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## Olanzapine As A Novel Treatment For Chemotherapy Induced Nausea

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# Olanzapine As A Novel Treatment For Chemotherapy Induced Nausea

## Abstract

OLANZAPINE AS A NOVEL TREATMENT FOR CHEMOTHERAPY-INDUCED  
NAUSEA AND VOMITING

Rosario B. Jaime-Lara

Bart C. De Jonghe

**Background:** Chemotherapy-Induced Nausea and Vomiting (CINV) is a prevalent adverse effect of chemotherapy, reducing quality of life and resulting in considerable healthcare resource utilization costs. Olanzapine, historically used as an antipsychotic, is now added to cancer antiemetic guidelines due to its effectiveness in preventing and managing CINV. While therapeutic options have improved CINV treatment, the literature highlights a substantial gap in knowledge regarding the pathophysiology of CINV and the biological basis of olanzapine's effect on CINV. The primary objective of this dissertation was to better understand how olanzapine alleviates CINV. These data can increase knowledge of the pathophysiology of CINV and help identify potential biological mediators to target for therapeutic interventions.

**Methods/Results:** To address our objective, we conducted a literature review of CINV with a focus on olanzapine and identified potential biological mediators of olanzapine's effects on CINV: ghrelin and serotonin g. Subsequently, we conducted a series of behavioral experiments in rats to study the antiemetic effects of olanzapine. Olanzapine in rats (as in humans) decreased chemotherapy-induced malaise/nausea, anorexia, and body weight loss. To test the effect of olanzapine on potential biological mediators of these chemotherapy-induced adverse effects, we assessed the effects of olanzapine on ghrelin and serotonin. We found that olanzapine decreases the number of cisplatin (a chemotherapeutic agent known to induce CINV in almost 100% of people and animals in the absence of antiemetic prophylaxis) activated neurons in the hindbrain and determined olanzapine's effect on chemotherapy-induced malaise is at least in part mediated by the hindbrain. Additionally, we found olanzapine counteracts cisplatin-induced decreases in circulating ghrelin and central ghrelin receptor [GHSR] gene expression six hours post chemotherapy. Lastly, we report olanzapine decreased serotonin 2C receptor [5-HT<sub>2C</sub>] gene expression in the hindbrain and the hypothalamus.

**Conclusion:** These findings suggest that olanzapine may alleviate CINV by counteracting the effects of cisplatin on the hindbrain, circulating ghrelin, ghrelin receptor expression, and 5-HT<sub>2C</sub> receptor gene expression in the hindbrain and hypothalamus. Future research should further define the role of these potential biological mediators in CINV and determine whether counteracting cisplatin-induced alterations of ghrelin and 5-HT<sub>2C</sub> signaling are sufficient to help control CINV.

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OLANZAPINE AS A NOVEL TREATMENT FOR CHEMOTHERAPY INDUCED NAUSEA  
AND VOMITING

Rosario B. Jaime-Lara

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in

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*Dedication page*

A mis padres, Saul y Teresa, por su apoyo y por todos los sacrificios que han hecho por mí- por tener la valentía y fortaleza de dejar atrás su tierra natal por proveernos una mejor educación.

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# ABSTRACT

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## CHAPTER I: Introduction

### Background

Chemotherapy-induced nausea and vomiting (CINV) persists alongside cancer treatment despite numerous advances in antiemetic drug development. CINV remains understudied, and research is needed to improve quality of life and reduce patient suffering during cancer treatment. In the United States alone, almost 650,000 people undergo chemotherapy annually. Although chemotherapy can lead to disease remission (Prevention, 2015), many chemotherapeutics are known to have severe adverse effects, including nausea and vomiting (P. J. Hesketh, 2008) which devastate quality of life (Bloechl-Daum, Deuson, Mavros, Hansen, & Herrstedt, 2006; P. Hesketh, 2005; Lindley et al., 1992; Osoba, Zee, Pater, et al., 1997) and may negatively impact treatment adherence (Markowitz & Rabow, 2008). Before the introduction of current antiemetic treatments (i.e., serotonin type 3 receptor antagonists [5-HT<sub>3</sub>RAs], neurokinin-1 receptor antagonists [NK-1RAs], and olanzapine), 20% of patients discontinuing chemotherapy identified CINV as a reason for terminating or postponing treatment (Markowitz & Rabow, 2008). Antiemetics have reduced the prevalence of CINV following chemotherapy from greater than 90% to 25% (vomiting) and 50% (nausea) (Grunberg et al., 2004; Hsieh et al., 2015). However, studies in oncology patients with different primary tumor sites and stages of cancer continue to identify CINV among the most distressing side effects of chemotherapy (Coates et al., 1983; Griffin et al., 1996). Thus, CINV is still a prominent clinical obstacle to cancer treatment and patient quality of life.

Olanzapine is a relatively novel agent in of the treatment of CINV. Historically used as an antipsychotic, olanzapine was added to cancer treatment antiemetic guidelines in 2014 after clinical trials demonstrated oral olanzapine effectively attenuates CINV in patients with different cancer diagnoses (Jackson & Tavernier, 2003; Navari et al., 2007; Navari et al., 2005; Navari,

Gray, & Kerr, 2011; S. D. Passik et al., 2003; S. D. Passik et al., 2002; Pirl & Roth, 2000; Srivastava, Brito-Dellan, Davis, Leach, & Lagman, 2003). Olanzapine is a particularly promising therapeutic option for multiple reasons. **1)** When it is used as a psychiatric drug, olanzapine (15.1mg  $\pm$  4.7mg/day) induces weight gain (an average of 4.2 kg) in greater than 90% of people within the first three months of initiating treatment, which could be valuable in reducing cancer-related weight loss (S. Gupta, Droney, Al-Samarrai, Keller, & Frank, 1999; Kinon, Basson, Gilmore, & Tollefson, 2001; Ratzoni et al., 2002). **2)** Additionally, prophylactic oral olanzapine (10 mg) reduces CINV during the first 24 hours following chemotherapy (from 35% to 14% compared to placebo) (Navari, 2014; Navari, Nagy, & Gray, 2013; Navari et al., 2016). **3)** Lastly, a single daily dose of oral olanzapine (10 mg) reduces the prevalence of CINV occurring 24+ hours following chemotherapy (from 75% to 58% compared to placebo) (Navari, 2014; Navari et al., 2013; Navari et al., 2016). While the anti-psychotic effects of olanzapine are thought to be mediated primarily by the blockade of serotonin and dopamine receptors (Reynolds, 2011; Seeman, 2002; Stahl, 2013), the molecular mechanisms that underlie olanzapine's effects on nausea and vomiting are largely unknown. However, olanzapine is known to impact energy balance through serotonin type 2C receptors [5-HT<sub>2C</sub>] and ghrelin (including circulating ghrelin and its receptor, growth hormone secretagogue [GHSR]) which overlap with known CINV pathways (Hattori, 2010; Murashita et al., 2005; van der Zwaal et al., 2012; Yakabi, Sadakane, et al., 2010). Identifying mediators that contribute to olanzapine's antiemetic properties may elucidate important details about mechanisms of alleviating CINV and can help identify new therapeutic targets.

### Significance of Chemotherapy-induced Nausea and Vomiting

#### *Prevalence of Chemotherapy-induced Nausea and Vomiting*

It is estimated that 14 million people worldwide and 1.6 million people in the United States are diagnosed with cancer every year (*Cancer Facts and Figures 2016*; Organization,

2013). Chemotherapy is one of the most commonly used types of cancer therapies (*Cancer Facts and Figures 2016*), with nearly four million people around the world receiving treatment every year (Research., 2016). Although chemotherapy can cure cancer, increase the rates of remission, and/or slow the growth of cancer cells, it is accompanied by side effects which can devastate quality of life and can lead to treatment discontinuation (Coates et al., 1983; Griffin et al., 1996; Lorusso et al., 2017; Markowitz & Rabow, 2008).

Nausea and vomiting are among the most prevalent side effects of chemotherapy (Coates et al., 1983; Griffin et al., 1996; Lorusso et al., 2017). Highly emetogenic chemotherapy (HEC) is characterized by induction of CINV in over 90% of patients in the absence of prophylaxis (5, 9, 48-50). Current antiemetics used to prevent CINV in patients receiving HEC include: dexamethasone, 5-HT3RAs, NK-1RAs, and olanzapine. Clinical guideline and medication regimens are discussed in detail in **Chapter II**. These medications have greatly reduced the prevalence of CINV, but even with optimal antiemetic therapy (including dexamethasone, 5-HT3RA, and NK-1RAs), 25% of patients continue to experience vomiting and over 50% continue to experience nausea (Grunberg et al., 2004; Hsieh et al., 2015). Notably, although nausea is more prevalent than vomiting, current antiemetics are more effective at controlling vomiting than at reducing nausea (Bloechl-Daum et al., 2006; Farrell, Brearley, Pilling, & Molassiotis, 2013; Fernandez-Ortega et al., 2012; Grassi et al., 2015). Thus, chemotherapy-induced nausea is not well managed by current antiemetic therapies and continues to be a significant adverse effect in over half of patients undergoing chemotherapy.

Additionally, the prevalence of delayed CINV (CINV occurring more than 24 hours following treatment) is greater than that of acute CINV (CINV occurring less than 24 hours following treatment). Studies report delayed CINV in up to 70% of patients with different types of cancer compared to 55% experiencing acute CINV (Bloechl-Daum et al., 2006; Farrell et al., 2013; Fernandez-Ortega et al., 2012; Grassi et al., 2015). However, most available antiemetics, with the exception of olanzapine and NK-1 antagonists, are better at treating acute side effects



(Bosnjak, Gralla, & Schwartzberg, 2017; Navari et al., 2016). Thus, despite the use of antiemetics, CINV - particularly delayed nausea - is not controlled and continues to cause severe distress in 50% of patients undergoing chemotherapy (Grunberg et al., 2004; Hsieh et al., 2015).

*Implications: human and financial consequences*

Uncontrolled CINV decreases quality of life (QOL), interfering with daily activities and compromising physical wellbeing (4, 23-25). In the context of this dissertation, QOL is defined as a multidimensional concept that includes subjective evaluations of physical and emotional wellbeing (CDC). In a recent study examining the effect of CINV on the quality of life of patients with different cancer diagnoses, after only 2 cycles of chemotherapy 64% of patients experiencing CINV had significant reductions in their QOL scores (Grassi et al., 2015). Intensity and duration of CINV are associated with greater decreases in quality of life (Ballatori et al., 2007; Fernandez-Ortega et al., 2012). Importantly, nausea has a stronger negative impact on QOL than vomiting (Bloechl-Daum et al., 2006; Farrell et al., 2013; Fernandez-Ortega et al., 2012; Grassi et al., 2015), interfering with daily activities of 89% of people experiencing severe nausea compared to 39.3% experiencing vomiting (Bloechl-Daum et al., 2006; Fernandez-Ortega et al., 2012). Furthermore, decreases in QOL associated with severe nausea can affect nutritional status. In a study by Farrell *et al.*, with a sample of 104 patients with breast, ovarian, or bladder cancer, 25% of Subjective Global Assessment scores (a measure of nutritional status- calculated by assessing weight changes, food intake, gastrointestinal symptoms, changes in functional capacity and physical signs of malnutrition) were indicative of malnutrition after just one cycle of chemotherapy (Farrell et al., 2013).

CINV has a severe impact on patients' perceptions of chemotherapy treatment and negatively impacts their emotional wellbeing with both nausea and vomiting continually identified among the most feared side effects of chemotherapy across gender and age groups (Coates et al., 1983; Lorusso et al., 2017; Ruhlmann et al., 2015). Importantly, the fear of CINV

was consistently high before treatment initiation and after multiple treatment cycles in patients with different types of cancer (breast, ovarian, lung, lymphoma, head, and neck ) (Coates et al., 1983; Lorusso et al., 2017; Ruhlmann et al., 2015). In a multinational (Australia, Italy and Spain) study by Grassi *et al.* of 302 participants with multiple cancer sites (gastrointestinal, breast, genitourinary, respiratory, and blood), those who reported CINV had higher levels of maladaptive coping (e.g., “hopelessness-helplessness” and “anxious preoccupation”) and emotional distress (Grassi et al., 2015). In a multinational study by Lorusso *et al.*, 50% of 373 participants with different primary tumor sites (including breast, colon, lung, and gastric cancer) identified CINV as the most intolerable adverse effects of chemotherapy (Lorusso et al., 2017). Thus, nausea and vomiting continue to be identified among the most distressing, intolerable, and feared side effects of chemotherapy (Griffin et al., 1996; Lorusso et al., 2017; Ruhlmann et al., 2015).

Qualitative studies also capture the negative impact of CINV on people’s physical and emotional wellbeing (Farrell et al., 2013; Molassiotis, Stricker, Eaby, Velders, & Coventry, 2008; Olver, Elliott, & Koczwara, 2014; Salihah, Mazlan, & Lua, 2016). Multiple studies in oncology patients with various types of cancer (including breast, lung, colon, and genitourinary), CINV was consistently identified and described as a disruption to daily functions (e.g., eating) (44-46). In a qualitative study by Malossiotis *et al.*, 17 patients (whose diagnoses included breast cancer, lung cancer, lymphoma, and sarcoma) receiving chemotherapy described CINV as distressing, “I’d rather be sick than have nausea...to be honest I’d rather have sickness because you can do something about it” (Molassiotis et al., 2008). In another study by Olver *et al.* 42 patients with nine cancer diagnoses (including breast cancer, testicular cancer, leukemia, and pancreatic cancer) identified CINV as interfering with their daily functions; “I feel less energized when the nausea is there...I don’t function when I’ve got that nausea feeling, at the moment it’s too hard, it might seem like a cop-out, but it just seems so hard...” (Olver et al., 2014). A study by Salihah *et al.* of 13 women with breast cancer also reported patients identified chemotherapy induced nausea as interfering with their eating patterns, “I eat less because of nausea, I felt terribly tired...”

(Salihah et al., 2016). In another study by Farrell *et al.* 104 participants with multiple primary tumor sites, perceived CINV to be an obstacle to healthy eating, “I don’t eat the right thing because the only things that stay down are crappy things. Like, healthy food makes me nauseous” (Farrell et al., 2013). From both quantitative and qualitative perspectives, these studies clearly indicate that CINV continues to negatively impact QOL by impacting physical and emotional wellbeing (Farrell et al., 2013; Molassiotis et al., 2008; Olver et al., 2014; Salihah et al., 2016).

Uncontrolled CINV has also been associated with considerable healthcare resource utilization and decreased productivity/lost work days (Burke, Wisniewski, & Ernst, 2011; Craver, Gayle, Balu, & Buchner, 2011; Tina Shih, Xu, & Elting, 2007). U.S.-based studies have found that CINV is associated with higher medical costs, including increased inpatient admissions, emergency room visits, and outpatient hospital visits (Burke et al., 2011; Tina Shih et al., 2007). Specifically, Burke *et al.* found CINV as a primary and/or secondary diagnosis accounts for 18% of health-related visits following the first cycle of chemotherapy (Burke et al., 2011). Although the estimates of CINV health related costs in the US vary, reports estimate direct medical costs of \$778.58 and \$1300 per patient during the first 5 days and the first 30 days following chemotherapy, respectively (Burke et al., 2011; Tina Shih et al., 2007). Notably, these costs were 30% higher than the health-related costs of patients with controlled CINV (Tina Shih et al., 2007). Uncontrolled CINV among working-age adults receiving highly or moderately emetogenic chemotherapy also have indirect costs of \$180 (\$112.49 for missed work and \$67.62 for reduced productivity) within just the first 5 days following chemotherapy initiation (Tina Shih et al., 2007). This amounts to an average of \$23.4 million nation-wide per day (Tina Shih et al., 2007). Thus, CINV poses a significant financial burden on people receiving chemotherapy, their families, and the US health care system.

#### *Nursing Science and CINV*

Despite its prevalence and severity, CINV receives little research attention. Nursing

research, as reflected in the National Institute of Nursing Research's (NINR) strategic plan, has identified the need to expand symptom science (NINR, September 2016). A symptom is defined as a subjective indicator of disease (e.g., pain) and may be accompanied by an objective "sign" of disease (e.g., bleeding). Mentioned by name are pain, fatigue, and stress, while nausea, although comparably prevalent, remains notably absent (NINR, September 2016). The absence of nausea and vomiting- with a focus on nausea as a standalone symptom - demonstrates that CINV continues to be neglected in the context of symptom management. Therefore, exploring the symptomatology of CINV is of utmost importance and its study is necessary to expand symptom science.

To guide research in symptom science that integrates behavioral and biological data, NINR scientists developed the NIH Symptom Science Model (NIH-SSM) (Cashion & Grady, 2015). The NIH-SSM model (**Figure 1.1**) describes a sequence for symptom science research, beginning with the identification of a complex symptom that holds a phenotype, followed by identification of biomarkers, and ultimately illuminates therapeutic and clinical interventions that lead to symptom reduction. Phenotypes, the observable physical or biochemical characteristics of an organism, are determined using biological, behavioral, and clinical data. Once the phenotype is determined, diverse methodologies (e.g. genomic data) are used to discover potential measures of biological processes and pharmacologic responses termed "biomarkers." These data can then be utilized to develop therapeutic interventions.

This model can be applied to chemotherapy-induced nausea (a symptom) which often proceeds chemotherapy-induced vomiting (a sign). This model has served as a foundation to the experiments described in this body of work. In this context, chemotherapy-induced nausea represents the complex symptom. Behavioral and biological data will be collected to identify observable physical and behavioral properties of nausea and vomiting (**Chapter III**). Immunohistochemistry and molecular experiments will be conducted to illuminate potential mediators of CINV (**Chapter IV**). These experiments can serve to identify potential mediators of

olanzapine's antiemetic properties, and further research can help establish the role of these potential mediators in CINV.

As recognized by NINR, "novel discoveries require the integration of behavioral and biologic data and the development of models to predict, treat, and manage the symptoms of diseases and treatments" (Cashion & Grady, 2015). Thus, understanding the pathophysiology of CINV is necessary to improve treatments. Our laboratory is among a small number of groups which examines the pathophysiology of CINV. Known details about the pathophysiology of CINV are described in **Chapter II**. To further define how olanzapine alleviates CINV, this dissertation focuses on studying its behavioral effects and identifying potential mediators of olanzapine's antiemetic properties. Determining the mechanism by which this drug alleviates CINV can lead to research that ultimately identifies better treatments of CINV and improves patient's quality of life.

#### Purpose and Specific Aims

The purpose of this body of work is to better understand how olanzapine alleviates CINV. These data can increase knowledge of the pathophysiology of CINV and help identify potential therapeutic targets that alleviate CINV and improve quality of life for people undergoing chemotherapy. Accordingly, the first aim is to identify gaps in the literature regarding CINV and olanzapine as an antiemetic. The second aim, is to determine the behavioral effects of systemic and central olanzapine following chemotherapy. Lastly, the third aim is to identify potential biological mediators of olanzapine's antiemetic properties during acute and delayed phases of CINV. These aims are summarized in **Table 1.1**.

To address the first aim, a literature review of the pathophysiology and clinical implications of CINV, with a focus on olanzapine, was performed (**Chapter II**). This review examines what is known regarding the pathophysiology of CINV, available therapeutic options,

clinical guidelines, olanzapine as an antiemetic, and potential biological mediators of olanzapine's antiemetic properties.

The second aim, to assess the behavioral effects of olanzapine (**Chapter III**), was addressed by measuring the effects of olanzapine on pica (consumption of non-nutritive material- an established proxy for malaise), feeding, and body weight post chemotherapy. To determine the behavioral effects of systemic and centrally administered olanzapine, both intraperitoneal (systemic) and intracerebroventricular (central) olanzapine were administered.

