Review

TOXICOLOGICAL ASPECTS OF CISPLATIN-INDUCED EMESIS WITH EMPHASIS ON SEROTONIN RELEASE

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I. INTRODUCTION

Therapeutic doses of metoclopramide which antagonizes the dopamine D₂ receptors do not inhibit the emesis induced by anticancer drugs such as cisplatin. However, a large dose of metoclopramide large enough to produce adverse reactions in the central nervous system inhibits the emesis induced by cisplatin; this antiemetic action appears to be due to an antagonistic action on the 5-HT₃ receptors (Miner and Sanger, 1986). The 5-HT receptor was classified by Bradley *et al.* (1986) and 5-HT₃ antagonists which selectively antagonize 5-HT₃ receptors have been developed. In the study of 5-HT₃ antagonists, ferrets were used as the animal model for emesis (Florczyk *et al.*, 1982). We have studied emesis using imported ferrets for the past 10 years.

Recent research using 5-HT₃ receptor antagonists has implicated 5-HT in the occurrence of anticancer drug-induced emesis (Miner and Sanger, 1986; Costall *et al.*, 1987). Emesis is an instinctive defense reaction caused by the somato-autonomic nerve reflex which is integrated in the medulla oblongata. Toxic substances produce an increase in 5-HT due to the stimulation of enterochromaffin cells; the increased 5-HT-

induced paraneuron stimulation may release VIP and diarrhea may occur. Although toxic substances may be ejected from the intestine via diarrhea, toxins which exist in the stomach and the upper digestive organs may not be eliminated. Simultaneously 5-HT may stimulate the adjacent vagal afferent nerves. This vagal afferent nerve excitation may evoke the vomiting reflex.

The precise role of 5-HT on the occurrence of vomiting, however, has not been fully elucidated. It is important to recognize that 5-HT₃ receptor antagonists are not universal antiemetics. For instance, 5-HT₃ receptor antagonists do not produce an antiemetic effect on emetic stimuli such as apomorphine, morphine or motion sickness. Because each antiemetic agent has its own pharmacological properties, each drug should be selected depending upon of emetic stimuli. This review describes the involvement of 5-HT on cisplatin-induced vomiting ... with special attention focused on 5-HT release from the enterochromaffin (EC) cells.

II. PRESENCE OF 5-HT

1. 5-HT in the gastrointestinal tract

5-HT exists at the neurons of the enteric plexuses. The source of most 5-HT is the intestinal mucosal EC cells (Erspamer, 1966). Mast cells contain 5-HT in both rats and mice. As 5-HT does not evoke vomiting in these rodents, the effect of 5-HT derived from the mast cells is not discussed in this text.

Over 80% of 5-HT in the body is localized in the gut and 90% of this 5-HT is derived from EC

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cells. Erspamer and Testinini (1959) reported that the human intestine contains 4.5 to 7.5 mg of 5-HT. In general, 5-HT is distributed mainly in the duodenum and the colon. Similar 5-HT stores at the large electron-dense granules of EC cells is characteristic of the distribution of 5-HT in humans, rats, ferrets (Endo *et al.*, 1990a), bulls, calves, sheep and horses (Faustini, 1955). 5-HT is synthesized by tryptophan hydroxylase which is a rate-limiting enzyme for 5-HT formation at the endoplasmic reticulum localized at the lumen site of the EC cells and the Golgi apparatus (Dey and Hoffpsuir, 1984; Nillson *et al.*, 1985). Synthetic 5-HT localizes at the basal surface of the EC cells, the release of synthetic 5-HT from the EC cells depends upon local stimulation (Racké and Schwörer, 1991). Some 5-HT secretes into the gut lumen, but its functional role has not been elucidated. The turnover of the 5-HT which is released into the mucus is relatively slow. The half-life of 5-HT in humans is 7 to 12 hours and in dogs is 6 to 8 hours (Erspamer and Testinini, 1959).

