin-induced vomiting. We demonstrated that the action of CART peptide may involve certain neurochemical circuitry in the hypothalamus and brainstem. Understanding the signaling pathway of the CART system allows us to evaluate whether it is a potential target for managing chemotherapy-induced vomiting.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
		Yes		Zengbing Lu, Sze Wa Chan* Bin Jiang, Dexuan Cui, Ichiro Sakata, Takafumi Sakai, Xiaofei Huang, Julia Yuen Hang Liu, Tak Wah Dominic Chan, John A Rudd	Action of cocaine- and amphetamine-regulated transcript (CART) peptide to antagonize cisplatin-induced emesis in *Suncus murinus* (house musk shrew)/ European Journal of Pharmacology	No	Yes (Attachment 1)	Yes	No

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered
(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
9 / 2021/ Virtual	Distribution and physiological effects of cocaine- and amphetamine-regulated transcript peptide in *S. murinus* (House Musk Shrew)	The American College of Clinical Pharmacology 2021 Annual Meeting	No	Yes (Attachment 2)	Yes	Yes
3 / 2022 / Virtual	The potential anti-emetic effect of cocaine- and amphetamine-regulated transcript peptide on cisplatin-induced emesis in *Suncus murinus*	The American Society for Clinical Pharmacology & Therapeutics 2022 Annual Meeting	No	Yes (Attachment 3)	Yes	Yes
3/ 2023 / Virtual	Central administration of cocaine- and amphetamine-regulated transcript peptide reduces cisplatin-induced emesis and c-Fos expression in *Suncus murinus*	The American Society for Clinical Pharmacology & Therapeutics 2023 Annual Meeting	No	Yes (Attachment 4)	Yes	Yes
5 / 2023 / United States	Investigation into the effect of *d*-amphetamine on cisplatin-induced emesis in *Suncus murinus*	Digestive Disease Week 2023	No	Yes (Attachment 5)	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

The research experience and new knowledge gained from this project have been integrated into the pharmacology curriculum, providing students with a deeper understanding of the mechanisms of emesis and feeding. Furthermore, my enhanced research proficiency has enabled me to offer specialized guidance to students working on their Final Year Projects.
This knowledge transfer has not only benefited the students but also contributed to the overall enhancement of our university's teaching and learning environment.

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
Nil			

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

Our collaboration with the School of Biomedical Sciences and Department of Chemistry at the Chinese University of Hong Kong and the Graduate School of Science and Engineering, Saitama University facilitated access to specialized facilities and expertise. These collaborations have had a broader impact, accelerating scientific progress and facilitating knowledge transfer and technological advancements.

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	1	4	0	0	Type	No.

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
Nil	Nil