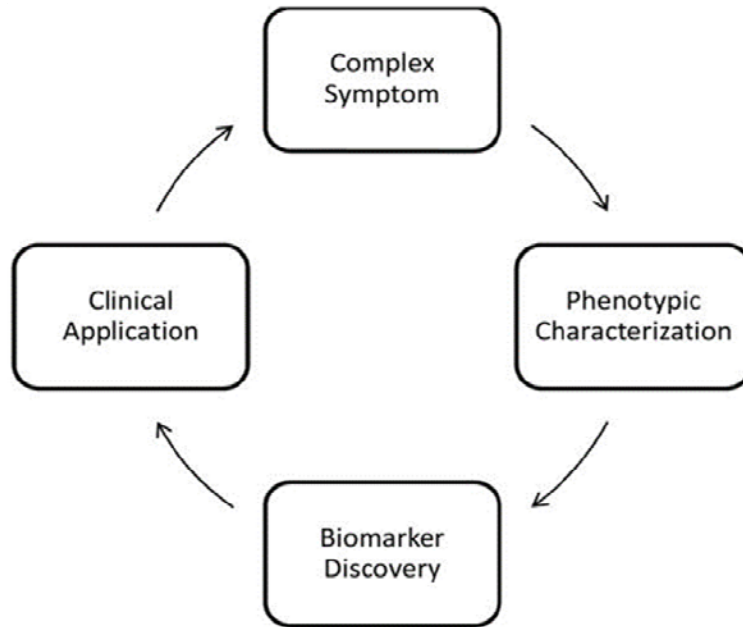
Building on the findings of our behavioral studies, the third aim was to identify potential biological mediators of olanzapine's effect on pica, anorexia, and body weight loss (**Chapter I**). First, immunohistochemical studies were conducted to assess olanzapine's site(s) of action by assessing its effect on neuronal activation at the Dorsal Vagal Complex (DVC), a brain region known to mediate CINV. Then, informed by our literature review (**Chapter II**), we tested two potential targets of olanzapine's therapeutic effect on CINV: serotonin and ghrelin/ghrelin. Accordingly, we tested ghrelin levels. Additionally, examined the effects of olanzapine on expression of serotonin receptors (HTR2C, HTR1A, HTR3, and HTR2A) and ghrelin receptor (GHSR) genes in the hypothalamus and the hindbrain (regions of the DVC).

### Summary

In summary, CINV is highly prevalent and understudied. Despite available antiemetic therapies, CINV continues to be cited among the most distressing, intolerable, and feared side effects of chemotherapy. However, despite its prevalence and severity, there is a gap in the literature regarding the pathophysiology of CINV. While the NINR supports the advancement of symptom science, CINV - and nausea as a standalone symptom - remain neglected in the context of care and research. Thus, it is imperative for research to focus on alleviating CINV. Particularly, studying olanzapine's antiemetic properties may shed light on important mediators of CINV and help identify therapeutic targets.

Figures

**Figure 1.1** The NIH Symptom Science Model



**Figure 1.1** The NIH-SSM. The NIH-SSM was developed to guide research. It begins with the presentation of a symptom; the symptom undergoes phenotypic characterization; then biomarkers are identified; and this ultimately leads to clinical applications, resulting in symptom reduction and improvement.

Tables

<b>Table 1.1 Specific Aims and How Each Was Addressed</b>	
<u>Specific Aim</u>	<u>How the aim was addressed</u>
Identify gaps in the olanzapine and CINV to identify potential biological mediators of olanzapine in the context of CINV	<ul style="list-style-type: none"> <li>• <b>Chapter II-</b> Chemotherapy induced nausea and vomiting: A literature review with a focus on olanzapine</li> </ul>
<p>Determine the behavioral effects of olanzapine on cisplatin-treated rats:</p> <ul style="list-style-type: none"> <li>• Does olanzapine decrease cisplatin-induced pica, anorexia, and weight loss?</li> </ul> <p><u>Hypothesis:</u> Both intraperitoneal and 4<sup>th</sup> ventricle olanzapine will decrease pica, anorexia, and weight loss in rats.</p>	<ul style="list-style-type: none"> <li>• <b>Chapter III-</b> olanzapine decreases chemotherapy-induced nausea, anorexia, and body weight loss in rats.</li> <li>a. <b>Experiment 1.1-</b> Effects of intraperitoneal olanzapine on cisplatin induced pica, anorexia, and body weight loss</li> <li>b. <b>Experiment 1.2-</b> Effects of fourth ventricle intracerebroventricular olanzapine on cisplatin-induced pica, anorexia, and body weight loss</li> </ul>
<p>Determine olanzapine’s effect on potential biological correlates of CINV and energy balance</p> <p><u>Hypothesis:</u> Olanzapine will decrease the number of c-Fos (marker of neuronal activation) positive neurons in the hindbrain (area postrema and nucleus of the solitary tract). Olanzapine will rescue cisplatin-related drops in circulating ghrelin. Olanzapine will decrease serotonin and ghrelin receptor expression in the</p>	<ul style="list-style-type: none"> <li>• <b>Chapter IV-</b> Olanzapine’s effect on biological mediators of CINV and energy balance</li> <li>a. <b>Experiment 2.1-</b> Assessment of olanzapine treatment on cisplatin-induced c-Fos in hindbrain (area postrema and nucleus of the solitary tract) neurons</li> <li>b. <b>Experiment 2.2-</b> Assessment of olanzapine treatment on cisplatin-induced reductions in plasma ghrelin</li> <li>c. <b>Experiment 2.3-</b> Assessment of olanzapine</li> </ul>



hypothalamus and the hindbrain.	treatment on cisplatin-induced alterations in central messenger RNA expression of ghrelin receptor and serotonergic receptors (5-HT2C, 5-HT2A, 5-HT3, 5-HT1A)
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CHAPTER II: CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING: A  
LITERATURE REVIEW WITH A FOCUS ON OLANZAPINE

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**Statement of Author Contributions:** Rosario B. Jaime-Lara, MSN, RN was the lead author on this article. She conceived of the study question, completed the analysis, drafted the article, and provided critical edits. Bart C. De Jonghe, PhD from the University of Pennsylvania School of Nursing, was the senior author of this article. Dr. De Jonghe was the PI of the parent study, and he provided strategic direction and critical edits for this article.

## Background

### *Defining emesis and CINV*

CINV is a major adverse effect of chemotherapeutic agents (Bloechl-Daum et al., 2006; P. Hesketh, 2005; Lindley et al., 1992; Osoba, Zee, Pater, et al., 1997). Vomiting, or emesis, refers to an involuntary response characterized in part by the forceful expulsion of contents from the upper gastrointestinal tract (P. Hesketh, 2005). The emetic response to chemotherapy and other stimuli is a complex polysynaptic neural pathway that is modulated by multiple sensory and motor systems to produce vomiting (P. L. B. P. G. S. Andrews, 1990; Horn, 2014b; D. Huang, Meyers, Henry, De la Torre, & Horn, 2011). The typical emetic response is a coordinated sequence of events where vagal and enteric input give rise to the emetic reflex. Prior to vomiting, a retrograde giant contraction (RGC) acts (under vagal control) to propel intestinal contents into the stomach for subsequent expulsion (P. L. Andrews, 1992; Lang, 2016). The RGC originates in the small intestine and travels toward the antrum, forcing chyme from the intestine to travel through the open pylorus and into the relaxed proximal stomach [*ibid*]. This is followed by the ejection phase comprised of two phases: 1) a retching phase and 2) a vomiting phase (P. L. Andrews, 1992; P. L. B. P. G. S. Andrews, 1990; Horn, 2014b; D. Huang et al., 2011). During retching, the diaphragm, abdominal muscles, and intercostal muscles contract, while the glottis remains closed. This movement results in a decrease in thoracic pressure and an increase in abdominal pressure (P. L. Andrews & Hawthorn, 1988; P. L. B. P. G. S. Andrews, 1990). Retching is believed to help prepare gastric content so that it is positioned for expulsion (P. L. B. P. G. S. Andrews, 1990). During the vomiting phase, there is an increase in both thoracic and abdominal pressure accompanied by a larger contraction of the stomach and the shortening of the esophagus (P. L. Andrews & Hawthorn, 1988; P. L. B. P. G. S. Andrews, 1990). This allows for the expulsion of the gastric contents [*ibid*].

In the context of this dissertation, nausea refers to the unpleasant sensation of malaise that often, but not always, precedes emesis (P. L. Andrews & Horn, 2006). However, it is important to note that unlike the definition of vomiting, there are multiple definitions of nausea found across the literature (Stern, 2011). The lack of consensus regarding the definition of nausea is likely due to the subjective nature of symptoms. Nausea (like other symptoms) is difficult to describe and detect in another person because it is a private sensation (Stern, 2011). This poses clinical challenges, including 1) variability in clinical assessment which could potentially alter treatment and 2) it makes it difficult to evaluate the efficacy of treatment. Nausea can be induced by the same stimuli as vomiting, and it often requires less intense stimulation (Horn, 2014a). However, it is not easier to treat or prevent nausea (Horn, 2007, 2008). Paradoxically, medications used to treat CINV are often able to treat/prevent vomiting better than nausea [*ibid*]. Furthermore, organisms are capable of experiencing vomiting without nausea and vice versa (Oman, 1987). For example, there are reports from astronauts in space that describe incidences of vomiting without prior sensation of nausea (Oman, 1987). This suggests that nausea may involve subtly independent pathways than those involved in emesis, and that although they may overlap, the two behaviors may be separate and distinctive (P. L. Andrews & Horn, 2006). Nonetheless, both of these phenomena are believed to serve as specialized warning systems that protect against toxic agents to ensure survival of the organism (Horn, 2014b).

### *Phases of CINV*

There are three distinct CINV phases: acute CINV, delayed CINV, and anticipatory nausea and vomiting (ANV) (Jacobsen & Redd, 1988; Kris et al., 1985; Moher, Arthur, & Pater, 1984; Roila et al., 1991). Acute CINV occurs within the first twenty-four hours following administration of a chemotherapeutic agent and has a peak in intensity at 5-6 hours post treatment (Kris et al., 1985; Roila et al., 1991). Delayed phase refers to CINV that takes place more than twenty-four hours post treatment (Kris et al., 1985). Delayed CINV peaks at 48-72 hours after

drug administration (Kris et al., 1985; Roila et al., 1991). Current “gold standard” antiemetics including, dexamethasone (an anti-inflammatory drug), serotonin type 3 receptor antagonists [5-HT<sub>3</sub>RAs], and neurokinin-1 receptor antagonists [NK-1RAs] have demonstrated variable efficacy in treating these phases. For example, 5-HT<sub>3</sub>RAs (e.g., ondansetron) are better at treating acute CINV, but not delayed CINV (Sanger & Andrews, 2006). Conversely, N-K1RAs (e.g., aprepitant and fosaprepitant) and olanzapine, demonstrate higher efficacy in treating delayed CINV (Campos et al., 2001; Cocquyt et al., 2001; Navari et al., 1999). This suggests that distinct chemical pathways may orchestrate these phases. The pathophysiology of CINV and the therapeutic options available for its treatment will be discussed in greater detail in subsequent sections.

ANV in response to chemotherapy refers to nausea and vomiting that occurs in “anticipation” of CINV and can occur in the absence of the chemotherapeutic agent (Jacobsen & Redd, 1988; Kamen et al., 2014). One theoretical mechanism of ANV is Pavlovian classical conditioning where a conditioned response occurs within/between multiple pairings of chemotherapy and a conditioned stimulus (e.g., hospital room, smells, healthcare personnel, etc.) result in the patient associating the stimuli to chemotherapy and its adverse effects (Jacobsen & Redd, 1988; Kamen et al., 2014). In other words, both chemotherapy and the conditioned stimuli can become associated with CINV [*ibid*]. Subsequently, the presence of the stimuli alone can be enough to induce nausea and/or vomiting (Kamen et al., 2014). For example, a patient can undergo multiple cycles of chemotherapy (accompanied by CINV) administered by a nurse. Seeing that nurse in future might induce nausea and vomiting even before, or independently of, the chemotherapy. This highlights the importance of psychosocial factors in patient care and suggests that the forebrain and other centers of cognition communicate with emetic centers to govern these associations.

There are numerous neuroanatomical regions involved in the genesis of CINV. A well-characterized anatomical site where the pathways controlling nausea and vomiting converge is in the caudal hindbrain within the brainstem. At this primitive and largely evolutionarily conserved area, there are neural circuits that integrate a wealth of information and organizational elements classically referred to as the “central pattern generator” (CPG) for emesis (Horn, 2007, 2014b). Although often referred to as a “vomiting center,” the CPG is not a “center” or discrete anatomical structure per se (P. L. Andrews & Hawthorn, 1988; Horn, 2007, 2014b; Miller & Wilson, 1983). Rather, it is a collection of nuclei in the brainstem which communicate in an interrelated fashion (Horn, 2007, 2014b). There are three main areas of physiological innervations to the CPG which have been shown to induce CINV when activated (Horn, 2014a, 2014b). These inputs include: (1) vagal-gastrointestinal, (2) the chemoreceptor trigger zone (CTZ), and (3) the forebrain (Horn, 2014a, 2014b), and may include elements of both the peripheral (vagal) and central nervous system (the CTZ, forebrain). Also, it is important to note that the CPG can be activated by different means and the circuits involved may interact with one another to induce or inhibit nausea and vomiting (17, 19, 35). The exact circuitry by which these inputs are integrated remains largely unknown. The sections below will provide a summary of the pathophysiology occurring within the three main physiological inputs listed above. **Table 2.1** provides a summary of abbreviation of terms and neuroanatomical regions and **Table 2.2** provides a summary of terms and neuroanatomical regions.

### *1) Vagal- gastrointestinal*

CINV has a strong gastrointestinal (GI) component where GI sensory stimuli are integrated by vagal afferents embedded in the peripheral nervous system (P. L. Andrews et al., 1990; P. L. Andrews & Hawthorn, 1988; P. L. Andrews & Horn, 2006; Horn, 2014b; Horn et al., 2014; Horn, Richardson, Andrews, & Friedman, 2004). Multiple studies across species (including- mice, rats, shrews, and ferrets) have provided evidence that the vagus nerve (cranial

nerve 10) plays a major role in CINV (P. L. Andrews et al., 1990; P. L. Andrews & Hawthorn, 1988; Fukui, Yamamoto, Sasaki, & Sato, 1993; Fukui, Yamamoto, & Sato, 1992; P. J. Hesketh, 2008). An intact vagus nerve is necessary to induce a maximal emetic response (P. L. Andrews et al., 1990; Fukui et al., 1993; Fukui et al., 1992). Additionally, studies in rats have shown that vagotomy of the common hepatic branch decreases neuronal activation and behavior indicative of chemotherapy-induced malaise (De Jonghe & Horn, 2008; Horn et al., 2004).

In the vagal-gastrointestinal pathway, free radicals are generated in the GI tract and other areas of the body after exposure to a chemotherapeutic agent [*ibid*]. This results in excessive exocytotic release of serotonin from enterochromaffin cells lining the small intestine (P. L. Andrews et al., 1990; P. J. Hesketh, 2008). Serotonin then binds to 5-HT<sub>3</sub> receptors at the vagal afferent terminals in the intestinal wall (P. L. Andrews et al., 1990; P. L. Andrews & Hawthorn, 1988; P. L. Andrews & Horn, 2006; Horn, 2014b; Horn et al., 2014; Horn et al., 2004). These afferents have axons which terminate in multiple regions of the brain implicated in emesis, including the CPG, dorsal vagal complex (discussed below), and have been shown to relay information to forebrain sites (Bayo et al., 2012; P. Hesketh, 2005; Inrhaoun, Kullmann, Elghissassi, Mrabti, & Errihani, 2012).

#### *Hindbrain and forebrain sites required for CINV*

##### *2) Chemoreceptor trigger zone (CTZ) within the dorsal vagal complex.*

The dorsal vagal complex (DVC) is a collection of nuclei within the hindbrain, including the nucleus of the solitary tract (NTS) and the area postrema (AP) (Bayo et al., 2012; "Dorsal Vagal Complex (DVC)," 2009; P. Hesketh, 2005). The DVC is located within the dorsomedial medulla oblongata and it receives input from visceral structures (described above) (Bayo et al., 2012; "Dorsal Vagal Complex (DVC)," 2009; P. Hesketh, 2005). Most of the visceral afferent signals terminate at the NTS (Travagli, 2007), which receives afferent signals from the gastrointestinal system via glutamatergic (excitatory neurotransmitter) synapses (Alhadeff &

Holland, 2015). Thus, the NTS integrates vital sensory information from cardiorespiratory and subdiaphragmatic organs of the gastrointestinal tract (Travagli, 2007). When the GI tract is exposed to noxious stimuli this information is relayed from the NTS to the CPG to induce emesis (P. Hesketh, 2005).

Another DVC structure that mediates emesis is the area postrema (AP). The AP is a circumventricular collection of neighboring nuclei that also receives input and innervates visceral structures ("Dorsal Vagal Complex (DVC)," 2009). The AP is known as the chemoreceptor trigger zone (CTZ) (Bayo et al., 2012; Inrhaoun et al., 2012), and as its name implies, it is an area known to have a role in chemical detection. It is positioned above the NTS at the inferior border of the fourth ventricle, an area that is relatively permeable and able to encounter and detect chemicals (Bayo et al., 2012; Zhou et al., 2015). At this site, capillaries are not tightly bound by glial cells and are easily permeated by chemical stimuli [*ibid*]. Thus, its anatomical features are consistent with its role in chemo-detection. Activation of this area is known to trigger CINV via two methods: 1) direct/indirect activation by vagal stimuli and 2) by detecting chemicals (endogenous chemicals or toxins) in the cerebrospinal fluid (P. L. Andrews & Hawthorn, 1988).

The vital role of the NTS and the AP is supported by dorsal vagal complex ablation studies (in rats and other species) and studies evoking emesis via chemical infusion into the cerebrospinal fluid (Beleslin & Strbac, 1987; Borison, Borison, & McCarthy, 1984; Coil & Norgren, 1981; Stewart, Burks, & Weisbrodt, 1977). Furthermore, animal studies utilizing immunohistochemical methods in both vomiting and non-vomiting species have shown c-Fos expression (a protein marker for neuronal activation) at the AP and NTS that are accompanied by behavioral signs of nausea following cisplatin (a potent chemotherapeutic agent known to induce CINV in almost 100% of people and animals in the absence of prophylaxis) treatment at multiple doses and across time periods treatment (Alhadeff & Holland, 2015; Billig, Yates, & Rinaman, 2001; De Jonghe & Horn, 2009; Holland, Leonard, Kensey, Hannikainen, & De Jonghe, 2014).



Another hindbrain region involved in emesis is the lateral parabrachial nucleus (PBN) (Bayo et al., 2012; "Dorsal Vagal Complex (DVC)," 2009; P. Hesketh, 2005). The PBN is located at the junction between the ventrolateral medulla and the pons [*ibid*]. Multiple studies demonstrate that cisplatin-chemotherapy activates neurons in the PBN (Alhadeff & Holland, 2015; Alhadeff et al., 2017; De Jonghe & Horn, 2009). Furthermore, recent studies conducted by Alhadeff *et al.* found that projections from the NTS to the lateral PBN may mediate cisplatin-induced nausea, anorexia, and weight loss (Alhadeff & Holland, 2015; Alhadeff et al., 2017). The lateral PBN serves as the secondary relay of visceral signals from NTS to forebrain regions, including the amygdala and the hypothalamus (discussed below) (Alhadeff & Holland, 2015; Alhadeff et al., 2017; De Jonghe & Horn, 2009; Yates, Catanzaro, Miller, & McCall, 2014).

### 3) Forebrain

Recent studies have suggested that the amygdala, a brain structure of the limbic system, is an important participant along the vagal-gastrointestinal CINV input described above (Alhadeff & Holland, 2015; De Jonghe & Horn, 2009; Holland et al., 2014; Horn et al., 2014). For example, in a 2014 study, Horn *et al.* utilized an anterograde tracer (a tracer that identifies axonal projections) to show that the NTS, AP, and the lateral parabrachial nucleus (IPBN) received input of axon terminals from vagal afferents known to induce emesis in musk shrews (Horn et al., 2014). Another 2014 study by Holland *et al.* in mice models demonstrated increased post-cisplatin expression of N-methyl-D-aspartic acid (NMDA) receptor subunit (a glutamate receptor subunit which is a major excitatory neurotransmitter) in the amygdala and other brain regions, including the DVC and PBN, (Holland et al., 2014). This expression was accompanied by increased neuronal activation at the DVC and PBN as well as behavioral changes (reduction in food intake and body weight) indicative of chemotherapy-induced malaise (Holland et al., 2014). A study by Alhadeff *et al.* identified a neural circuit (NTS→IPBN→CeA) activated by cisplatin-chemotherapy in which glutamate signaling (modulated by gene expressions of  $\alpha$ -amino-3-

hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA glutamate receptor subunits of neurons in the amygdala) is required for full expression of cisplatin-induced nausea, anorexia, and weight loss in rats (Alhadeff & Holland, 2015). More recently Alhadeff *et al.*, demonstrated that most NTS→IPBN and IPBN→CeA projections co-express vesicular glutamate transporter 2 (VGLUT2) (Alhadeff *et al.*, 2017). Together these data outline a neuronal circuit (NTS→IPBN→CeA) that is required for full expression of CINV and identify the phenotype of neurons within this circuit (neurons with excitatory projections that are modulated by glutamate receptors and VGLUT2).