2. 5-HT in the brain stem

It is generally accepted that the vomiting center is located in the area surrounding the chemoreceptor trigger zone (CTZ), although concrete evidence for the localization of this vomiting center has not been established. In several species, 5-HT is distributed at the dorsal vagal complex, area postrema (AP), NTS and fibers or varicosities of the dorsal motor vagal nucleus of the brain stem (Leslie, 1985). With regard to the sources of 5-HT in the AP, numerous possibilities exist: (1) the neuronal cell bodies of the AP itself, (2) the vagal afferent fibers which terminate at the AP, (3) the raphe which is a major 5-hydroxytryptaminergic nucleus, especially the adjacent fibers originated from raphe which are found in the area of reticular formation.

3. Platelet 5-HT

5-HT released from the intestine is transported to the liver; of the released 5-HT, 70% was metabolized into 5-HIAA (Erspamer and Testinini, 1959). Although circulating platelets can not synthesize 5-HT by themselves, they can

take it up. This 5-HT uptake by the platelets appears to be a protection mechanism against rapid 5-HT degradation (Thomas and Vane, 1967). Platelets transport large quantities of circulatory 5-HT. If humoral stimuli could be made to release 5-HT during the passage of the platelets through the vasculature of the AP and/or NTS, 5-HT₃ receptor excitation would occur at these areas.

III. ROLE OF 5-HT IN THE GUT

What is the functional role of the 5-HT in the EC cells? With regard to the functional role of mucosal 5-HT, the role of 5-HT has been reviewed in respects to cholera toxin, food allergens and the responsiveness of the vagal afferent nerves (Andrews, 1991; Sanger, 1992). Large quantities of 5-HT exist in the epithelia of the gastrointestinal tract, airway and skin. The presence of 5-HT might be an indication of defensive or protective reactions of the body to the outer environment. The vomiting or diarrhea caused by 5-HT seems to be a defense reaction; Vomiting and diarrhea both purge the body of ingested toxins. Afferent vagal terminals are located in areas adjacent to the EC cells. Therefore, the EC cell-vagal afferent nerve unit appears to be the anatomical mucosal chemoreceptor of the gastrointestinal tract (Newson *et al.*, 1982). In fact, vagal afferent nerves were affected by the luminal environment of the gut (osmolarity, pH and temperature) as well as by particles of food which passed through it (Andrews, 1986).

IV. RELEASE OF 5-HT

1. Mucosal levels of 5-HT

It has been reported that total body irradiation by X-ray produced a decrease in the 5-HT levels of the intestinal mucosa of the mice, rats (Penttilä and Kormano, 1971) and ferrets (Andrews, unpublished observations). This decrease in the 5-HT level appears to be associated with an increased release of 5-HT from the intestinal mucosa. The release of 5-HT from the EC cells induced by radiation and cytotoxic drugs

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may be related to a triggering of the secretory mechanism of the EC cells. The most probable mode of 5-HT release may depend upon calcium ion influx (Racké and Schwörer, 1991), although the mechanism has not been clarified. Our group reported that ileal mucosal tryptophan hydroxylase activity increased in cisplatin- or cyclophosphamide-treated ferrets as compared with that in non-drug control ferrets. Monoamine oxidase activity, on the contrary, was inhibited in cisplatin- or cyclophosphamide-treated ferrets as compared with that in non-drug control ferrets (Endo *et al.*, 1993). This increased tryptophan hydroxylase activity appears to be due to the activation of ileal 5-HT biosynthesis induced by the cytotoxic drugs. Through this mechanism, the life span of 5-HT might be prolonged. Cisplatin-treated ferrets showed an increase in ileal 5-HT, 5-HIAA and norepinephrine concentrations, but no increase in their dopamine level (Gunning *et al.*, 1987; Endo *et al.*, 1990a, 1990b). Our group also clarified that intragastric copper sulfate administration produced an increase in ileal 5-HT levels (Endo *et al.*, 1991). Cisplatin increased 5-HT release from both isolated guinea pig ileum (Schwörer *et al.*, 1991) and isolated cat ileum (Milano *et al.*, 1991).

In summary, anticancer therapy activates 5-HT release from the mucosa in the upper gastric intestinal tract. Released 5-HT may activate locally the vagal afferent nerve terminal which is adjacent to the basal surface of the EC cells. On the other hand, 5-HT may release into the circulation at the hepatic portal vein. And 5-HT may activate afferents within the liver.