Other brain regions that likely mediate chemotherapy-induced symptoms are the bed nucleus of the stria terminalis (BNST) and the hypothalamus (Gungor & Pare, 2016). The BNST is located in the basal forebrain and is known to mediate anxiety-like responses (elicited by different stimuli than amygdala-induced anxiety) to environmental threats (Gungor & Pare, 2016). Cisplatin induces c-Fos expression (48 hours post cisplatin administration) and increases in AMPA and NMDA subunit expression (24 hours post cisplatin treatment) at the BNST in rats and mice (Holland *et al.*, 2014; Horn, Ciucci, & Chaudhury, 2007). Furthermore, the hypothalamus regulates important functions, including energy balance, thermoregulation, and circadian rhythms (Olszewski, Grace, Billington, & Levine, 2003). A study by Yamamoto *et al.* found that intraperitoneal cisplatin (30 mg/kg) increases glutamate release in the hypothalamus 1 hour following cisplatin treatment and this rise is associated with emesis (Yamamoto *et al.*, 2009). This study also found that 5-HT<sub>3</sub>RA (known to reduce CINV) inhibited hypothalamic glutamate levels (Yamamoto *et al.*, 2009). Furthermore, decreases in hypothalamic ghrelin receptor expression are associated with CINV and chemotherapy-induced anorexia (Olszewski *et al.*, 2003; Yakabi, Kurosawa, *et al.*, 2010; Yakabi, Sadakane, *et al.*, 2010). The role of hypothalamic ghrelin is discussed in further detail below.

The limbic system and the amygdala are also known to play a role in anticipatory CINV. The amygdala is known to be an integrative center for emotions (Desmedt, Marighetto, Richter-

Levin, & Calandreau, 2015; P. Hesketh, 2005). It receives and interprets sensory and visceral stimuli to produce intense emotional responses, such as fear. Pathways from the thalamus to the amygdala are important in emotional learning and fear conditioning (Desmedt et al., 2015; P. Hesketh, 2005). This mechanism is believed to play a role in the conditioning that is characteristic of anticipatory CINV (P. Hesketh, 2005; Horn, 2014b). For example, anticipatory CINV can develop as a learned response to chemotherapy after patients are exposed to multiple treatment cycles (Bernstein, 1985; Mattes, 1994; Schwartz, Jacobsen, & Bovbjerg, 1996). In fact, the strongest predictor of nausea or vomiting in subsequent chemotherapy cycles is the presence or absence of CINV in the first cycle of chemotherapy (Warr, 2014).

#### The Use of Pre-Clinical Models in the Study of CINV

As is reflected by existing knowledge of the pathophysiology of CINV, pre-clinical animal models - including: mice, rats, house musk shrews, and ferrets- have been instrumental to the study of CINV. For example, ferret models played an important role in the discovery of 5-HT<sub>3</sub> receptor antagonists (Christie, 2007; Leslie et al., 1990). However, the use of animal models in the study of CINV poses some challenges, as some animals do not possess the capacity to vomit, and nausea and other symptoms (e.g. pain and fatigue) are self-reported sensations that cannot be directly verbalized by non-human animals (Horn, 2014a). It is important to note that this challenge (measuring a subjective sensation in a non-verbal animal) also applies to humans who are unable to verbalize their symptoms (e.g. infants, patients suffering from dementia, patients with speech impairments following a stroke, etc.).

Each species demonstrates unique physiological and behavioral responses, some of which are similar to humans. For example, although rats and mice lack a vomiting reflex, they demonstrate similar brainstem and limbic forebrain activation in response to chemotherapy compared to vomiting species such as ferrets and shrews (Billig et al., 2001; De Jonghe & Horn,

2009; Holland et al., 2014; Horn et al., 2007). Importantly, although rodents do not vomit, they also exhibit behavior, pica (consumption of non-nutritive substances), that serves a similar evolutionary function to eliminate toxic substances. Rodents ingest non-nutritive substances (such as clay) that bind to toxins and are eliminated by the gastrointestinal system through the feces (De Jonghe, Lawler, Horn, & Tordoff, 2009; Mitchell, Krusemark, & Hafner, 1977; Mitchell et al., 1976; N. Takeda, Hasegawa, Morita, & Matsunaga, 1993). Antiemetics used to treat CINV are also effective at reducing pica, and peaks in chemotherapy-induced pica in rodents mirror peaks in CINV observed in humans (Malik, Liu, Cole, Sanger, & Andrews, 2007; Yamamoto, Matsunaga, Matsui, Takeda, & Yamatodani, 2002). Thus, despite their inability to vomit, these “lower” mammalian species are useful models for studying central nausea circuits found in “higher” mammalian species. The translational value of the animal-model data must be independently assessed and compared to other animals/human parameters.

Although animals and non-verbal humans cannot directly communicate or report sensation (i.e., nausea), nausea may be inferred through the quantification of observed behaviors and measurement of physiological parameters associated with nausea (P. L. Andrews & Horn, 2006; Horn, 2014b; J. E. Smith, Friedman, & Andrews, 2001). Some examples of behavioral measures in rodents include: pica (consumption of non-nutritive material- such as clay- presumably to dilute toxins in the body), conditioned taste aversion, decreased food consumption, and decreased movement (P. L. Andrews & Horn, 2006; De Jonghe et al., 2009; Stern, 2011). Examples of potential biological markers include plasma vasopressin (a hormone that plays an important role in fluid regulation) (Billig et al., 2001) and gastric dysrhythmias which precede nausea/emesis (Koch, 2014; Ueno & Chen, 2004). Hawthorn *et al.*, found that administration of apomorphine (a potent emetic agent) dramatically increased levels of vasopressin in ferrets (Hawthorn, Andrews, Ang, & Jenkins, 1988). Additionally, a study conducted by Xu *et al.*, found that severity of nausea (induced by motion) correlated with increases in plasma vasopressin in healthy adults (Xu et al., 1993). Multiple studies have found that gastric dysrhythmias are

associated with nausea and vomiting (Koch, 2014; J. Liu, Qiao, & Chen, 2006; Ueno & Chen, 2004). For example, Ueno *et al.* found that gastric dysrhythmias preceded vomiting induced by gastric electrical stimulation in dogs (Ueno & Chen, 2004). Additionally, a study by Liu *et al.* found that gastric electrical stimulation for gastric dysrhythmias reduced nausea and vomiting in dogs (J. Liu et al., 2006). Thus, studies suggest that gastric dysrhythmias are an objective biomarker for nausea and a potential therapeutic target for antiemetic treatment.

Animal studies can have a smaller sample size compared to human studies because environmental variations and variability between subjects are controlled, and thus these studies do not require a large sample size to achieve statistical power (Horn, 2014a). Another benefit of utilizing pre-clinical models is that studies conducted on humans are limited by stricter ethical principles. For example, use of chemotherapeutic agents outside of the context of patient care is prohibited in humans. Moreover, animal studies are required before trials escalate to clinical phases. Thus, there are advantages and challenges in utilizing animal models and experimental design should aim to achieve a balance between efficiency (decreased costs and decreased ethical/time constraints) and translatability (Acton & Winter, 2002).

## Clinical Guidelines for the Prevention and Management of CINV

### *Chemotherapy emetic risk categories*

Antiemetic prophylaxis and treatment depend on the emetogenic potential of the chemotherapeutic agent and the type of CINV (acute, delayed, or anticipatory) (Inrhaoun et al., 2012; Jordan, Gralla, Jahn, & Molassiotis, 2014; Jordan, Jahn, & Aapro, 2015). There are 4 categories of emetogenicity: 1) highly emetic (HEC), 2) moderately emetic (MEC), 3) low emetic (LEC), and 4) minimally emetic. These categories correspond with 90% risk or greater, 30-90% risk, 10-30% risk, and less than 10% risk of emesis respectively.

### *Antiemetic regimen by emetic risk category*

Current guidelines, including those set forth by the Multinational Association of Supportive Care in Cancer (MASCC) and the American Society of Clinical Oncology (ASCO), utilize emetogenicity risk to guide CINV management (Inrhaoun et al., 2012; Jordan et al., 2014; Jordan et al., 2015). For regimens involving more than one chemotherapeutic agent, the emetogenic category is determined by the most emetogenic drug (P. Hesketh, 2015). 5-HT<sub>3</sub> receptor antagonists [5-HT<sub>3</sub>RAs], olanzapine, and NK-1 receptor antagonists [NK-1RAs], and dexamethasone are among the pharmacological agents recommended by current clinical guidelines for CINV prevention ((NCCN), 2017; Jahn, Jordan, & Rizzi). The prophylactic regimen for acute CINV induced by HEC is comprised of: 1) 5-HT<sub>3</sub>RAs, 2) dexamethasone, and 3) an NK-1RAs or olanzapine, while only dexamethasone and an NK-1RAs or olanzapine are recommended for delayed CINV induced by HEC (Basch et al., 2011; P. Hesketh, 2015; Roila et al., 2010). To prevent acute CINV caused by MEC, guidelines recommend: 5-HT<sub>3</sub>RAs and glucocorticoids (e.g. dexamethasone) to be administered, whereas for delayed CINV for MEC either dexamethasone or 5-HT<sub>3</sub>RAs alone are recommended [*ibid*]. To prevent acute CINV caused by LEC, dexamethasone is recommended as prophylaxis; no preventative measures are recommended for delayed CINV for LEC (Basch et al., 2011; P. Hesketh, 2015; Roila et al., 2010). Lastly, guidelines do not suggest any preventative measures prior to minimally emetogenic chemotherapy [*ibid*].

Multiple studies have determined that guideline-consistent CINV prophylaxis is associated with decreased CINV and improved patient outcomes (Jordan et al., 2015). This highlights the importance of implementing and adhering to treatment guidelines. Despite the literature supporting the benefit of following clinical guidelines, adherence to treatment guidelines is suboptimal (Clark-Snow, Affronti, & Rittenberg, 2017; Jahn et al.; Jordan et al., 2015). A 2017 nationwide study by Clark-Snow *et al.* found adherence to antiemetic guidelines was low (73% for acute and 25% for delayed phases), especially during the delayed phase (Clark-

Snow et al., 2017). Thus, it is important for clinicians to integrate clinical guidelines into their practice. Nurses should be familiar with guidelines, so they can advocate for patients to receive optimal treatments which better prevent and treat CINV.

### Therapeutic Options for the Treatment of CINV

The introduction of chemotherapy in the late 1950s was revered by the medical community, because it was the first therapeutic option known to cure cancer (Institute). However, antineoplastic agents, such as the HEC cisplatin, induce severe nausea and vomiting (P. J. Hesketh, 2008). The introduction of antiemetics including dexamethasone, 5-HT<sub>3</sub>RAs, and NK-1RAs reduced the incidence of vomiting from 90% to 50% in people undergoing chemotherapy (Rojas, Raje, Tsukamoto, & Slusher, 2014). Dexamethasone and newer antiemetic agents are described in detail below.

#### *Dexamethasone*

Dexamethasone is a synthetic steroid that is used as an anti-inflammatory, anti-allergic, and antiemetic drug (P. Hesketh, 2005). Dexamethasone mimics endogenous glucocorticoids, which are secreted by the adrenal cortex and stimulate the release of cortisol (a hormone that regulates metabolism and immune response) (P. Hesketh, 2005; Morrow, Hickok, Andrews, & Stern, 2002). In the 1980s dexamethasone was reported to be effective in the prevention of CINV (Chu et al., 2014). Subsequently, dexamethasone and other glucocorticoids were shown to successfully prevent CINV, radiotherapy-induced nausea and vomiting, and post-operative nausea and vomiting (Chu et al., 2014). Currently, dexamethasone is widely used in combination with other agents to prevent CINV (Basch et al., 2011; P. Hesketh, 2015; Roila et al., 2010). However, dexamethasone's antiemetic mechanisms are not fully understood.

A recent review suggests that dexamethasone prevents CINV, through its anti-inflammatory effect, its direct effect on the NTS, its effect on serotonin, and its regulation of the

HPA axis (Chu et al., 2014). Dexamethasone and other glucocorticoids are anti-inflammatory and can attenuate cisplatin-induced production of eicosanoids (inflammatory mediators- e.g. prostaglandins, thromboxanes, and lipoxins) (Chu et al., 2014). Dexamethasone can also decrease the transcription of inflammatory cytokines (TNF- $\alpha$ , IL-1, -2, -3, etc.) and NK-1 and NK-2 receptors (Barnes, 1998; Chu et al., 2014). Secondly, dexamethasone may act directly at the NTS reducing CINV; a study by Ho *et al.* in cats found that microinjection of dexamethasone (100 nanoliters) into the bilateral NTS decreased xylazine-induced emesis (Ho, Ho, Wang, Tsai, & Chai, 2004). The third proposed mechanism is that glucocorticoids reduce CINV by acting on serotonin receptors (Chu et al., 2014; Suzuki, Sugimoto, Koyama, Mashimo, & Uchida, 2004). An electrophysiology study by Zusuki *et al.*, in xenopus oocytes found that dexamethasone directly inhibited the effect of 5-HT<sub>3</sub> receptors (known to mediate CINV) by decreasing serotonin electric current (a measure of electrical activity in neurons) by 50% (Suzuki et al., 2004). Lastly, dexamethasone's antiemetic effects may be related to its effect on the hypothalamic pituitary adrenal (HPA) axis, where it alters cortisol and adrenocorticotrophic hormone (ACTH), a hormone secreted by the anterior pituitary gland that mediates cortisol release (C. Huang et al., 2012; Morrow et al., 2002). Chemotherapy alters HPA axis activity, with studies demonstrating that chemotherapy reduces serum cortisol and ACTH in patients with different cancer diagnoses (Chu et al., 2014; C. Huang et al., 2012; Morrow et al., 2002). Thus, chemotherapy may induce CINV by impeding normal physiological function of HPA axis activity, and dexamethasone may revert these changes.

#### *5-HT<sub>3</sub> Receptor Antagonists [5-HT<sub>3</sub>RAs]*

The introduction of 5-HT<sub>3</sub>RAs is considered one of the most important advances in CINV research (P. J. Hesketh, 2008). They are currently the single most effective pharmacological agent for the prevention of acute CINV (P. Hesketh, 2015). First generation receptor antagonists work through competitively inhibiting the binding of serotonin to the 5-HT<sub>3</sub> receptor [*ibid*]. There are



currently five first-generation 5-HT<sub>3</sub>RAs -dolasetron, granisetron, ondansetron, ramosetron, and tropisetron- available for the treatment of CINV (P. Hesketh, 2015). All five agents, in both oral and intravenous formulations, have demonstrated equal effectiveness in preventing CINV (Gandara et al., 1998; R. J. Gralla et al., 1998; P. Hesketh, 2015; Perez et al., 1998). However dolasetron and ondansetron carry a risk of QTc prolongation (a measure of heart electrical activity indicating ventricular tachyarrhythmias and increasing risk of cardiac arrest/sudden death), and thus the FDA has placed restrictions on their use (P. Hesketh, 2015).

In 2003, the first and only second-generation agent, palonosetron, came to market (Rojas et al., 2014). Compared to first generation agents, palonosetron has a higher affinity for 5-HT<sub>3</sub> receptors and has a longer half-life (Aapro, 2005; Zhou et al., 2015). Presumably due to these more favorable pharmacological properties, palonosetron has not been associated with QTc prolongation and has been found to be effective in preventing both acute and delayed CINV (Aapro, 2005; Eisenberg et al., 2003; R. Gralla et al., 2003; Zhou et al., 2015). Unlike first generation agents that act through competitively inhibiting serotonin binding to 5HT<sub>3</sub> receptors, palonosetron is an allosteric receptor antagonist (it binds to a site other than the receptor active site resulting in a conformational change that inhibits binding) (Rojas et al., 2014). This difference in binding suggests palonosetron could have a unique effect on receptor function. It was suggested that this second-generation agent works through internalization of the 5-HT<sub>3</sub> receptors. Four findings support this theory (Rojas et al., 2014). First, palonosetron has a longer half-life than first generation agents CINV (Aapro, 2005; Eisenberg et al., 2003; R. Gralla et al., 2003). This would be expected since reversing receptor internalization would require more time than removing competitive inhibitors. Second, the antagonizing effects of palonosetron are more resistant to extracellular proteases compared to drugs that act upon cell-surface receptors (Rojas et al., 2014). This suggests that its effect on 5-HT<sub>3</sub> receptors occurs within the cell, not on the cell surface. Third, cells treated with palonosetron have decreased extracellular surface area, suggesting receptors (which would increase surface area) are not located on the cell surface

(Rojas et al., 2014). Lastly, visualization with florescent microscopy has further confirmed the internal location of 5-HT<sub>3</sub> receptors on cells treated with palonosetron [*ibid*]. Thus, palonosetron offers new and promising properties to better manage CINV.

#### *NK-1 Receptor Antagonists [NK-1RAs]*

Neurokinin-1 receptor antagonists inhibit CINV by blocking the binding of Substance P to NK-1 receptors in both central and peripheral locations (P. J. Hesketh, 2008; Navari, 2013; Rojas et al., 2014). Aprepitant, its prodrug fosaprepitant, and rolapitant are among the only NK-1RA currently approved for the prevention of highly emetogenic chemotherapy (HEC) or moderately emetogenic chemotherapy (MEC) (Bosnjak et al., 2017; Institute; Rapoport et al., 2016). Unlike first generation 5-HT<sub>3</sub> receptor antagonists alone, NK-1RAs in combination with palonosetron are more effective at treating delayed CINV (Bosnjak et al., 2017; Roscoe et al., 2012). A study by Aapro *et al.* showed that aprepitant and fosaprepitant given in conjunction with 5-HT<sub>3</sub>RAs and dexamethasone were more effective than ondansetron alone and dexamethasone for controlling delayed emesis and reduced the need for rescue medication (Aapro et al., 2015). These two drugs are also well tolerated and have fewer adverse effects than ondansetron and metoclopramide (Van Belle et al., 2002). Other studies have demonstrated that the rate of complete response (no emesis or rescue treatment following chemotherapy) was higher on days 1-5 for patients that had received aprepitant compared to 5-HT<sub>3</sub>RAs and dexamethasone alone (P. J. Hesketh et al., 2003; Poli-Bigelli et al., 2003). Thus, aprepitant and fosaprepitant are now included in current clinical guidelines ((NCCN), 2017; Roila et al., 2010).

Another drug combination including netupitant, a highly selective NK-1RA, was recently approved in the United States for the treatment of highly emetogenic chemotherapy (P. J. Hesketh, Palmas, & Nicolas, 2017). This drug combination of netupitant plus palonosetron (NEPA) has proven to have comparable efficacy and safety compared to aprepitant (P. Hesketh, 2015; P. J. Hesketh et al., 2017). Furthermore, NEPA given in combination with dexamethasone

was found to be superior in efficacy compared to oral palonosetron plus dexamethasone (Jordan et al., 2015). In addition, patients receiving NEPA also reported fewer decreases in function than patients receiving palonosetron alone (Jahn et al.). Lastly a 2017 study by Hesketh *et al.* found that NEPA was effective and well tolerated in lung cancer patients receiving their first cycle of HEC across both acute and delayed phases of CINV and across treatment cycles (P. J. Hesketh et al., 2017). Thus, this new drug combination has become a promising new treatment for CINV.

### Olanzapine as a Novel Agent for the Prevention and Management of CINV

Olanzapine was recently added to CINV antiemetic guidelines after case studies, chart reviews, and proceeding clinical trials demonstrated oral olanzapine effectively attenuates CINV in patients with different primary cancer sites (Jackson & Tavernier, 2003; Navari et al., 2007; Navari et al., 2005; Navari et al., 2011; S. D. Passik et al., 2003; S. D. Passik et al., 2002; Pirl & Roth, 2000; Srivastava et al., 2003). Olanzapine is an atypical antipsychotic that has broad spectrum binding characteristics known to block multiple serotonergic, histaminic, muscarinic, and dopaminergic receptors. Common side effects of olanzapine include sedation and weight gain (S. Gupta et al., 1999; Ratzoni et al., 2002). Since the early 1970s, olanzapine has been used to treat schizophrenia and bipolar disorder (Jordan et al., 2015). However, in the early 2000s, multiple case studies reported olanzapine effectively attenuates nausea (Jackson & Tavernier, 2003; S. D. Passik et al., 2003; S. D. Passik et al., 2002; Pirl & Roth, 2000; Srivastava et al., 2003). The first case study, presented by Pirl and Roth reported olanzapine (5mg/day) improved nausea in a patient suffering from chronic nausea (Pirl & Roth, 2000). Another set of six case studies by Jackson and Tavernier also found olanzapine (2.5 mg/day titrated to 5mg/day) was effective in treating intractable nausea in palliative care patients diagnosed with different types of cancer, heart disease, or Alzheimer's disease (Jackson & Tavernier, 2003). A pilot study by Passik *et al.* found that in 15 patients with advanced cancer (with different primary tumor sites),

three doses of oral olanzapine (2.5 mg/day, 5.0 mg/day, and 10 mg/day) given for two days significantly reduced nausea (full control was achieved in over 90% of patients compared to a baseline of less 40%) and were associated with improvements in quality of life for all dosage conditions (S. D. Passik et al., 2002). A follow up retrospective chart review of 28 patients (with different primary cancer sites and stages of cancer) who had received olanzapine (2.5 mg or 5 mg twice daily) found it reduced the incidence of delayed CINV in patients receiving MEC and HEC to 20% (compared to an incidence of 50% in patients receiving standard antiemetics-dexamethasone, 5-HT3, and/or NK-1RAs) (S. D. Passik et al., 2003).

These case studies led to the implementation of a series of clinical trials testing the efficacy of oral olanzapine in the prevention and management of CINV. A phase I study conducted by Passik *et al.* using an olanzapine dose escalation (5mg to 10mg prior to chemotherapy and on days 0-7), reported that olanzapine was effective in fully controlling CINV (in 100% of patients receiving MEC and in 67% of patients receiving HEC) in 15 patients with varied primary cancer sites (Steven D. Passik et al., 2004). Phase II trials found olanzapine (5 mg/day prior to chemotherapy and on day 1-4) given in combination with dexamethasone and 5-HT3RAs was safe and very effective (full control was achieved in over 80% of the 30 participants) at controlling both acute and delayed CINV in patients with different forms of cancer (Navari et al., 2007; Navari et al., 2005). A phase III clinical trial (251 participants) found olanzapine (10 mg/day prior to chemotherapy and on day 1-4) had superior efficacy in the prevention of nausea after HEC compared to NK-1RAs (full control in up to 86% vs 65% of participants), reduced CINV costs, and was well tolerated by oncology patients with different types and stages of cancer (Bosnjak, Dimitrijevic, & Djordjevic, 2016; Navari et al., 2007; Navari et al., 2005). In another phase III clinical trial (276 participants) olanzapine (10 mg/day for 3 days) was also found to be superior in managing breakthrough CINV compared to metoclopramide (full control in 70% vs 30% of participants) in patients with different cancer diagnoses (Navari, 2013). Thus, olanzapine's superiority and efficacy in reducing CINV led to

the addition of olanzapine (10 mg orally prior to chemotherapy and on days 1-4 following chemotherapy) to National Cancer Comprehensive Network (NCCN) antiemetic guidelines in 2014 as an adjunctive therapy to 5-HT3RAs and dexamethasone ((NCCN), 2017; NCCN, 2014). Since 2014 and as recently as July 2017, other antiemetic guidelines, including the American Society of Clinical Oncology (ASCO) and the Multinational Association of Supportive Care In Cancer (MASCC) guidelines have been modified to include olanzapine to prevent CINV and/or to treat breakthrough CINV (Cancer, 2016; Oncology, 2017).

In addition to its efficacy as an antiemetic, olanzapine is a particularly attractive pharmacotherapy for its potential to induce weight gain (Kim et al., 2008; Naing et al., 2015; Q. H. Zhang, M. Lian, J. Deng, C. Huang, X., 2012). More than 90% of people taking olanzapine for the treatment of schizophrenia and bipolar disorder gain weight within the first 3 months of initiating treatment (S. Gupta et al., 1999; Ratzoni et al., 2002). Olanzapine is also associated with more weight gain compared to all other second-generation antipsychotics (Lett et al., 2012; Zhu et al., 2017). However, few studies have been conducted to test the effect of olanzapine on body weight in cancer patients (Naing et al., 2015; Navari & Brenner, 2010). A study by Naing *et al.* found oral olanzapine (doses ranging from 2.5 to 10 mg/day for 8 weeks) had a modest effect on weight, with a trend towards the reduction of weight loss in patients with advanced (stage III and stage IV) cancer with diverse tumor types (Naing et al., 2015). Another study by Navari *et al.* found oral olanzapine (5 mg/day for 4 weeks) when given with megestrol acetate, significantly improved weight and appetite in 43% of patients with advanced (stage III and stage IV) gastrointestinal or lung cancer (compared to megestrol acetate alone)(Navari & Brenner, 2010). Further studies must be conducted to establish olanzapine's efficacy in improving weight loss in oncology patients.

While the anti-psychotic effects of olanzapine are thought to be mediated primarily by the blockade of serotonin and dopamine receptors (Reynolds, 2011; Seeman, 2002; Stahl, 2013), the molecular mechanisms that underlie its effects on nausea and vomiting are largely unknown.

However, olanzapine is known to impact energy balance through serotonin type 2c receptors [5-HT<sub>2</sub>CRs] and ghrelin (including circulating ghrelin and its receptor- growth hormone secretagogue receptor) and these metabolic pathways overlap with known CINV pathways (Hattori, 2010; Murashita et al., 2005; van der Zwaal et al., 2012; Yakabi, Sadakane, et al., 2010). Identifying mediators that contribute to olanzapine's antiemetic properties can elucidate important details about mechanisms of alleviating CINV and can help identify therapeutic targets.

#### *Potential Mediators of Olanzapine's Antiemetic properties*

Potential mediators of olanzapine's therapeutic effect on CINV include 5-HT<sub>2</sub>CRs, circulating ghrelin, and growth hormone secretagogue receptor (GHSR). Olanzapine and cisplatin-chemotherapy are known to have opposite effects on serotonin and ghrelin systems (Hattori, 2010; Murashita et al., 2005; van der Zwaal et al., 2012; Yakabi, Sadakane, et al., 2010). Olanzapine is known to antagonize 5-HT<sub>2</sub>CRs (Bymaster et al., 1996; Fuller & Snoddy, 1992), increase plasma ghrelin (Murashita et al., 2005; van der Zwaal et al., 2012; Weston-Green, Huang, & Deng, 2011), and increase GHSR1 expression (Q. Zhang et al., 2014; Q. H. Zhang, M. Lian, J. Deng, C. Huang, X, 2012), while cisplatin is known to stimulate 5-HT<sub>2</sub>CR expression (Yakabi, Sadakane, et al., 2010) and decrease plasma ghrelin (Hiura, Takiguchi, Yamamoto, Kurokawa, et al., 2012; H. Takeda et al., 2008). Thus, olanzapine may work by counteracting the effects of cisplatin by decreasing 5-HT<sub>2</sub>CR expression, increasing circulating ghrelin, and increasing GHSR.

#### *5-HT<sub>2</sub>C Receptors and Their Potential Role in Chemotherapy-induced Symptoms*

Olanzapine has a high affinity for 5-HT<sub>2</sub>CRs and is known to antagonize these receptors (Bymaster et al., 1996; Kirk, Glazebrook, Grayson, Neill, & Reynolds, 2009). 5-HT<sub>2</sub>C is a serotonin receptor subtype (Bymaster et al., 1996), that is located in high concentrations in the brain at regions known to mediate CINV: the NTS, CeA, and hypothalamus (Wright, Seroogy,

Lundgren, Davis, & Jennes, 1995) (Alhadeff & Holland, 2015; De Jonghe & Horn, 2009; Holland et al., 2014). Thus, 5-HT<sub>2</sub>CRs' location makes them likely candidates for involvement in CINV. Additionally, 5-HT<sub>2</sub>CRs' functional roles make these receptors particularly attractive for the study of CINV. 5-HT<sub>2</sub>CRs have been linked to nausea and other GI symptoms- including loss of appetite (Fujitsuka et al., 2009; Okada, Saito, & Matsuki, 1995; Silenieks, 2014; S. R. Smith et al., 2009; Yakabi, Sadakane, et al., 2010). Specifically, olanzapine is known to cause weight gain via serotonin receptor 2C antagonism (X. F. Huang, Weston-Green, & Yu, 2017; Lord et al., 2017) and inhibition of 5-HT<sub>2</sub>CRs has been linked to decreases in nausea (Okada et al., 1995; H. Takeda et al., 2008). Conversely, cisplatin treatment has been associated with increased activation of 5-HT<sub>2</sub>CRs (Y. K. Gupta & Sharma, 2002; Hattori, 2010; Okada et al., 1995; H. Takeda et al., 2008) and increases the expression of 5-HT<sub>2</sub>CRs in the brain (Yakabi, Sadakane, et al., 2010). This stimulation of 5-HT<sub>2</sub>CRs in the brain is linked to GI side effects- including nausea (Y. K. Gupta & Sharma, 2002; H. Takeda et al., 2008). Thus, olanzapine may decrease CINV by antagonizing 5-HT<sub>2</sub>CRs in the brain.

#### *Ghrelin and Its Potential Role in Chemotherapy-Induced Symptoms*

Ghrelin is an orexigenic hormone that is mainly secreted by the stomach during fasting and shortly before meals (Inui, 2001; Inui et al., 2004). Ghrelin is a high affinity ligand for the growth hormone secretagogue receptor 1 (GHSR1), which is highly expressed in the DVC and in the hypothalamus (Inui, 2001; Kojima et al., 1999; Yakabi, Sadakane, et al., 2010). Olanzapine increases GHSR expression, potentiates ghrelin-mediated GHSR1 activity, and increases circulating ghrelin levels (Tagami et al., 2016; Weston-Green et al., 2011; Q. Zhang et al., 2014). These findings combined with pre-clinical and clinical evidence demonstrating ghrelin-mediated decreases in CINV, anorexia, and cachexia (Hiura, Takiguchi, Yamamoto, Takahashi, et al., 2012; Y. L. Liu, Malik, Sanger, & Andrews, 2006), suggest that OLZ's antiemetic/anti-anorectic effect may be mediated by the ghrelin system. However, the effect of olanzapine on plasma

ghrelin and GHSR expression in the brain, have not been studied post chemotherapy. Determining if olanzapine decreases CINV through potentiating ghrelin can help clarify the role of ghrelin in CINV. It also highlights ghrelin enhancement as a potential treatment for CINV.

#### *5-HT2CR and GHSR Interaction*

Multiple studies have reported an interaction between the serotonin and ghrelin signaling pathways in the brain (Aoki et al., 2016; Dryden, Frankish, Wang, Pickavance, & Williams, 1996; Hansson et al., 2014; Schellekens et al., 2015). Studies have shown that administration of 5-HT2CR agonists inhibit ghrelin-induced orexigenic effects, and GHSR1 activity decreases in response to serotonin (Currie, John, Nicholson, Chapman, & Loera, 2010; Schellekens et al., 2015). Furthermore, centrally administered ghrelin increases 5-HT2CR expression in the amygdala and dorsal raphe, while centrally administered 5-HT2CR agonists decrease ghrelin-induced food intake in rats (Hansson et al., 2014; Schellekens et al., 2015). Additionally, 5-HT2CR agonists decreases hypothalamic neuropeptide Y (NPY), a GHSR1 downstream orexigenic signal, expression in the hypothalamus and its subsequent ghrelin-induced food intake (Aoki et al., 2016; Dryden et al., 1996). Furthermore, 5-HT2CR and GHSR1 colocalize in hypothalamic neurons (Schein et al., 1970). Thus, these studies suggest there is a 5-HT2CR, GHSR1a, ghrelin, and NPY interaction in the brain.

Most recently, this interaction was established through experiments showing that 5-HT2CR and GHSR1 form a heterodimer that inhibits the effects of ghrelin (Schellekens et al., 2015; Schellekens, van Oeffelen, Dinan, & Cryan, 2013). Flow cytometry fluorescence resonance energy transfer (fcFRET) studies demonstrate there is a direct interaction between the 5-HT2CR and GHSR1a, which translates into a biologically significant inhibition of ghrelin-induced food intake (Schellekens et al., 2015). 5-HT2CR/GHSR1 dimerization can lead to co-internalization, which prevents GHSR activity on the cellular surface and subsequently blocks ghrelin's orexigenic effects (Schellekens et al., 2015; Schellekens et al., 2013). Importantly, olanzapine



reduces hypothalamic 5-HT<sub>2C</sub>R/GHSR1 dimerization in a dose-dependent manner (X. F. Huang et al., 2017). Since, olanzapine is a 5-HT<sub>2C</sub>R agonists and upregulates GHSR1, its blockade of 5-HT<sub>2C</sub>R may interfere with its inhibition of GHSR, blocking GHSR's constitutive effects on food intake and CINV. However, olanzapine's effect on 5-HT<sub>2C</sub>R/GHSR dimerization in relationship to CINV has not been studied.

### Summary

Great advancements in our knowledge of the pathophysiology of CINV have been made within the last 30 years, and this knowledge has led to improvements in prevention, treatment, and management of CINV (Institute). Pharmacological discoveries such as the identification of 5-HT<sub>3</sub>RAs, NK-1RAs, and olanzapine have improved the prevention and management of CINV and are reflected in current clinical guidelines. Despite these advances, CINV continues to be prevalent (Bloechl-Daum et al., 2006; Lindley et al., 1992; Osoba, Zee, Warr, et al., 1997). Better understanding of the mediators of effective pharmacotherapies -specifically olanzapine- can help in identifying therapeutic targets to better alleviate CINV.

## Tables

**Table 2.1 Index of Abbreviations**

<b>Abbreviation</b>	<b>Term</b>
<b>5-HT</b>	Serotonin
<b>5-HT2CR</b>	serotonin type 2 C receptor
<b>5-HT3RAs</b>	serotonin 3 receptor antagonist
<b>ACTH</b>	adrenocorticotrophic hormone
<b>AMPA-receptor subunit</b>	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit
<b>ANV</b>	anticipatory nausea and vomiting
<b>AP</b>	area postrema
<b>ASCO</b>	American Society of Clinical Oncology
<b>CeA</b>	central amygdala
<b>CINV</b>	chemotherapy-induced nausea vomiting
<b>CPG</b>	central pattern generator
<b>DMSO</b>	dimethyl sulfoxide
<b>DVC</b>	dorsal vagal complex
<b>ELISA</b>	enzyme-linked immunosorbent assay
<b>fcFRET</b>	floctometry fluorescence resonance energy transfer
<b>FDA</b>	Food and Drug Administration
<b>GHSR1</b>	growth hormone secretagogue receptor 1
<b>GI</b>	Gastrointestinal
<b>GRC</b>	giant retrograde contraction
<b>HEC</b>	highly emetogenic chemotherapy
<b>HPA</b>	hypothalamic pituitary axis
<b>ICV</b>	Intracerebroventricular
<b>IL</b>	Interleukin

<b>IP</b>	Intraperitoneal
<b>MASCC</b>	Multinational Association of Supportive Care in Cancer
<b>MEC</b>	moderately emetogenic chemotherapy
<b>NCCN</b>	National Comprehensive Cancer Network
<b>NEPA</b>	netupitant plus palonosetron
<b>NIH-SSM</b>	NIH Symptom Science Model
<b>NK-1RAs</b>	neurokin-1 receptor antagonists
<b>NMDA receptor subunit</b>	N-methyl-D-aspartic acid receptor subunit
<b>NPY</b>	neuropeptide Y
<b>NTS</b>	nucleus of the solitary tract
<b>OLZ</b>	Olanzapine
<b>PBN</b>	parabrachial nucleus
<b>QOL</b>	quality of life
<b>QTc</b>	corrected QT (measure between the Q and T wave in the heart's electrical cycle) interval
<b>RGC</b>	retrograde giant contraction
<b>SAL</b>	Saline
<b>TNF</b>	tumor necrosis factor

**Table 2.2 Summary of Terms and Neuroanatomical Regions**

<b>Term</b>	<b>Definition/ Context</b>
<b>Acute CINV</b>	CINV occurring within the first 24 hours following chemotherapy
<b>Anticipatory CINV</b>	Nausea and vomiting that occurs in “anticipation” of CINV.
<b>Area Postrema (AP)</b>	Circumventricular collection of nuclei (neuronal/cell nuclei) within the DVC. Positioned above the NTS at the inferior border of fourth ventricle and plays a role chemo detection.
<b>AMPA &amp; NMDA receptor subunits</b>	Glutamate receptor subunits. Modulate glutamate signaling required for full expression of cisplatin-induced nausea, anorexia, and weight loss in rats.
<b>Cisplatin</b>	Potent chemotherapeutic agent known to induce CINV in almost 100% of people and animals in the absence of prophylaxis
<b>CPG for emesis “vomiting center”</b>	Collection of nuclei in the brainstem which communicate in an interrelated fashion to produce vomiting. The CPG is activated during CINV
<b>Dorsal Vagal Complex (DVC)</b>	Region of the hindbrain known to mediate CINV and energy balance. It includes the NTS and the AP
<b>Delayed CINV</b>	CINV occurring 24+ hours following chemotherapy
<b>Highly Emetogenic Chemotherapy</b>	Chemotherapeutic agents that induced CINV in over 90% of people/animals receiving chemotherapy
<b>Nucleus of the Solitary Tract (NTS)</b>	Region of the DVC that received afferent signals from the gastrointestinal system (most visceral signals terminate at the NTS).
<b>Parabrachial Nucleus</b>	Region of the hindbrain that is activated by cisplatin-chemotherapy. It receives projections from the NTS- relaying information from visceral organs to forebrain regions- may mediate CINV
<b>Pica</b>	Consumption of non-nutritive material (e.g. kaolin clay, chalk, dirt, etc.). In rodents, pica (like vomiting in humans) is believed to be an evolutionary adaptation to help eliminate toxic substances and is an established proxy for malaise.

CHAPTER III: OLANZAPINE DECREASES CHEMOTHERAPY-INDUCED  
NAUSEA, ANOREXIA, AND BODY WEIGHT LOSS IN RATS

(To be Submitted for Publication: Journal of Neuropharmacology)

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**Statement of Author Contributions:** Rosario B. Jaime-Lara, MSN, RN from the University of Pennsylvania was the lead author on this article. She conceived of the study question, completed the analysis, drafted the article, and provided critical edits. Dr. Borner and Ruby Holland provided strategic direction and critical edits for this article. Bart C. De Jonghe, PhD from the University of Pennsylvania School of Nursing, was the senior author of this article. Dr. De Jonghe was the PI of the parent study, and he provided strategic direction and critical edits for this article.

**Key Words:** Nausea, antiemetics, olanzapine, cisplatin, antineoplastic agents

## Introduction

Over 1.6 million people in the United States are diagnosed with cancer every year, with greater than a third of these individuals undergoing chemotherapy (*Cancer Facts and Figures 2016*). Given that for many patients chemotherapy is critical to survival, it is concerning that these agents produce severe side effects including vomiting, nausea, and anorexia, which decrease quality of life and can lead to treatment attrition or discontinuation (Coates et al., 1983; Griffin et al., 1996; Molassiotis et al., 2008). Within the last 30 years, antiemetic pharmacotherapies have improved control of chemotherapy-induced vomiting (Aapro, 2005; Aapro et al., 2015; Chawla et al., 2003; Gandara et al., 1998; P. J. Hesketh, Aapro, Street, & Carides, 2010). However, these drugs have been less effective in the management of chemotherapy-induced nausea and associated long-term malaise (P. L. Andrews & Hawthorn, 1988; Hsieh et al., 2015; Olver et al., 2014). CINV is particularly important as CINV can lead to weight loss (decreased muscle mass and decreased adiposity) that aggravates cancer-related cachexia (Ottery, 1994; Tisdale, 2002; Warren, 1932). Given the gravity and prevalence of CINV, improved understanding of the chemical mediators and the pathophysiology of CINV is necessary to promote the development of more effective pharmacotherapies.