2. Platelet 5-HT

In order to clarify the correlation between the time course changes of released 5-HT derived from the EC cells and the pattern of emesis, we measured the actual 5-HT release by *in vivo* microdialysis. Our group monitored the free 5-HT concentration from the portal vein using this technique (Yoshioka *et al.*, 1992). The portal vein 5-HT concentration fluctuated markedly. After dialysis, the probe membrane showed platelet adhesion and aggregation under electron microscope. These results indicated

that using microdialysis as a method for determination of the portal vein 5-HT concentration is not accurate due to the contamination of the probe membrane and cannula with platelet 5-HT.

The administration of platelet-activating factor (PAF) into the dog trachea produced an increase in 5-HT in venous blood (Murphy *et al.*, 1989). If platelet 5-HT is involved in emesis, then PAF should also produce emesis. Cubeddu *et al.* (1992) reported that no difference in platelet 5-HT levels was observed between healthy subjects and cancer patients. Platelet 5-HT did not increase during cisplatin- or cyclophosphamide-induced emesis. These studies suggest that platelet 5-HT is not involved in the emetic response induced by anticancer therapy.

3. Blood and urinary levels of 5-HT

An experiment involving isolated vascularly perfused guinea-pig small intestine showed that cisplatin produced a dose-dependent increase in both 5-HT and 5-HIAA (Schwörer *et al.*, 1991). Interesting evidence revealed by Schwörer's experiment was that the cisplatin-induced 5-HT release was blocked by tetrodotoxin, hexamethonium and/or scopolamine. These findings suggest that the cisplatin-induced EC cell effects may not be due to direct action, but may be due to indirect action via a cholinergic mechanism such as the stimulation of the enteric neurons by cisplatin to release ACh. This released ACh produces an increase in 5-HT release from the EC cells (Racké and Schwörer, 1991). Cubeddu *et al.* measured plasma and urinary 5-HT concentrations in numerous cancer patients. They proposed that the determination of urinary 5-HIAA concentration, a major metabolite of 5-HT, might be an indicator of gut mucosal 5-HT release. A large dose of cisplatin produced an marked increase in urinary 5-HIAA levels in patients, and the increased urinary 5-HIAA levels paralleled the occurrence of vomiting. This suggests that urinary 5-HIAA might be a convenient index. Thus, using urinary 5-HIAA concentration, the severity of acute emesis and the degree of delayed emesis may be predictable. Urinary 5-HIAA levels might be useful for predicting anticipatory emesis in female and for determining

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whether anticipatory emesis occurs due to psychological phenomenon or occurs due to the disease itself or to anticancer drugs. The urinary 5-HIAA determination might also be useful in the analysis of mechanism of pregnancy sickness.

As aforementioned, 5-HT release from the EC cells may be the initial trigger for vomiting. It is uncertain how cytotoxic drug-induced 5-HT release is brought about. Intracerebroventricular administration of cisplatin produced an emesis within approximately 4 minutes; intravenous or intraperitoneal injection of cisplatin produced vomiting 90–120 minutes after drug administration (Minami *et al.*, 1991). The difference in anticancer drugs may be related to different time latencies for vomiting. It is possible that cisplatin may transform emesis-producing substances via drug biotransformation in the intestine and the liver and that these emesis-producing substances may cause the vomiting. With regard to the mechanism of cisplatin-induced emesis, involvement of free radicals has also been proposed.

Torii *et al.* found that the free radical formation of cisplatin produced emesis without a time-lag in *Suncus murinus*. They also reported that both antioxidant and 5-HT₃ receptor antagonists abolished the vomiting induced by a radical generator, pyrogallol (Torii *et al.*, 1992, 1993). Our group also found that the phosphatidylcholine hydroperoxide levels were reduced compensationally in the intestine of ferrets which received two administrations of cisplatin (Minami *et al.*, unpublished data).