Olanzapine, historically used as an antipsychotic, was recently added to antiemetic guidelines due to its efficacy in reducing CINV ((NCCN), 2017; Navari, 2014). Olanzapine not only attenuates both acute and delayed phases of CINV ((NCCN), 2017; Navari, 2014), but it also induces hyperphagia and weight gain in healthy subjects (Kim et al., 2008; Naing et al., 2015; Q. H. Zhang, M. Lian, J. Deng, C. Huang, X, 2012), which make it a promising agent in reducing chemotherapy-related anorexia and weight loss. While the anti-psychotic effects of olanzapine are thought to be mediated primarily by a combinatorial blockade of serotonin (5-HT) and dopamine receptors (Reynolds, 2011; Seeman, 2002; Stahl, 2013), the molecular mechanisms

that underlie olanzapine's effects on emesis remain largely unknown. Furthermore, there are few pre-clinical studies examining olanzapine's antiemetic properties.

The present work aimed to evaluate olanzapine's effectiveness against chemotherapy-dependent reductions in food intake/body weight and increases in pica behavior (a well-established proxy for malaise/nausea in rats- quantified by kaolin clay consumption) (Mitchell et al., 1976; N. Takeda et al., 1993; Yamamoto et al., 2002). To evaluate the potential site of action of olanzapine on food, body weight change, and pica, we administered olanzapine both systemically and directly into the hindbrain prior to cisplatin treatment.

## Methods

### *Animals and housing conditions*

Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were housed individually in hanging, wire-bottom cages in a 12 h light, 12 h dark cycle and had ad libitum access to pelleted chow (Purina Rodent Chow, 5001) and water except when noted. Rats were adapted to the housing conditions for 7 days before the start of the experiments. Rats in behavioral studies had ad libitum access to kaolin (Research Diets, K50001) for 3 days prior to the start of the experiment. All procedures conformed to the institutional standards of the University of Pennsylvania Institutional Animal Care and Use Committee.

### *Drugs*

Cisplatin (*cis*-diammineplatinum dichloride, Sigma-Aldrich) was dissolved in 0.9% saline and administered at a dose of 6 mg/kg. The cisplatin solution was sonicated and vortexed immediately before intraperitoneal (IP) injection for each experiment. Intraperitoneally

administered olanzapine (olanzapine, Tocris) was dissolved in saline. The time-line of drug administration is depicted in **Figure 3.1 (A)** and **Figure 3.2 (A)**. Centrally infused olanzapine was dissolved in 100% dimethyl sulfoxide (DMSO, injected volume 2 $\mu$ L).

#### ***Fourth Ventricle Intracerebroventricular (ICV) Surgeries***

Rats received intraperitoneal anesthesia (ketamine, 90 mg/kg; Butler Animal Health Supply), xylazine (2.7 mg/kg; Anased), and acepromazine (0.64 mg/kg; Butler Animal Health Supply) and subcutaneous analgesia (2.0 mg/kg Metacam; Boehringer Ingelheim Vetmedica) for all surgeries. For intracerebroventricular infusions, a 26-gauge guide cannula (8 mm 81C3151/Spc, Plastics One,) directed at the fourth ventricle was implanted (bregma -11.6 mm, lateral 0.0 mm, and dorsoventral -7.2 mm) and affixed to the skull with screws and dental cement as previously published (Schmidt et al., 2016). All rats were given one week for recovery from surgery. To confirm cannula placement, blood glucose was measured following 5-thio-D-glucose (210  $\mu$ g in 2  $\mu$ L of artificial cerebrospinal fluid) infusion as described previously (Hayes et al., 2011).

#### ***Experiment 1: Effects of intraperitoneal (IP) olanzapine on cisplatin-induced kaolin intake, anorexia, and body weight loss***

We evaluated the ability of olanzapine to ameliorate cisplatin-induced anorexia, kaolin intake and body weight loss using pseudo-counterbalanced approach described as published (Alhadeff & Holland, 2015). On day 1, body weight-matched rats (n=16; 350-450 g, n=8/group) received IP injections of olanzapine (2 mg/kg) or saline followed by IP saline. On day 6, the 2 groups received IP saline or olanzapine (2mg/kg) followed by IP cisplatin. All animals were injected between 0900 h (one hour before dark onset) and 1000 h. Food and kaolin intake measurements were taken as previously described (De Jonghe et al., 2012) at 2 h, 6 h, 24 h, 48 h,



and 72 h post cisplatin injections. Body weights were taken daily immediately before the onset of the dark cycle. Following 72 h measurements, rats were euthanized.

***Experiment 2: Effects of fourth ventricle intracerebroventricular (4<sup>th</sup> ICV) olanzapine on cisplatin-induced kaolin intake, anorexia, and body weight loss***

Rats (n=16; 350-450 g) were habituated to experimental procedures identical to Experiment 1 except on day 1, two groups of weight matched animals (n=8/group) received 4<sup>th</sup> ventricle infusions of olanzapine (125 µg/2 µL) or 100% DMSO followed by IP saline. Six days later, the 2 groups received 4<sup>th</sup> ventricle infusions of olanzapine or DMSO followed by IP cisplatin. All animals were infused between 0900 h and 1000 h. Food and kaolin intake measurements were taken as in Experiment 1. Following 72 h measurements, rats were euthanized.

***Statistics and data evaluation***

All data is expressed as means ± SEM. For all experiments, two-way ANOVAs were performed to evaluate the two drug treatments (main effects: cisplatin/saline and olanzapine/vehicle) for food intake, body weight change, mRNA expression, and immunofluorescence. For the dose response study in Experiment 1 and Experiment 2, a one-way ANOVA was performed to evaluate the effect of each dose on food intake. In Experiment 1 and Experiment 2, kaolin intake, food intake, and body weight were calculated by subtracting cumulative measurements at each time point from measurements taken at 0 h.

Post hoc analyses were conducted with Tukey's multiple comparison test. For all statistical tests, a p-value less than 0.05 was considered significant. For each experiment, statistical differences between mean values were calculated using PRISM (GraphPad Inc.).

## Results

### ***Experiment 1: Systemic olanzapine attenuates cisplatin-induced pica***

A significant interaction between pretreatment with IP OLZ (2 mg/kg) and IP cisplatin (6 mg/kg) on kaolin intake was observed at 24, 48, and 72 hours (**Fig. 1B**,  $F_{(1,25)} \geq 4.41$ ;  $p < 0.05$ ). There was also significant interaction between OLZ and cisplatin treatments on body weight at 24 and 48 hours and food intake at 72 hours (Fig. 1C-D, all  $F_{(1,25)} \geq 4.34$ ;  $p < 0.05$ ). Cisplatin had a significant main effect on kaolin intake at 24, 48, and 72 hours (**Fig. 1C**, all  $F_{(1,25)} \geq 4.41$ ;  $p < 0.05$ ). Cisplatin also had an effect on food intake, and body weight at 24, 48, and 72 hours (**Fig. 1D and E**, all  $F_{(1,25)} \geq 41.03$ ;  $p < 0.05$ ). Post hoc analyses revealed a significant attenuation of cisplatin-induced kaolin intake in animals pre-treated with OLZ at 72 hours (**Fig. 1B**, all  $p < 0.05$ ). However, OLZ pre-treatment failed to attenuate body weight loss induced by cisplatin (**Fig. 1B and C**). As expected, there was no difference in kaolin intake, food intake, and body weight in IP OLZ vs IP saline pre-treated animals following cisplatin treatment (**Fig. 1**).

### ***Experiment 2: Centrally administered (ICV) olanzapine decreased cisplatin-induced pica, anorexia, and weight loss***

A significant OLZ-cisplatin interaction was observed on kaolin intake and body weight at 48, and 72 hours (**Fig. 2C-E**, all  $F_{(1,18)} \geq 4.79$ ;  $p < 0.05$ ). Cisplatin had a significant main effect on kaolin intake, food intake, and body weight at 48 and 72 hours (**Fig. 2**, all  $F_{(1,18)} \geq 33.56$ ;  $p < 0.01$ ). Cisplatin also had a significant effect on kaolin intake at 2, 6, and 24 hours and food intake at 24 hours (**Fig. 2**, all  $F_{(1,18)} \geq 17.00$ ;  $p < 0.001$ ). Post hoc analyses showed significant decreases in kaolin intake and body weight loss in animals pre-treated with OLZ (OLZ-cisplatin vs saline-cisplatin) at 48, and 72 hours (**Fig. 2C**, all  $p < 0.05$ ). No difference in kaolin intake, food intake, and body weight were observed between ICV OLZ and ICV DMSO pre-treated animals (**Fig. 2**)

## Discussion

Current clinical guidelines recommend administration of oral OLZ to prevent and manage CINV ((NCCN), 2017). OLZ is a particularly promising drug because it is known to increase body weight and it is more effective than 5-HT3 receptor antagonists and N-K1 receptor antagonists in reducing delayed CINV (Navari et al., 2007; Navari et al., 2005; Navari et al., 2016). Although numerous pre-clinical studies have been conducted to study OLZ's psychotropic and metabolic effects (Lieberman et al., 2005; Navari et al., 2016), only one pre-clinical study has been conducted to examine OLZ's antiemetic properties, and the results were inconclusive (Machida et al., 2015). The current set of studies sought to examine the behavioral effects of OLZ on cisplatin-induced malaise and energy balance in rats.

Here we show that prophylactic IP OLZ reduces chemotherapy-induced pica and anorexia at 72 hours post cisplatin administration, and via 4<sup>th</sup> ICV OLZ infusion, reduced pica and weight loss 48 and 72 hours following cisplatin treatment. Thus, both systemic and central OLZ reduce cisplatin-induced malaise. Although the mechanism of OLZ on malaise/nausea is not clear, the reduction in pica following ICV OLZ suggest that OLZ's antiemetic properties are sufficient to induce malaise/nausea. Importantly, OLZ's reduction in cisplatin-induced malaise correspond with the peak of delayed CINV (48 -72 hours). This has significant clinical implications since other medications, such as 5-HT3 receptor antagonists, do not effectively control delayed CINV (Baba et al., 2017; Chelkeba et al., 2017; Feyer & Jordan, 2011; Hsieh et al., 2015). Thus, as reflected in clinical guidelines, OLZ can complement other antiemetics to better prevent and manage delayed CINV (Abe et al., 2016; Navari et al., 2011).

Furthermore, IP OLZ also reduced cisplatin-induced anorexia at 72 hours. This is important since cisplatin-induced anorexia can lead to a state of negative energy balance and can further aggravate cancer-related cachexia (Ottery, 1994; Tisdale, 2002; Warren, 1932). Cancer-related cachexia is characterized by an involuntary loss of lean body mass with or without loss of

fat mass and is associated with poor responses to chemotherapy and decreased survival (Aoyagi, Terracina, Raza, Matsubara, & Takabe, 2015; Dewys et al., 1980). Anorexia is one of multiple mechanisms involved in the development of cachexia. Thus, IP OLZ's ability to counteract anorexia may be important in decreasing aggravation of cancer-related cachexia. Importantly, we did not observe an attenuation of cisplatin-induced anorexia in animals receiving ICV OLZ. This suggests that OLZ effect on anorexia is not mediated by the hindbrain, OLZ effect on feeding may be mediated by other regions of the brain involved in feeding, such as the hypothalamus.

Unexpectedly, the effects of IP OLZ on anorexia and malaise were not paralleled by an attenuation of body weight loss. One possible explanation is that dose of OLZ was insufficient to elicit an effect on all aspects of energy balance. The dose of IP OLZ was chosen because it had no effect on energy balance under non-pathological conditions. Thus, the dose of IP OLZ may have also been insufficient to elicit an effect.

Unlike the effects on feeding observed after IP administration, ICV OLZ did not significantly reduce chemotherapy-induced anorexia, but it reduced weight loss. This suggests that centrally infused OLZ may cause metabolic changes, such as decreasing energy expenditure, that were not elicited following peripheral administration. Therefore, the effects of systemic and central OLZ administration may partially occur through distinct mechanisms, which eventually lead to changes in body weight but not necessarily impact feeding behavior. This is consistent with other studies conducted in rats showing olanzapine induced metabolic changes, including weight gain, hypothermia, and decreased locomotor activity, in the absence of hyperphagia (Cooper et al., 2007; Evers, Calcagnoli, van Dijk, & Scheurink, 2010). However, the mechanisms underlying OLZ's metabolic effects in pre-clinical models has provided contradictory results, and require further investigation and standardization of doses, sex, and experimental time periods (Cooper et al., 2007; Evers et al., 2010; van der Zwaal, Janhunen, la Fleur, & Adan, 2014).

In summary, we found that OLZ reduces malaise, anorexia, and weight loss following chemotherapy in rats. Our findings suggest olanzapine may decrease pica through hindbrain actions and that feeding changes may be mediated by other brain regions (non-hindbrain). Together, these data help to identify potential mediators of OLZ's antiemetic properties, contribute to better understanding CINV, and provide the opportunity for the development of more effective and better tolerated antiemetic pharmacotherapies.

Figures

Figure 3.1

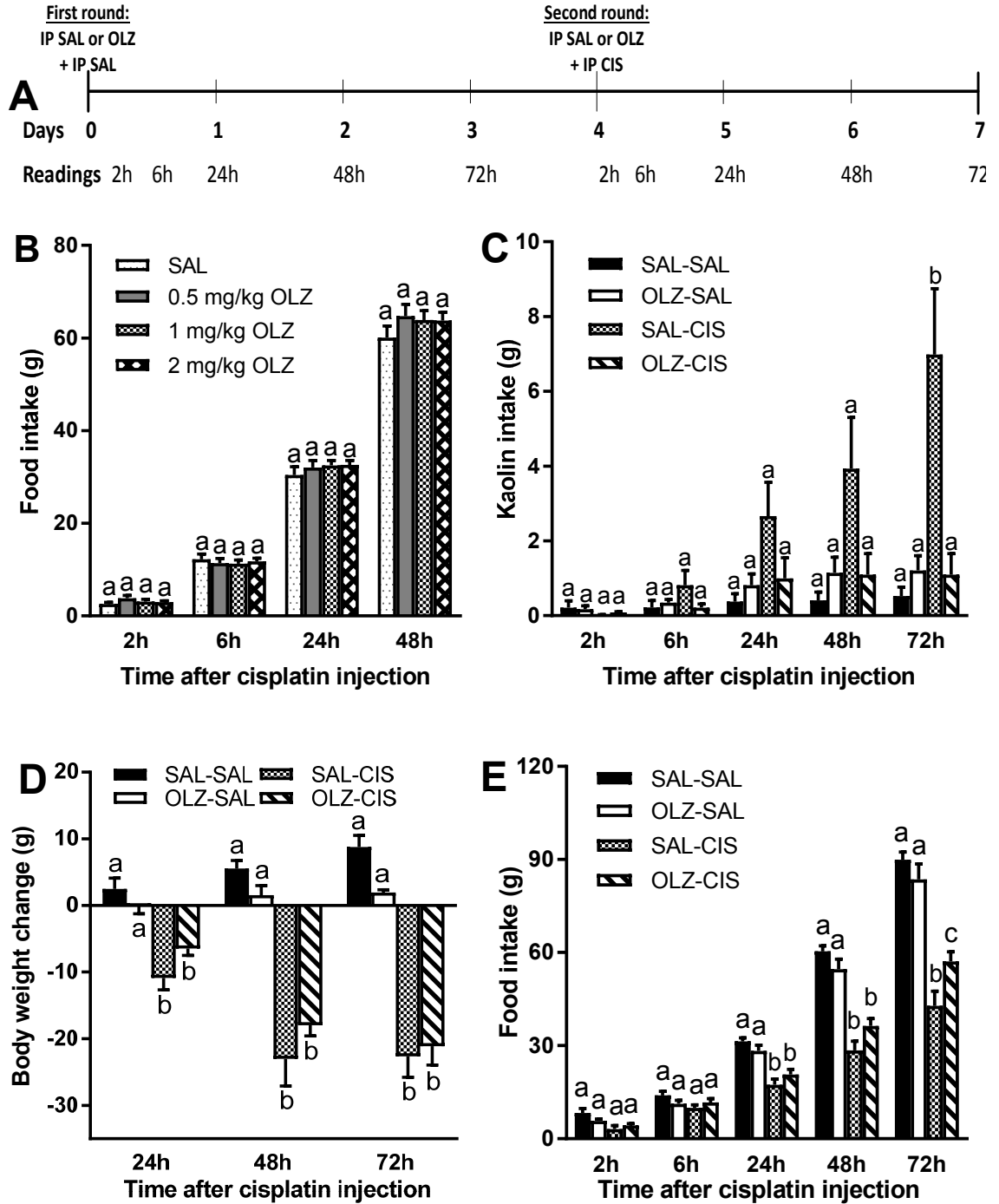
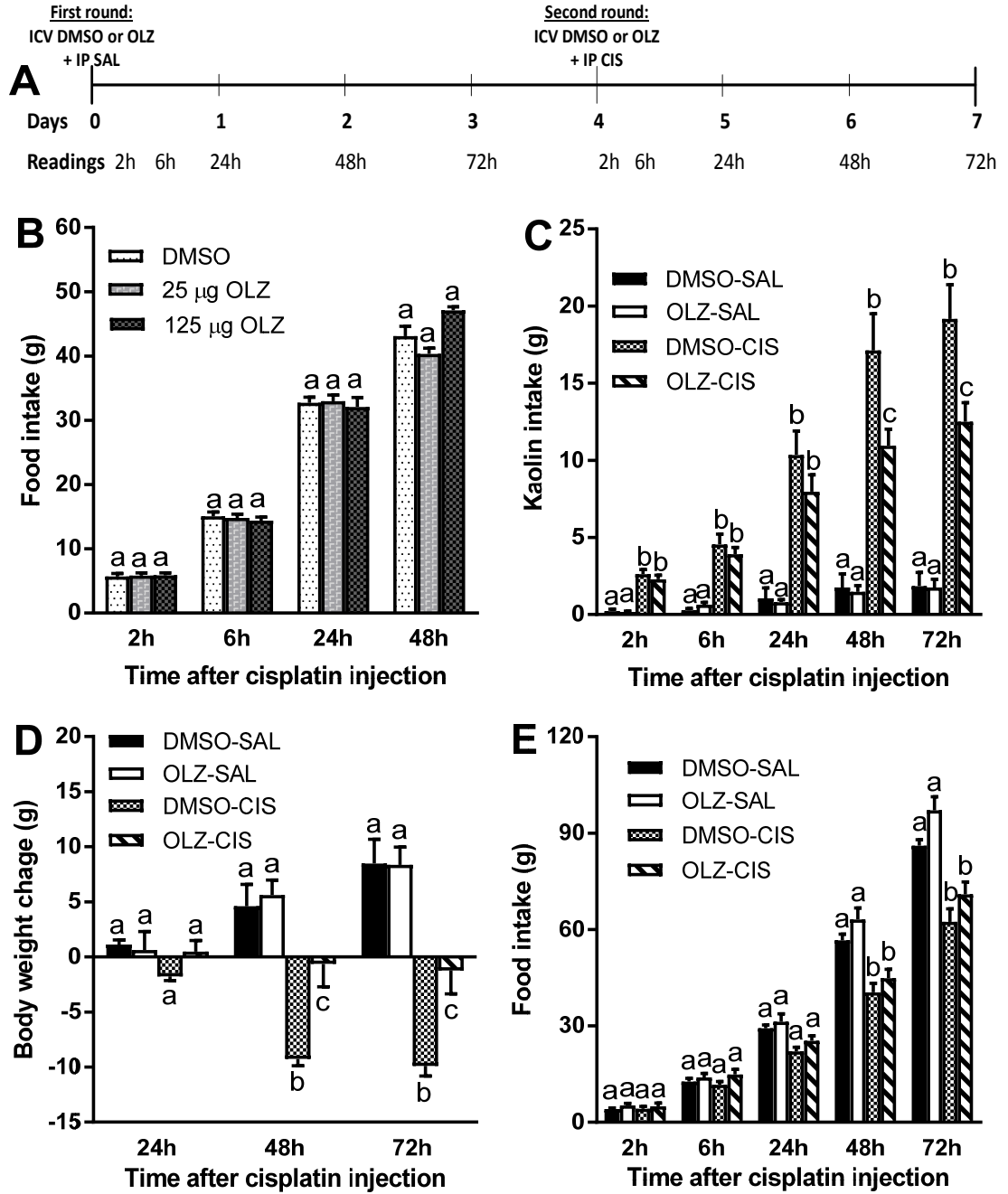


Figure 3.2



## Figure Legends

**Figure 3.1.** (A) Timeline of injections in Experiment 1. (B) Dose response experiment found no significant difference between doses of IP OLZ (0.5 mg/kg, 1 mg/kg, and 2 mg/kg) on feeding (C) IP OLZ (2 mg/kg) decreased cisplatin induced pica at 72 hours. (D) Cisplatin significantly increased weight loss. IP OLZ did not significantly affect weight loss. (F) IP OLZ decreases cisplatin-induced anorexia. Data are expressed as mean  $\pm$  SEM. Different lower-case letters represent statistically significant differences (Tukey HSD,  $P < 0.05$ ).