4. Central 5-HT

Cytotoxic drugs cause 5-HT release from the EC cells. This released 5-HT stimulates the vagal afferent nerve fibers in areas adjacent to the EC cells. Stimulation of afferent vagal nerve fibers appears to be related to the increased 5-HT levels in the area postrema. In fact, the electrical stimulation of abdominal vagal afferent nerves produced an increase in the area postrema-5-HT levels and evoked vomiting in anesthetized ferrets (Endo *et al.*, in press). Our group reported that 5-HT levels of the area post-

rema and adjacent structures increased significantly after cisplatin or cyclophosphamide administration (Endo *et al.*, 1992).

Our group measured the rate-limiting enzyme (TPH) activity of 5-HT synthesis, of the area postrema. We found that TPH activity increased significantly in cisplatin-treated ferrets as compared with that in non-drug control ferrets (Minami *et al.*, in press). Our group reported that after abdominal vagotomy, cisplatin did not increase the area postrema-5-HT level, and the cisplatin produced a significant decrease in vomiting (Endo *et al.*, 1993). It has been reported that area postrema-5-HT release occurred while platelets passed through the area postrema; abdominal vagotomy clearly inhibited the area postrema-5-HT increase induced by cisplatin. These findings suggest that an area postrema-5-HT increase induced by cisplatin is due to emetic stimuli originating from the vagal afferent nerves. Area postrema-5-HT levels correlated well with the frequency of emesis (Fig. 1).

In ferrets, the area postrema contained a large amount of 5-HT₃ receptors and the area postrema-5-HT levels increased after cytotoxic drug administration. Ondansetron, moreover, diminished the area postrema-5-HT increase induced by cytotoxic drugs. Therefore, the area postrema appears to perform a functional role in relaying signals between the visceral afferents and the vomiting center. Pretreatment with ondansetron diminished the area postrema-5-HT increase, but did not alter the increased ileum 5-HT levels (Endo *et al.*, 1992).

These findings suggest that the site of action of 5-HT₃ receptor antagonists are the 5-HT₃ receptors and that the primary pharmacological action is an antagonistic action for 5-HT. Namely, the increased 5-HT levels in the vomiting center which were induced by anticancer drugs were inhibited by the chemical vagotomy caused by 5-HT₃ receptor antagonists. Oral administration of 5-HT₃ receptor antagonists produced a strong antiemetic action as compared with the intraperitoneal administration of equivalent doses of 5-HT₃ receptor antagonists (Endo *et al.*, 1993). A possible explanation of this difference between oral and intraperitoneal effects involves the large

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Fig. 1 Correlation between area postrema-5-HT levels and the frequency of emesis.

distribution of the 5-HT₃ receptor antagonists at 5-HT₃ receptor sites on afferent vagal nerve endings.

Abdominal vagotomy is effective for the emetic stimuli which release 5-HT from the gut and stimulate the vagal afferent nerves. On the other hand, abdominal vagotomy tends to be ineffective for emesis in which humoral agents excite the area postrema or the CTZ. Anticancer drugs congregate at rapid turnover tissues such as the gastrointestinal tract, and therefore, impair epithelium of the mucous membrane. Increased endotoxin levels in the gastrointestinal tract correlated with the frequency of vomiting. Endotoxin stimulates prostaglandin synthesis and endotoxin facilitates the secretion of β -endorphin; prostaglandin and β -endorphin appear to be related to emesis (Carr *et al.*, 1982).

After endotoxins produced hyperthermia, brain β -endorphin was released into cerebrospinal fluid, after which β -endorphin was detected in the circulation. Endotoxin-induced emesis accompanied with hyperthermia was ameliorated by naloxone (Carr *et al.*, 1982).

The central terminal of the abdominal vagal afferent nerves projects into the area postrema. The vagal afferent nerve itself does not release 5-HT, but activates the 5-hydroxytryptaminergic neurons. One of the 5-HT-containing neuronal groups in the brain stem is the raphe nucleus.

The NTS receives input from the raphe nucleus. However, it is uncertain whether the NTS-raphe nucleus pathway is an important component of emetic response (Leslie *et al.*, 1992). The neurons within the area postrema were excited not only by 5-HT, but also by gastrin, histamine, neurotensin, substance P and enkephalin (Carpenter *et al.*, 1983; Borison *et al.*, 1984). All these substances increased in the gastrointestinal tracts.