**Figure 3.2.** (A) Timeline of injections in Experiment 2. (B) Dose response experiment found no significant difference between doses of ICV OLZ (pure DMSO, 25  $\mu$ g, and 125  $\mu$ g) on feeding (C) ICV OLZ (125  $\mu$ g) decreased cisplatin induced pica at 48 and 72 hours. (D) Cisplatin significantly increased weight loss at all time points, and ICV OLZ significantly reduced weight loss at 48 and 72 hours. (F) Cisplatin significantly increased anorexia. ICV OLZ did not significantly improve anorexia. Data are expressed as mean  $\pm$  SEM. Different lower-case letters represent statistically significant differences (Tukey HSD,  $P < 0.05$ ).



CHAPTER IV: OLANZAPINE COUNTERACTS CISPLATIN-INDUCED CHANGES  
IN CIRCULATING GHRELIN, GHRELIN RECEPTOR EXPRESSION, AND  
SEROTONIN RECEPTOR EXPRESSION IN RATS

(To be Submitted for Publication: Journal of Neuropharmacology)

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**Statement of Author Contributions:** Rosario B. Jaime-Lara, MSN, RN from the University of Pennsylvania was the lead author on this article. She conceived of the study, completed the analyses, drafted the article, and provided critical edits. Dr. Borner and Ruby Holland from the University of Pennsylvania provided strategic direction and critical edits for this article. Brianna Brooks assisted in the analyses and drafting of the article. Bart C. De Jonghe, PhD from the University of Pennsylvania School of Nursing, was the senior author of this article. Dr. De Jonghe was the PI of the parent study, designed the study, and provided strategic direction and critical edits for this article.

**Key works:** Nausea, antiemetics, olanzapine, cisplatin, antineoplastic agents

## Introduction

Despite the administration of appropriate pharmacologic methods of prevention, a significant percentage of patients continue to exhibit chemotherapy-induced nausea, vomiting (CINV), and anorexia especially during the delayed phase (>24 h) following treatment. The antipsychotic drug olanzapine was recently added to antiemetic guidelines due to its efficacy in reducing CINV. The activity of olanzapine at multiple serotonin (5-HT) receptors, particularly the 5-HT<sub>2C</sub> and 5-HT<sub>3</sub> receptors, which both play a role in the central mediation of nausea and vomiting, suggests that it might have clinically significant antiemetic properties by modulating central serotonergic signaling. Moreover, olanzapine may also stimulate ghrelin release and sensitivity, which might contribute to its antiemetic and anti-anorectic effects.

Olanzapine's antiemetic effect may be mediated by its effect on serotonin (Brafford & Glode, 2014; Navari, 2014). Serotonin is known to play a key role in emesis (Y. K. Gupta & Sharma, 2002; Johnston, Lu, & Rudd, 2014; Rojas et al., 2014), with serotonin 3 receptor antagonists being among the most widely used antiemetics ((NCCN), 2017; Aapro, 2005; P. Hesketh, 2015). Olanzapine is a strong antagonist of serotonin receptors, including serotonin 2C, serotonin 2A and serotonin 3 receptors (Bymaster et al., 1996; Selent, Lopez, Sanz, & Pastor, 2008; Stahl, 2013). Notably, olanzapine has especially high affinity for serotonin 2C receptors (5-HT<sub>2CRs</sub>) which are located in high concentrations in the hindbrain at regions known to mediate CINV, the nucleus of the solitary tract and the area postrema (Wright et al., 1995) (Alhadef & Holland, 2015; De Jonghe & Horn, 2009; Holland et al., 2014). Additionally, 5-HT<sub>2CRs</sub>' functional roles make these receptors particularly attractive for the study of CINV. Specifically, olanzapine is known to cause weight gain via serotonin receptor 2C antagonism (X. F. Huang et al., 2017; Lord et al., 2017) and inhibition of 5-HT<sub>2CRs</sub> has been linked to decreases in nausea (Okada et al., 1995; H. Takeda et al., 2008). Conversely, cisplatin treatment has been associated with increased activation of 5-HT<sub>2CRs</sub> (Y. K. Gupta & Sharma, 2002; Hattori, 2010; Okada et

al., 1995; H. Takeda et al., 2008) and increased expression of 5-HT<sub>2</sub>CRs in the brain (Yakabi, Sadakane, et al., 2010). This stimulation of 5-HT<sub>2</sub>CRs in the brain is linked to GI side effects including anorexia and nausea (Y. K. Gupta & Sharma, 2002; H. Takeda et al., 2008). Thus, olanzapine may decrease CINV by counteracting cisplatin-induced effects on 5-HT<sub>2</sub>CRs in the brain.

Another potential mechanism by which olanzapine stimulates food intake and alleviates CINV is via modulation of the ghrelin system. Ghrelin is an orexigenic hormone that is mainly secreted by the stomach during fasting and shortly before meals (Inui, 2001; Inui et al., 2004). Ghrelin is a high affinity ligand for the growth hormone secretagogue receptor (GHSR), which is highly expressed in the hindbrain (Inui, 2001; Kojima et al., 1999). Cisplatin decreases hypothalamic GHSR and circulating ghrelin (Hiura, Takiguchi, Yamamoto, Kurokawa, et al., 2012; Tanaka et al., 2008; Yakabi, Sadakane, et al., 2010). Conversely, olanzapine increases GHSR expression, potentiates ghrelin-mediated GHSR activity, and increases circulating ghrelin levels (Tagami et al., 2016; Weston-Green et al., 2011; Q. Zhang et al., 2014). These findings combined with pre-clinical and clinical evidence demonstrating ghrelin-mediated decreases in CINV, anorexia and cachexia (Hiura, Takiguchi, Yamamoto, Takahashi, et al., 2012; Y. L. Liu et al., 2006), suggest that olanzapine may mediate olanzapine's antiemetic/anti-anorectic effect by counteracting the effects of cisplatin on the ghrelin system.

The present work aimed to determine olanzapine's effect on potential biological correlates of CINV and energy balance. Immunohistochemistry was conducted to determine changes in the number of c-Fos (a marker of neuronal activation) positive neurons in the area postrema (AP) and the nucleus of the solitary tract, regions known to mediate CINV (Beleslin & Strbac, 1987; Borison et al., 1984; Coil & Norgren, 1981; Stewart et al., 1977). Additionally, circulating ghrelin as well as central expression levels of GSHR and serotonin receptor subunits in the hypothalamus, dorsal vagal complex (DVC), central amygdala (CeA), and parabrachial nucleus (PBN) were examined following cisplatin administration.

## Methods

### ***Animals and housing conditions***

Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were housed individually in hanging, wire-bottom cages in a 12 h light, 12 h dark cycle and had ad libitum access to pelleted chow (Purina Rodent Chow, 5001) and water except when noted. Rats were adapted to the housing conditions for 7 days before the start of the experiments. Rats in behavioral studies had ad libitum access to kaolin (Research Diets, K50001) for 3 days prior to the start of the experiment. All procedures conformed to the institutional standards of the University of Pennsylvania Institutional Animal Care and Use Committee.

### ***Drugs***

Cisplatin (*cis*-diammineplatinum dichloride, Sigma-Aldrich) was dissolved in 0.9% saline and was administered at a dose of 6 mg/kg. The cisplatin solution was sonicated and vortexed immediately before intraperitoneal (IP) injection for each experiment. Intraperitoneally administered olanzapine (Tocris) was dissolved in saline.

### ***Experiment 1: Assessment of olanzapine treatment on cisplatin-induced c-Fos in DVC neurons***

Body weight-matched rats (n=16; 250-300 g) were assigned to one of four conditions (n=4/group). Half of the groups received IP olanzapine (2 mg/kg) and the other half received saline followed (15 minutes later) by IP cisplatin (6 mg/kg) or saline in a 2 x 2 between subjects design. To avoid feeding-related changes in c-Fos expression between groups, all animals were food deprived until samples were collected 6 h following cisplatin treatment. Briefly, rats were deeply anesthetized with IP anesthesia [ketamine, (90 mg/kg; Butler Animal Health Supply), xylazine (2.7 mg/kg; Anased), and acepromazine (0.64 mg/kg; Butler Animal Health Supply)]

and transcardially perfused with 0.1 M PBS (phosphate buffered saline, Boston Bioproducts), pH 7.4, followed by 4% PFA (Paraformaldehyde, Boston Bioproducts). Brains were removed and post fixed in 4% PFA for 4 h and then stored in 30% sucrose for two days. Brain sections containing the DVC were processed for c-Fos as published (De Jonghe & Horn, 2009; Holland et al., 2014). The brains were sectioned coronally and collected serially in triplicate starting at bregma -14.3 mm and ending at bregma -13.3 mm. Immunohistochemistry was generally conducted according to previously described procedures (Holland et al., 2014). Briefly, sections were washed with 0.1 M PBS. Brain sections were incubated in 5% normal donkey serum for 1 h, followed by an overnight (16 h) incubation with the polyclonal rabbit anti-Fos primary antibody (1:1000, s2250; Cell Signaling). Sections were washed and incubated with the secondary antibody donkey anti-rabbit Alexa Fluor 594 (1:500; Jackson Immuno Research Laboratories), mounted onto glass slides, and coverslipped using Fluorogel (Electron Microscopy Sciences). The c-Fos positive neurons were visualized and quantified using fluorescence microscopy (Nikon 80i, NIS Elements AR 3.0) at 20X magnification.

***Experiment 2: Assessment of olanzapine treatment on cisplatin-induced reductions in plasma ghrelin***

Rats (n=16; 300-350 g) (n=4/group) received IP injections of olanzapine (2 mg/kg) or saline followed by IP cisplatin (6 mg/kg) or saline in a between subject design. All animals were injected 1 hour before dark onset. Six hours after the last injection, rats were decapitated, and trunk blood was collected into heparin-coated tubes containing protease inhibitor, p-hydroxymercuribenzoic acid (Bertin Pharma, final concentration of 1 mM). Blood was centrifuged to collect plasma and stored at -80 °C until assayed. Plasma was assayed using an acylated ghrelin ELISA kit and an unacylated ghrelin ELISA kit (Bertin Pharma, Montigny-le-

Bretonneux, France) according to the manufacturer's instructions. Plasma samples was analyzed in duplicate.

***Experiment 3: Assessment of olanzapine treatment on cisplatin-induced alterations in central mRNA expression of GHSR and serotonergic receptors (5-HT2C, 5-HT2A, 5-HT3, 5-HT1A)***

Rats (n=32; 300-350 g) received IP olanzapine or saline followed by IP cisplatin or saline (n=8/group) and food was removed. At 6 h, rats were euthanized via decapitation. Whole brains were collected, and flash frozen in isopentane. Whole hypothalamus and bilateral micropunches (1 mm in diameter) containing regions of the DVC, PBN, and CeA were collected according to previously published methods (Holland et al., 2014). The micropunches of each tissue corresponded to the following coordinates: DVC (-14.3 mm from bregma ~ 1.0 mm long), IPBN (beginning at -10.04 mm from bregma and were ~ 1.5 mm long), and CeA (beginning at -3.0 mm from bregma and were ~ 1.0 mm long).

For quantitative real-time polymerase chain reaction (qPCR), RNA was extracted from samples using Trizol (Invitrogen) and RNeasy Kit (Qiagen) according to manufacturer's instructions. cDNA was generated from extracted RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The qPCR was performed using pre-designed Taqman probes (Applied Bioscience). Rat beta actin (Cat. 4352340E) was used as a housekeeping reference gene and probes were used to quantify relative mRNA levels of Growth Hormone Secretagogue Receptor (RN00821417), Serotonin Receptor 2C (Rn00562748), Serotonin Receptor 2A (Rn00567856), Serotonin 3A (Rn00567878) and Serotonin Receptor 1A (Rn00569876). Samples were run in duplicate and relative mRNA expression was calculated using the comparative delta-delta Ct Method as described previously (Holland et al., 2014).

***Statistics and data evaluation***

All data are expressed as means  $\pm$  SEM. For all experiments, two-way ANOVAs were performed to evaluate our two drug treatments (main effects: cisplatin/saline and olanzapine/vehicle). For Experiment 1, c-Fos counts of three sections from each animal were averaged at each plate level. In Experiment 2, sample absorbance measurements were used to determine concentration by linear regression from standard curves. To determine relative mRNA expression in Experiment 3, average delta Ct values of beta actin were subtracted from average delta Ct values of the gene of interest. Post hoc analyses were conducted with Tukey's multiple comparison test. For all statistical tests, a p-value less than 0.05 were considered significant. For each experiment, statistical differences between mean values was calculated using PRISM (GraphPad Inc.)

## Results

### ***Experiment 1: Olanzapine decreased cisplatin-induced c-Fos in the area postrema and the nucleus of the solitary tract***

Representative immunofluorescent sections from the hindbrain (the area postrema and the nucleus of the solitary tract) 6 hours post cisplatin administration are depicted in **Fig. 4.1**. OLZ (2 mg/kg) pretreatment and cisplatin (6 mg/kg) displayed a significant interaction (all  $F_{(1,12)} \geq 7.56$ ;  $p < 0.05$ ) on c-Fos positive neurons in nucleus of the solitary tract (NTS) neurons at the level of the obex (-14.3 mm from bregma) and mid-DVC (-13.8 mm from Bregma). Cisplatin had a main effect at the level of the obex, mid-DVC, and 4<sup>th</sup> ventricle (-13.3 mm from Bregma) (all  $F_{(1,12)} \geq 5.09$ ;  $p < 0.05$ ). Post hoc tests showed that cisplatin increased c-Fos counts at the level of the obex and mid-DVC (all  $p < 0.05$ ). OLZ pretreatment significantly reduced cisplatin-induced increases in c-Fos counts in nucleus of the solitary tract (NTS) neurons at the level of the obex and mid-DVC (olanzapine-cisplatin vs saline-cisplatin;  $p < 0.05$ ). Cisplatin induced an increase in the number of c-Fos positive cells in the area postrema (AP) and at the level of the 4<sup>th</sup> ventricle.

However, olanzapine did not significantly reduce cisplatin-induced increases in the number of immunofluorescent cells in the AP and at the level of the 4<sup>th</sup> ventricle.

### ***Experiment 2: Olanzapine blocks cisplatin-induced reduction in circulating ghrelin levels***

There was a significant interaction between OLZ and cisplatin treatments (**Fig. 4.2A**, all  $F_{(1,12)} \geq 10.35$ ;  $p < 0.01$ ) on acylated (active) ghrelin levels 6 hours post cisplatin treatment. Data also showed there was a significant interaction between OLZ and cisplatin on the acylated to unacylated ghrelin ratio (**Fig. 4.2B**,  $F_{(1,12)} = 11.09$ ;  $p < 0.005$ ). Post hoc tests showed that cisplatin decreased acylated ghrelin and this effect was blocked in animals pre-treated with OLZ (**Fig. 4.2A**,  $p < 0.05$ ). The ratio of acylated ghrelin to unacylated ghrelin was also significantly lower in cisplatin treated animals, and this effect was blocked by pretreatment with OLZ (**Fig. 4.2C**,  $p < 0.05$ ). There was no significant difference in unacylated ghrelin levels between groups (**Fig. 4.2B**). In addition, post hoc analyses revealed that OLZ in healthy animals (OLZ-saline) did not change acylated ghrelin levels. There was no significant difference in acylated to unacylated ghrelin between OLZ-cisplatin treated animals and control groups.

### ***Experiment 3: Olanzapine decreased 5-HT2C gene expression in the DVC and hypothalamus following cisplatin treatment***

A significant interaction between IP OLZ (2 mg/kg) and IP cisplatin (6 mg/kg) was observed on 5-HT2C expression in the DVC and hypothalamus (**Fig. 4.3A-B**, all  $F_{(1,23)} \geq 4.73$ ;  $p < 0.05$ ). Cisplatin also had a main effect on 5-HT2C in the DVC and hypothalamus (**Fig. 4.3A-B**, all  $F_{(1,23)} \geq 30.5$ ;  $p < 0.0001$ ). Post hoc analysis showed cisplatin increased expression of 5-HT2C in the DVC and hypothalamus (**Fig. 4.3A-B**, all  $p < 0.05$ ). There was no difference in 5-HT2C expression between OLZ treated animals and saline treated controls in any tissue sample analyzed. Additionally, the saline-cisplatin treatment group had decreased GHSR expression in the hypothalamus compared to OLZ-cisplatin treated animals (**Fig. 4.3B**, all  $p < 0.05$ ). There was



no significant difference between HTR2A, HTR1A, and HTR3 expression in the DVC, or HTR2A expression in hypothalamic tissue. Finally, no significant differences in gene expression of HTR2C and GHSR were noticed in the PBN and CeA.

## Discussion

Olanzapine was added to antiemetic guidelines in 2014 after case studies, pilot studies, and clinical trials found that it is effective at preventing and managing CINV ((NCCN), 2014). The current set of studies sought to examine the neuronal, and molecular effects of OLZ on cisplatin-treated rats. The current studies showed IP OLZ reduced cisplatin-induced neuronal activation in the DVC and rescued cisplatin-induced decreases in circulating ghrelin 6 hours post cisplatin administration. Additionally, molecular experiments suggested OLZ counteracts cisplatin related changes in hindbrain and hypothalamic 5-HT<sub>2C</sub> and GHSR expression 6 hours post chemotherapy.

The central sites of action contributing to OLZ's antiemetic side effects are not fully defined. A recent electrophysiological study demonstrated that OLZ decreases the excitability of neurons located in the hindbrain and reduced vagal output to subdiaphragmatic organs (Anwar, Miyata, & Zsombok, 2016). Conversely, cisplatin increases neuronal activation in the dorsal vagal complex of the hindbrain (Horn, 2009). This effect is dependent on vagal input, and these effects contribute to nausea (Horn, 2009). Thus, Experiment 1 examines the effects of OLZ in the NTS and AP following cisplatin treatment. To our knowledge, this is the first immunohistochemical study that has been conducted to study the effect of OLZ on neuronal activation in the hindbrain and the first to study cisplatin-induced activation following olanzapine pretreatment. Our finding corroborates studies conducted in rats, mice, shrews, and other species, which show that cisplatin increases the number of c-Fos positive NTS neurons (Alhadeff et al., 2017; De Jonghe & Horn, 2009; Holland et al., 2014; Horn, 2009; Ray, Griggs, & Darmani, 2009). Previous research studies have shown OLZ increases the number of c-Fos expressing

neurons in the forebrain including the locus coeruleus (Dawe et al., 2001). However, in the present study OLZ decreases cisplatin-related increases in neuronal activation in NTS neurons at the level of the obex and mid-dorsal vagal complex 6 hours post chemotherapy. The reduction in neuronal activation could indicate that OLZ could have an inhibitory effect on neuronal activation in the NTS. This experiment also showed that OLZ alone did not significantly increase c-Fos expression in the NTS or the AP.

Recent studies have demonstrated the positive effects of ghrelin or its analogs in the prevention of CINV (Hiura, Takiguchi, Yamamoto, Takahashi, et al., 2012; Y. L. Liu et al., 2006; Rudd et al., 2006). However, some ghrelin-based clinical trials have failed to demonstrate significant attenuation of CINV, anorexia, weight loss, and cachexia (Currow et al., 2017; Temel et al., 2016). This discrepancy may be caused by partial ghrelin resistance, which may limit the effectiveness of ghrelin-based therapy in cancer patients. OLZ has been reported to increase ghrelin levels in psychiatric patients (Murashita et al., 2005; Q. Zhang, Deng, & Huang, 2013). Our data show that IP OLZ reverses cisplatin-induced reductions in circulating ghrelin. Additionally, we observed cisplatin-induced decreases in hypothalamic GHSR expression were rescued by pretreatment with OLZ. This supports studies demonstrating cisplatin-induced decreases in hypothalamic GHSR (Yakabi, Kurosawa, et al., 2010). Furthermore, GHSR agonists reduce CINV and anorexia (Yakabi, Kurosawa, et al., 2010; Yakabi, Sadakane, et al., 2010; Yoshiya et al., 2016). These data highlight the possibility that OLZ's antiemetic properties may be partially mediated by ghrelin, specifically by reversing cisplatin-induced reduction in circulating ghrelin and ghrelin receptor expression. Further studies are required to establish the role of ghrelin in relation to OLZ's antiemetic properties and in the prevention of CINV. Nevertheless, OLZ may be able to counteract ghrelin-resistance, highlighting the possibility of combination therapy of OLZ and ghrelin may be more effective at managing CINV.

To further define the molecular effects of OLZ, we examined whether OLZ had an effect on serotonin receptors. Since OLZs antipsychotic properties are mediated by serotonin receptors

(Brafford & Glode, 2014; Reynolds, 2011; Seeman, 2002), and serotonin is also known to play an important role in CINV, we hypothesized that serotonin may also mediate OLZ's antiemetic properties. Indeed, we found that IP OLZ reversed cisplatin-induced increases in 5-HT<sub>2</sub>CR expression. Since increases in 5-HT<sub>2</sub>CR have been associated with nausea and anorexia (Kirk et al., 2009; Silenieks, 2014; H. Takeda et al., 2008), these results indicate that OLZ's antiemetic effect may be mediated by 5-HT<sub>2</sub>CR expression. Further studies should be conducted to examine whether OLZ's antiemetic effects are dependent on 5-HT<sub>2</sub>CR signaling and whether 5-HT<sub>2</sub>CR antagonism is sufficient to effectively manage CINV.

In summary, we found that OLZ reversed cisplatin-induced changes in serotonin and ghrelin. Specifically, OLZ reduced the number of activated neurons in the nucleus of the solitary tract, rescued drops in circulating ghrelin, decreased 5-HT<sub>2</sub>C expression in the DVC and hypothalamus, and increased hypothalamic GHSR expression. Our findings suggest 5-HT<sub>2</sub>C and circulating ghrelin may mediate OLZ's antiemetic properties. Together, these data help to identify potential mediators of OLZ's antiemetic properties, contribute to better understanding CINV, and provide the opportunity for the development of more effective and better tolerated antiemetic pharmacotherapies.

Figures

Figure 4.1

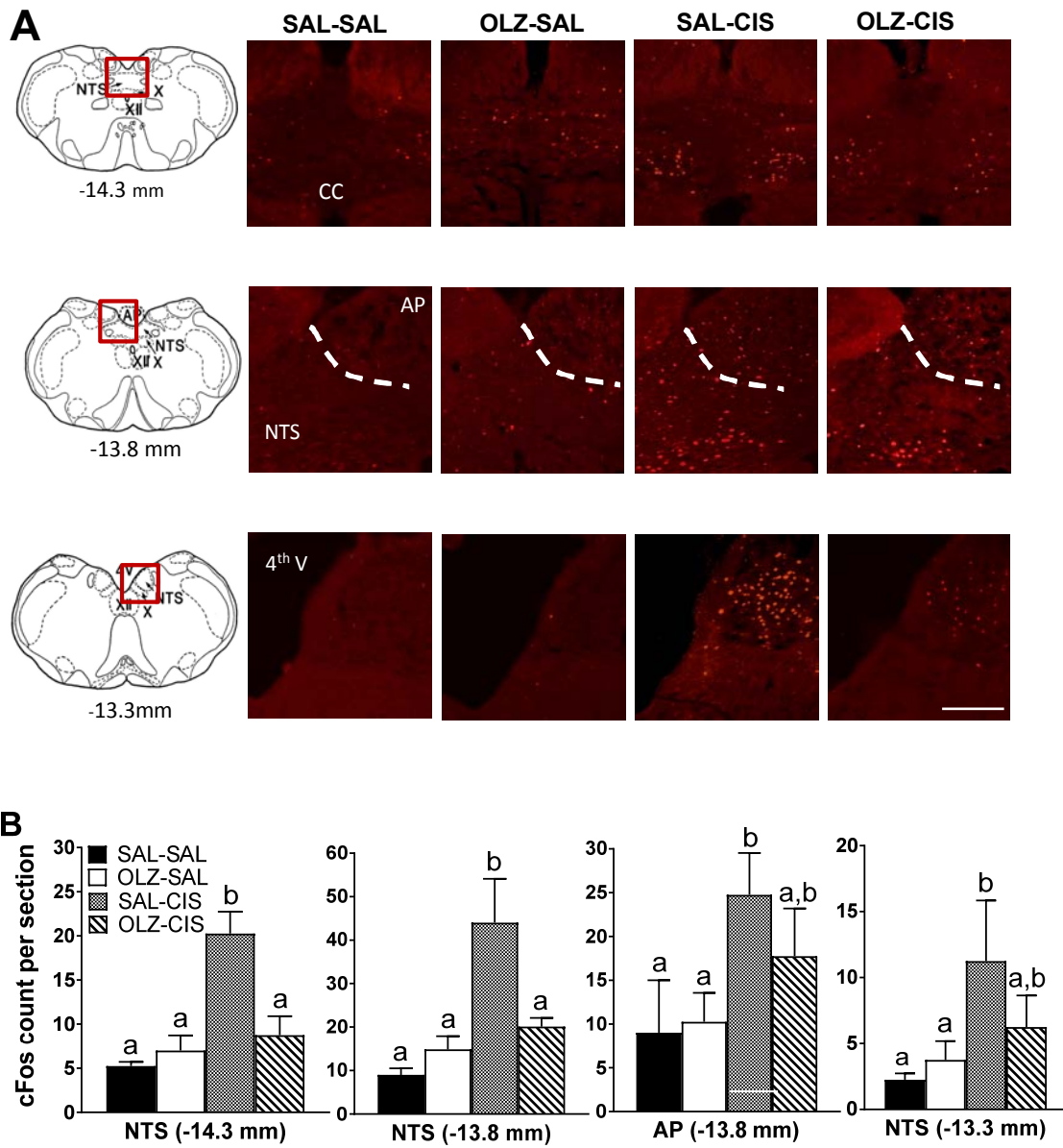


Figure 4.2

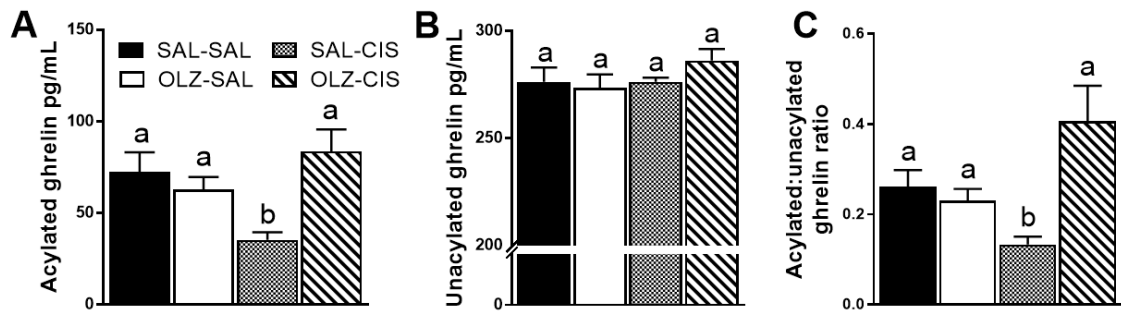
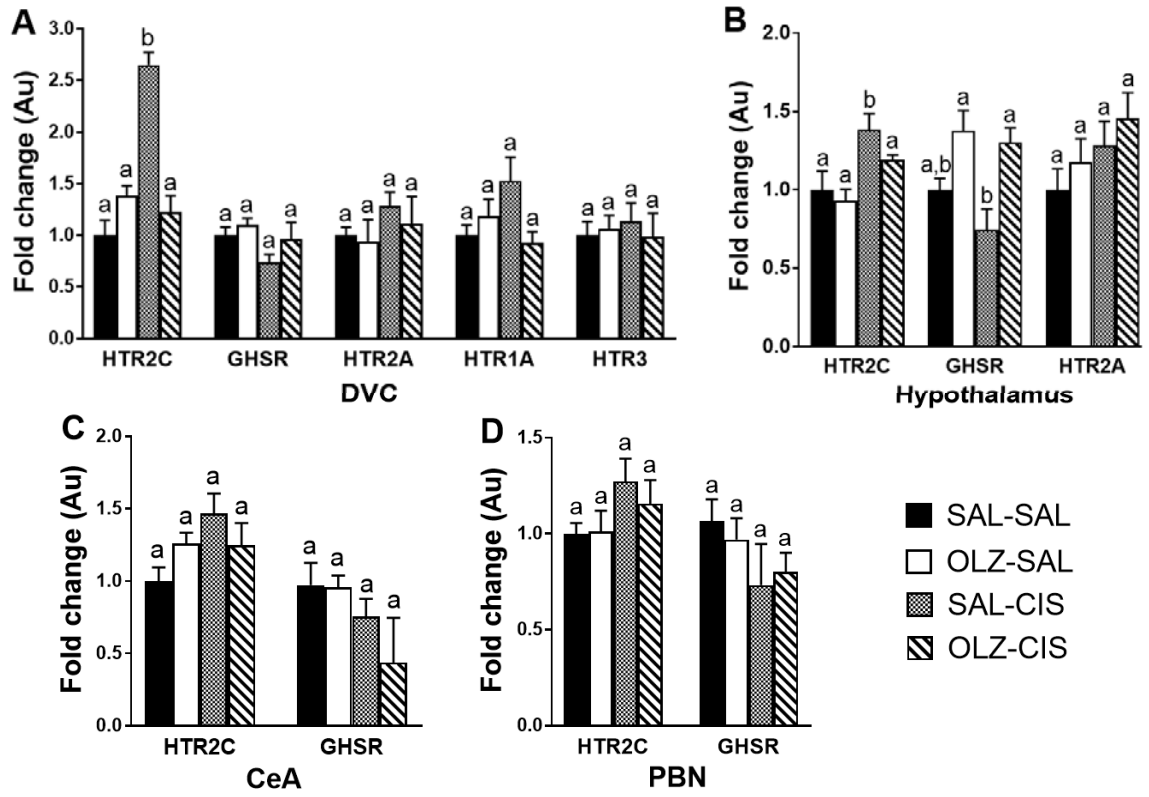


Figure 4.3



## Figure Legends

**Figure 4.1.** Representative immunofluorescent images of NTS neurons in the DVC at the level of the obex (-14.3 mm), of NTS and AP neurons at the caudal medial DVC (-13.8 mm), and the NTS at the level of the fourth ventricle (-13.3 mm). Coordinates correspond to distance from Bregma. **(A)** At all levels, cisplatin induced increases the number c-Fos positive NTS/AP neurons. IP OLZ (2 mg/kg) reduced c-Fos immunofluorescence in NTS neurons at the level of the obex and at the caudal medial NTS in cisplatin treated rats (OLZ-CIS vs SAL-CIS). **(B)** Mean  $\pm$  SEM c-Fos positive cell counts in the NTS and AP are shown. Different lower-case letters represent statistically significant differences (Tukey HSD,  $P < 0.05$ ).

**Figure 4.2** Concentration (pg/mL) of circulating acylated and unacylated ghrelin were calculated for each treatment group. **(A)** Cisplatin decreases levels of acylated ghrelin compared to controls. IP OLZ rescued acylated ghrelin levels. **(B)** There were no significant differences in unacylated ghrelin levels between treatment groups. **(C)** Cisplatin decreased the ratio of acylated to unacylated ghrelin ratio. IP OLZ Olanzapine increased cisplatin-induced decrease in the ratio of acylated to unacylated ghrelin. Data are expressed as mean  $\pm$  SEM. Different lower-case letters represent statistically significant differences (Tukey HSD,  $P < 0.05$ ).

**Figure 4.3** Changes in serotonin (HTR2C, HTR2A, HTR1A, and HTR3) and ghrelin (GHSR) subunit expression in the DVC, hypothalamus, CeA, and PBN were examined. **(A)** HT2C receptor subunit expression was increased in the DVC of cisplatin-treated rats. **(B)** HT2C expression was increased and GHSR receptor subunit expression was reduced in the hypothalamus. No significant changes on HTR2C and GHSR expression were observed at the CeA **(C)** or at the PBN **(D)**. Data are expressed as mean  $\pm$  SEM. Different lower-case letters represent statistically significant differences (Tukey HSD,  $P < 0.05$ ).

## Chapter V: Summary and Conclusion

### Introduction

CINV continues to be highly prevalent, is an economic burden, and negatively impacts patient quality of life. Thus, it is important to better understand and improve treatments that alleviate CINV. Olanzapine is an atypical antipsychotic commonly used in the treatment of schizophrenia (Stahl, 2013) recently added to antiemetic guidelines due to its effectiveness in preventing and managing CINV ((NCCN), 2017; Navari, 2014). It is known to act on multiple receptor families, including dopaminergic, serotonergic, muscarinic, and histaminic receptors (Bymaster et al., 1996; Stahl, 2013). It was introduced as an antiemetic after multiple case studies reported that patients receiving olanzapine had a lower incidence of CINV (Jackson & Tavernier, 2003; S. D. Passik et al., 2003; Pirl & Roth, 2000; Srivastava et al., 2003). Subsequent clinical trials confirmed its effectiveness in the prevention and management of CINV (Navari et al., 2005; Navari et al., 2011; Steven D. Passik et al., 2004). Although, the precise mechanism by which olanzapine reduces CINV is unknown, olanzapine is known to have an impact on serotonin and ghrelin receptor expression in the brain, which are known to play a role in CINV (Hiura, Takiguchi, Yamamoto, Takahashi, et al., 2012; Lord et al., 2017; Yakabi, Kurosawa, et al., 2010; Q. Zhang et al., 2013). Thus, olanzapine's reduction of chemotherapy-induced side effects may be correlated with changes in serotonergic and ghrelin systems.

This body of work sought to better understand how olanzapine alleviates chemotherapy-induced side effects. The specific aims of this body of work were 1) to identify gaps in the literature regarding olanzapine as an antiemetic and identify potential biological correlates associated with olanzapine's effect on CINV and energy balance 2) determine the behavioral effects of olanzapine in rats and 3) test the effects of olanzapine on potential biological mediators of CINV and energy balance. Each specific aim was met, and the findings contribute to our



knowledge of olanzapine in relation to CINV. These data provide guidance for future programs of research.

### Summary of Principal Findings

The first aim was to identify gaps in the literature regarding the use of olanzapine as an antiemetic and identifying olanzapine's effect on biological correlates of CINV and energy balance. Our literature review revealed that there is scant literature studying the effect of olanzapine as an antiemetic in pre-clinical models. Only one study has been conducted to examine the effects of olanzapine on chemotherapy-induced malaise in rats (Machida et al., 2015). Additionally, the literature highlighted serotonin and ghrelin as important players in olanzapine-induced metabolic changes, including weight gain and increased feeding (Kirk et al., 2009; Silenieks, 2014; Q. Zhang et al., 2013; Q. Zhang et al., 2014). Specifically, olanzapine has opposite effects on serotonin (5-HT) and ghrelin compared to cisplatin (Kirk et al., 2009; Silenieks, 2014; Yakabi, Kurosawa, et al., 2010; Yakabi, Sadakane, et al., 2010; Q. Zhang et al., 2013; Q. Zhang et al., 2014). While cisplatin is known to increase activation and expression of serotonin 2C receptors (5-HT<sub>2</sub>CRs) in the brain (H. Takeda et al., 2008), olanzapine is known to increase food intake and body weight through antagonizing 5-HT<sub>2</sub>CRs (X. F. Huang et al., 2017; Lord et al., 2017). Furthermore, cisplatin has been reported to decrease circulating ghrelin and hypothalamic ghrelin receptor (GHSR) expression (Yakabi, Kurosawa, et al., 2010; Yakabi, Sadakane, et al., 2010). Conversely, olanzapine can increase circulating ghrelin (Q. Zhang et al., 2014) (Murashita et al., 2005). However, olanzapine's effect on serotonin and ghrelin signaling in relation to CINV had not been explored. A summary of the principal findings is provided in **Table 5.1**. These findings informed the experimental questions and experimental designs of the primary data included in this body of work.

The second aim of this dissertation was to identify the effects of olanzapine on cisplatin-induced pica, anorexia, and weight loss in rats. The principal findings are summarized in Table

**5.2.** As mentioned above, since olanzapine was introduced based on clinical observations, its effect on pre-clinical models for the study of CINV remains unexplored. Thus, in Chapter II we tested the effects of intraperitoneal and fourth ventricle olanzapine administration. These experiments show that olanzapine decreases cisplatin-induced pica, anorexia, and weight loss. Our observation that IP olanzapine decreases malaise/nausea and anorexia 72 hours post cisplatin treatment in rats aligns with the effect of olanzapine in humans. Additionally, fourth ventricle olanzapine decreased, cisplatin-induced pica and weight loss at 48 and 72 hours. Notably, these behavioral changes were observed at 48 and 72 hours, a time period corresponding to the peak of delayed CINV. Thus, in rats, as in humans, olanzapine is particularly effective at reducing delayed CINV. This suggests that rats are a useful model in the study of olanzapine's effect on CINV.

The third aim of this body of work was to determine olanzapine's effect on the hindbrain and on potential biological correlates of CINV. To address this aim, we conducted a series of experiments to determine 1) does olanzapine have an effect on the hindbrain, and if so, is olanzapine's effect on the hindbrain sufficient to reduce cisplatin-induced side effects? and 2) does olanzapine counteract cisplatin-induced changes in circulating ghrelin and serotonin and ghrelin receptor expression? We found that IP olanzapine decreases the number of cisplatin-activated neurons in the NTS (region of the hindbrain known to mediate CINV) 6 hours post cisplatin-treatment. Thus, olanzapine acts on the hindbrain, reducing the number of activated neurons in the NTS. To determine if olanzapine's effect on the hindbrain is sufficient to reduce cisplatin-induced pica, anorexia, and weight loss, olanzapine was administered directly into the fourth ventricle. Olanzapine infusion into the fourth ventricle attenuated cisplatin-induced pica and body weight loss, but not anorexia. Thus, olanzapine's effect on the hindbrain is sufficient to attenuate pica and weight loss, but olanzapine's effect on feeding is likely mediated by other brain regions (not the hindbrain). Additionally, these experiments suggest that olanzapine's reduction of weight loss, is not directly caused by changes in feeding. Rather, olanzapine may

decrease weight loss via other changes in energy balance such as decreased locomotion and reduced thermogenesis.

To determine if olanzapine counteracts cisplatin-induced changes in serotonin and ghrelin systems we measured circulating ghrelin levels, changes in ghrelin receptor (GHSR1) expression, and in serotonin receptor expression (5-HT2C, 5-HT2A, 5-HT1A, and 5-HT3). We found olanzapine reverses cisplatin-induced decreases in circulating acylated ghrelin (active ghrelin) and that olanzapine treated animals showed increased hypothalamic GHSR1 expression compared to cisplatin treated animals. Thus, olanzapine has an opposite effect on circulating ghrelin and ghrelin receptor expression compared to cisplatin. Olanzapine did not have an effect on GHSR in the DVC, the central amygdala, or the PBN. Lastly, olanzapine counteracted cisplatin-induced increases in 5-HT2CR expression, but did not change 5-HT2A, 5-HT1A, or 5-HT3 receptor expression. Together these experiments show that olanzapine counteracts cisplatin-induced: decreases in circulating ghrelin, decreased hypothalamic GHSR1 expression, and increases in 5-HT2CR expression. These principal findings are summarized in Table **5.3**.

Together the primary data suggest that, in the rat, olanzapine decreases, cisplatin-induced pica, anorexia, and weight loss during the delayed phase of CINV. Thus, rats can be a useful model to study the effects of olanzapine on CINV and energy balance. Additionally, intraperitoneal olanzapine decreases the number of activated neurons in the AP and the NTS six hours post-cisplatin treatment, suggesting olanzapine has an effect on the hindbrain. Indeed, fourth ventricle administration of olanzapine reduced pica, demonstrating that olanzapine's effect on the hindbrain is sufficient to reduce pica. Furthermore, fourth ventricular administration of olanzapine reduced weight loss but did not reduce cisplatin-induced anorexia. This suggests that olanzapine's effects on feeding are mediated by non-hindbrain regions. Olanzapine may have eating-independent mechanisms of reducing weight loss (e.g. reduced thermogenesis, locomotor activity). Lastly, the data show olanzapine counteracts cisplatin-induced: decreases in circulating

ghrelin, decreased hypothalamic GHSR1 expression, and increases in 5-HT2CR expression.

**Figure 5.1** provides a summary of these integrated findings.

#### NIH Symptom Science Model

The NIH Symptom Science Model (NIH-SSM) served to guide the experiments described in this body of work. As mentioned previously, this model can be applied to chemotherapy-induced nausea (a symptom). The behavioral experiments were conducted to characterize the phenotype (determining whether olanzapine decreases cisplatin-induced pica, anorexia, and weight loss). The data obtained in this body of work suggests olanzapine does attenuate cisplatin-induced, pica, anorexia, and weight loss. Immunohistochemistry and molecular experiments were conducted to identify the effect of olanzapine on potential biological mediators of CINV and energy balance dysregulation. These experiments suggest that olanzapine may act by counteracting the effects of cisplatin on 5HT2CR expression, decreasing cisplatin-induced increases in 5-HT2CR expression. The studies also suggested that olanzapine may counteract decreases in ghrelin, by counteracting cisplatin-induced reductions in circulating ghrelin and rescuing cisplatin-induced decreases in hypothalamic GHSR expression. Further research is required to establish whether these changes in serotonin and ghrelin are sufficient to reduce CINV, anorexia, and weight loss. Better understanding the biological basis of olanzapine's action of CINV, could help identify more specific therapeutic targets that improve chemotherapy-induced symptoms while reducing side effects. Thus, the specific aims of the studies presented in this dissertation align with NINR's recognition that "novel discoveries require the integration of behavioral and biological data in the development of models to predict, treat, and manage symptoms of diseases and treatments (NINR, September 2016)."

#### Limitations

The experiments described in this body of work suggest that olanzapine may alleviate CINV, anorexia, and weight loss by counteracting the effects of cisplatin on the hindbrain,

circulating ghrelin, ghrelin receptor expression, and 5HT<sub>2C</sub> receptor expression. However, these changes may interact with other factors or may be mediated by confounding factors. For example, emetogenic risk in response to chemotherapy varies by sex and olanzapine's effect on body weight also varies by sex and (Davey et al., 2012; Nyman et al., 2007; Roila et al., 1991; J. P. Zhang et al., 2013). Thus, estrogen or other hormones that affect sexual development or reproduction may also play an important role in olanzapine's effect on malaise and energy balance. Further research is needed to determine if olanzapine's effect on chemotherapy-induced changes in ghrelin and serotonin is sufficient to attenuate CINV, anorexia, and weight loss and to identify/study other factors that may also contribute to decreased malaise behavior.

Additionally, water intake data may help clarify the effect of olanzapine on body weight changes. In addition to inducing anorexia, cisplatin is also known to reduce water intake during the first 48-72 hours (De Jonghe & Horn, 2008; De Jonghe et al., 2009; Malik et al., 2006), which likely contributes to cisplatin-induced body weight loss. As is discussed above, our studies suggest that olanzapine's attenuation of cisplatin-induced anorexia is mediated by hindbrain independent mechanisms as supported by our findings that ICV olanzapine (hindbrain specific) was not sufficient to decrease anorexia. However, the hindbrain (including the area postrema, the nucleus of the solitary tract and the parabrachial nucleus, and other areas known to mediate CINV) is known to be important in regulating water intake (Johnson & Thunhorst, 1997; Ramsay & Booth, 2012). Thus, although ICV olanzapine may not be sufficient to reduce anorexia, ICV olanzapine may be particularly effective at reverse cisplatin-induced decreases in water intake. An increase in water intake following ICV olanzapine could increase weight and explain the observed reductions in weight loss in the absence of increased food intake. Future experiments should examine the effect of ICV olanzapine on water intake.

Additionally, the experiments in this body of work were conducted on healthy rodents and, as described below, future research should examine the effects of olanzapine on malaise behavior of tumor bearing models. As described in chapter two, cancer treatment is often

determined based on tumor type, stage, and location and treatments have different emetogenic risks ((NCCN), 2017; Baba et al., 2017). Thus, cancer pathology also impacts response cancer treatment and to antiemetics and should be considered in CINV research. Furthermore, we also observed improvement in cisplatin-induced malaise, anorexia, and weight loss during the delayed phase of CINV. However, our biological data was collected during the acute phase (6 hours post cisplatin treatment) and future research should also examine changes in the number of hindbrain activated neurons, circulating ghrelin, and GHSR/5HT2CR expression in the delayed phase (24 hours+ post cisplatin treatment).

#### Recommendations for Future Research

##### *Expanding the Study of the of Olanzapine as an Antiemetic in Pre-Clinical Models*

Although multiple pre-clinical studies have been conducted to examine the metabolic effects of olanzapine, there is scant literature examining olanzapine as an antiemetic in pre-clinical models (Machida et al., 2015). Only one previous study has been conducted to investigate the effects of cisplatin-induced pica in rats (Machida et al., 2015). In this study, intraperitoneal olanzapine (2 mg/kg and 10 mg/kg.) decreased cisplatin-induced pica, but this change was not significant. The data described in this body of work builds upon these findings and shows that olanzapine does significantly reduce cisplatin-induced pica in rats. Future research studies can utilize rats as a pre-clinical model for the study olanzapine in the context of CINV and energy balance. Future research should also be conducted on tumor bearing pre-clinical models to determine olanzapine's effect on chemotherapy-induced malaise behaviors and to account for the complexity of cancer pathology.

##### *Better Defining the Role of 5-HT2CR, GHSR, and circulating ghrelin in CINV*

The experiments described in this in this body of work show olanzapine decreases pica, anorexia, and weight loss. They also suggest that olanzapine may counteract cisplatin-induced 1) increases in 5-HT<sub>2</sub>CR expression (in the dorsal vagal complex and hypothalamus), 2) decreases in hypothalamic GHRS expression, and 3) decreases in circulating ghrelin. Future experiments should test whether serotonergic antagonism and ghrelin agonism are sufficient to reduce pica, anorexia, and weight loss. To test this relationship, future studies can investigate the effects of olanzapine on CINV and energy balance regulation in ghrelin signal-deficient models (e.g. ghrelin-knockout or GHSR-knockout animals). Additionally, animals could be administered an antagonist specific to 5-HT<sub>2</sub>CR and/or a GHSR agonist to determine if this is sufficient to reduce CINV. Translating these results into clinical applications, such as developing drugs that target 5-HT<sub>2</sub>C and ghrelin signaling systems, warrants further research.

#### *Standardizing Measures for the Study of the Metabolic and Antiemetic Effects of Olanzapine in Rodents*

As mentioned previously, pre-clinical studies have been conducted to examine the metabolic effects of olanzapine in rodents, including its effect on food intake and body weight. Additionally, pre-clinical studies have examined the effects of olanzapine on circulating ghrelin levels. However, as literature reviews have highlighted, olanzapine's effect on body weight, food intake, and ghrelin are not consistent across the literature. This may be due to variations in doses, route of administration, measurement times, and study duration. A recent review, examining conflicting data regarding the effects of olanzapine on circulating ghrelin suggested that the differences might be due to a triphasic effect, with an initial upregulatory effect followed by a downregulatory negative feedback phase, and a final increase effect in the long term (Q. Zhang et al., 2014). Standardizing experimental conditions to study the metabolic and antiemetic effects of olanzapine in rodents would allow for inter-study comparisons that clarify the effects of olanzapine on serotonin and ghrelin signaling systems.

## Recommendations for Clinical Practice

### *Understanding the Biological Mediators and Mechanisms of Drugs Used in the Clinical Setting*

There are numerous medications with unknown biological mechanisms that like olanzapine, are used because of their observed clinical efficacy. However, it is important to understand the biological basis of their clinical effects to improve scientific knowledge about the pathological condition(s) they treat. Better understanding the biological effects of these drugs can also help in the development of more specific drug targets that improve health while minimizing adverse effects. Drugs like olanzapine lack specificity, acting on multiple receptors throughout the body and this lack of specificity can lead to increased adverse effects. This body of work highlights the gaps in the literature regarding the biological basis of olanzapine's use as an antiemetic and the need to better understand how olanzapine alleviates CINV. Thus, the studies presented in this dissertation support the need to better understand drugs that are utilized to treat and manage disease conditions or side effects of treatment, aligning with the NINR's recognition that integration of behavioral and biological data is crucial in the study of symptoms.

### *Integrating Updated Clinical Guidelines in to Clinical Practice*

As was evident in the literature (Chapter II) and in this body of work, olanzapine is very effective and superior to other antiemetics in the management of CINV, particularly in alleviating delayed CINV. For example, olanzapine when used in combination with dexamethasone and 5-HT<sub>3</sub> receptor antagonists provides superior control (compared to a combination of NK-1 receptor antagonists, dexamethasone, and 5-HT<sub>3</sub> receptor antagonists) of delayed CINV reducing delayed nausea from 62% to 31% (Navari et al., 2011). This 50% reduction in the incidence of delayed nausea, translates to improvement in symptom management for a potentially large number of people undergoing cancer treatment. Thus, it is important for clinicians to integrate clinical guidelines into their practice to ensure patients' symptoms are better controlled.



## Conclusion

The purpose of this body of work was to better understand how olanzapine alleviates CINV. Reviews of the literature highlighted the need to better understand the biological basis of olanzapine's antiemetic effect and led to the identification of ghrelin and serotonin systems as potential mediators of olanzapine's effect on malaise and energy balance. Behavioral data showed olanzapine decreases pica, anorexia, and body weight loss in rats, suggesting that rats are a useful pre-clinical model in the study of olanzapine. Experimental data in rats provided evidence suggesting olanzapine's reduction in chemotherapy-induced malaise/nausea is at least partially mediated by the hindbrain. Lastly, biological assays and molecular techniques demonstrated that olanzapine counteracts cisplatin-induced 1) neuronal activation in the dorsal vagal complex 2) decreases in plasma ghrelin and hypothalamic ghrelin receptor expression and 3) increases in 5-HT<sub>2C</sub> expression in the dorsal vagal complex and the hypothalamus. In summary, these results suggest that olanzapine may alleviate CINV by counteracting the effects of cisplatin on the hindbrain, on circulating ghrelin, ghrelin receptor expression, and 5HT<sub>2C</sub> receptor expression. Further research is needed to establish whether rescuing chemotherapy-induced changes in ghrelin and serotonin signaling is sufficient to attenuate CINV.

Tables

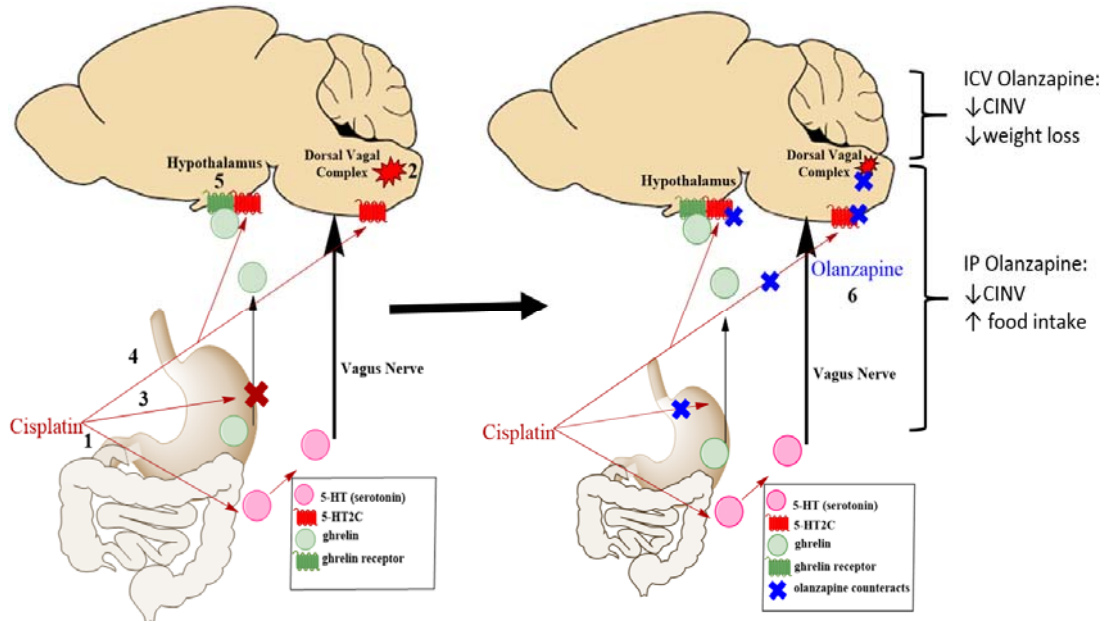
<b>Table 5.1 Principle Findings: Specific Aim 1</b>	
<b>Specific Aim 1: Identify gaps in the literature regarding the use of olanzapine in CINV</b>	
<b>Studies</b>	<b>Principal Findings</b>
Identify gaps in the literature regarding olanzapine and CINV	<p>1) Olanzapine’s mechanism(s) of reducing CINV are unknown.</p> <p>2) Few preclinical studies have examined olanzapine as an antiemetic</p>
Identify potential biological mediators of olanzapine in the context of CINV	<p>1) Olanzapine may alleviate CINV by counteracting cisplatin-induced decreases in circulating ghrelin and ghrelin receptor expression</p> <p>2) Olanzapine may alleviate CINV by counteracting cisplatin-induced increases in 5HT2C expression</p>

<b>Table 5.2</b> Principle Findings: Specific Aim 2	
<b>Specific Aim 2:</b> Identify behavioral effects of olanzapine in cisplatin treated rats	
Studies	Principal Findings
Determine the effects of intraperitoneal olanzapine on cisplatin-induced pica, anorexia, and weight loss	<p>1) IP olanzapine decreases cisplatin-induced pica 72 hours following cisplatin administration</p> <p>2) IP olanzapine decreases cisplatin-induced anorexia 72 hours post cisplatin administration</p> <p>3) IP olanzapine does not decrease cisplatin-induced weight loss</p>
Determine the effects of fourth ventricle olanzapine infusion on cisplatin-induced pica, anorexia, and weight loss	<p>1) 4<sup>th</sup> ICV olanzapine decreased cisplatin-induced pica 48 and 72 hours post chemotherapy</p> <p>2) 4<sup>th</sup> ICV olanzapine decreases cisplatin-induced weight loss 48 and 72 hours following chemotherapy</p> <p>3) 4<sup>th</sup> ICV olanzapine does not decrease cisplatin-induced anorexia</p>

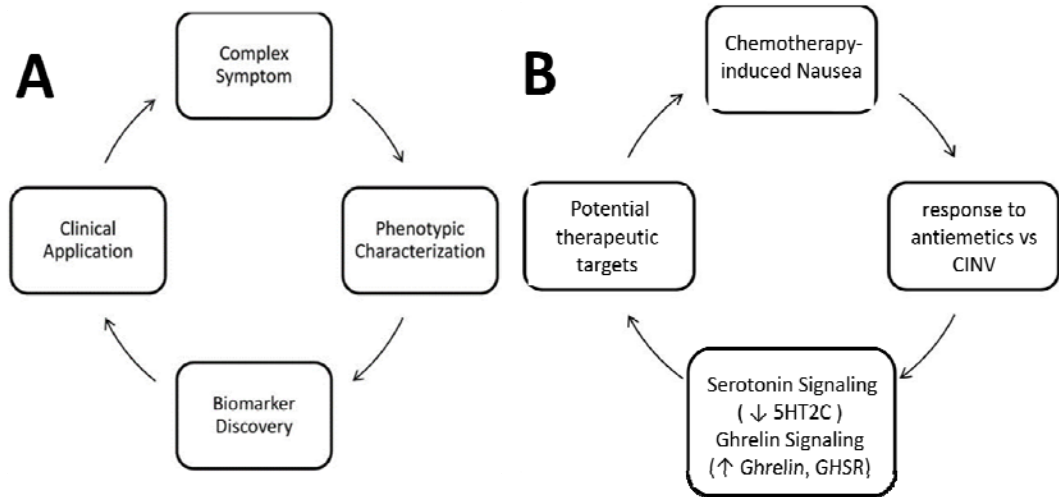
Table 5.3 Principle Findings: Specific Aim 3	
Specific Aim 1: Determine olanzapine's effect on potential biological correlates of CINV.	
Studies	Principal Findings
Assessment of olanzapine treatment on cisplatin-induced c-Fos in DVC neurons	1) IP olanzapine decreases the number of neurons that are activated by cisplatin in the area postrema and the nucleus of the solitary tract 6 hours post cisplatin treatment
Determine the effects of intraperitoneal and fourth ventricle olanzapine on cisplatin-induced pica, anorexia, and weight loss	1) Olanzapine's effect on the hindbrain is sufficient to induce pica 72 hours post chemotherapy 2) Hindbrain olanzapine reduced cisplatin-induced weight loss 72 hours post chemotherapy 3) 4 <sup>th</sup> ICV olanzapine did not reduce cisplatin-induced anorexia
Assessment of olanzapine and cisplatin on ghrelin	1) IP olanzapine rescues cisplatin-induced decreases in plasma acylated ghrelin 6 hours post cisplatin treatment
Assessment of olanzapine on cisplatin-induced alterations in central mRNA expression of GHSR and serotonergic receptors (5-HT <sub>2C</sub> , 5-HT <sub>2A</sub> , 5-HT <sub>1A</sub> , and 5-HT <sub>3</sub> )	1) Olanzapine reduces cisplatin-induced increases in 5-HT <sub>2CR</sub> expression in the hypothalamus and dorsal vagal complex 6 hours post cisplatin treatment 2) Cisplatin-treated animals had lower GHSR expression compared to cisplatin treated animals 6 hours post cisplatin treatment

Figures

Figure 5.1



**Figure 5.2**



*Note. Based on the NIH Symptom Science Model (NIH-SSM; Cashion & Grady, 2015)*

## Figure Legends

**Figure 5.1-** **1)** Cisplatin stimulates the release of serotonin from enterochromaffin cells in the small intestine and a signal is relayed by the vagus nerve to the hindbrain, a known mediator of CINV. **2)** These afferent signals from the vagus nerve, terminate at the hindbrain, activating neurons in the dorsal vagal complex. **3)** Cisplatin reduces circulating ghrelin levels. **4)** Cisplatin increases expression of 5-HT<sub>2C</sub>. **5)** Cisplatin treated animals have decreased hypothalamic GHSR expression compared to cisplatin-treated animals. **6)** Olanzapine counteracts the effects of cisplatin, antagonizing 5-HT<sub>2C</sub> receptors, decreasing the number of cisplatin-activated neurons in the dorsal vagal complex, decreasing 5-HT<sub>2C</sub> receptor expression in the hypothalamus and DVC, and increasing hypothalamic GHSR compared to cisplatin-treated animals.

**Figure 5.2-** **1)** Cisplatin stimulates the release of serotonin from enterochromaffin cells in the small intestine and a signal is relayed by the vagus nerve to the hindbrain, a known mediator of CINV. **2)** These afferent signals from the vagus nerve, terminate at the hindbrain, activating neurons in the dorsal vagal complex. **3)** Cisplatin reduces circulating ghrelin levels. **4)** Cisplatin increases expression of 5-HT<sub>2C</sub>. **5)** Cisplatin treated animals have decreased hypothalamic GHSR expression compared to cisplatin-treated animals. **6)** Olanzapine counteracts the effects of cisplatin, antagonizing 5-HT<sub>2C</sub> receptors, decreasing the number of cisplatin-activated neurons in the dorsal vagal complex, decreasing 5-HT<sub>2C</sub> receptor expression in the hypothalamus and DVC, and increasing hypothalamic GHSR compared to cisplatin-treated animals.

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