V. MODULATION OF 5-HT RELEASE

1. Autoreceptors

5-HT₃ receptors: The fact that 5-HT₃ receptor antagonists prevent cisplatin-induced emesis suggests that they may be associated with 5-HT release. According to a study by Cubeddu *et al.* (1990, 1992), cisplatin-treated patients receiving ondansetron did not show a decrease in urinary 5-HIAA levels. In our ferret study (Endo *et al.*, 1990a, 1990b, 1991), simultaneous administration of cisplatin and ondansetron did not change ileum 5-HT levels. Schwörer *et al.* (1991) reported that 5-HT₃ receptor antagonists such as ondansetron, tropisetron and MDL72222 produced a dose-related reduction of cisplatin-evoked 5-HT release in the isolated vascularly perfused guinea-pig intestine. Schwörer's results are controversial, however, because a threshold exists be-

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tween 10^{-14} to 10^{-13} M of ondansetron and tropisetron. Moreover, Schwörer used guinea-pigs which have a lower affinity for 5-HT₃ receptor antagonists (Butler *et al.*, 1990; Newberry *et al.*, 1991).

Regulation of 5-HT release from the intestinal mucosa involves a very complex mechanism (Racké and Schwörer, 1991). The cisplatin-induced 5-HT release from the guinea-pig intestine was prevented by tetrodotoxin, hexamethonium and scopolamine (Schwörer and Racké, 1989). The study suggests that 5-HT release from the EC cells was regulated by a positive feedback loop mediated by cholinergic interneurons. Gebauer *et al.* (1993) reported that 5-HT release decreased after the administration of granisetron, tropisetron and MDL72222. Compared to granisetron, tropisetron and MDL72222, ondansetron possesses a lower affinity to the 5-HT₃ receptors of the EC cells in the guinea pig intestine. Our group found that granisetron produced a significant decrease in the 5-HT release induced by 5-HT₃ receptor agonist, 2-methyl-5-HT (Minami *et al.*, unpublished data). Ondansetron selectively antagonized neuronal 5-HT₃ receptors (Butler *et al.*, 1990), but did not modify the cisplatin-induced 5-HT release from the isolated ferret ileum (Endo *et al.*, 1993). The fact that ondansetron and granisetron elicit different responses on 5-HT release at the 5-HT₃ receptor sites appears to support the existence of different species response and different 5-HT₃ receptor subtypes (Butler *et al.*, 1990).

Neurokinin A (NKA) and neurokinin B (NKB) are known as tachykinins. Substance P induces physiological activity via the NK1 receptors; NKA induces physiological activity via the NK2 receptors; NKB induces physiological activity via the NK3 receptors (Ramírez *et al.*, 1994). It has been reported that substance P may be associated with the depolarization of the 5-HT₃ receptors, and that NKB may excite 5-HT₄ receptors (Ramírez *et al.*, 1994).

5-HT₄ receptor: A selective 5-HT₄ receptor agonist, 5-methoxytryptamine, produced a dose-dependent increase in vomiting. We found that 5-methoxytryptamine produced a dose-dependent increase in 5-HT release from the isolated rat

ileum (Minami *et al.*, unpublished data). Our experiment did not use tetrodotoxin pretreatment because we had the future clinical use of 5-HT₄ receptor agonists and antagonists in mind.

Using vascularly perfused guinea-pig small intestine, Gebauer *et al.* (1993) studied the regulation of 5-HT release under tetrodotoxin-treated conditions. They reported that 5-HT₄ receptor stimulation inhibited 5-HT release and that the administration of tetrodotoxin eliminated the possibility of neuronally mediated 5-HT release (Gebauer *et al.*, 1993). Racké and Schwörer (1991) reported that tetrodotoxin blocked the neuronal input to the EC cells and that tetrodotoxin did not prevent receptor mediated 5-HT release. These experiments were carried out to explore species difference with and without tetrodotoxin pretreatment. Further study is needed to elucidate whether the EC cell-5-HT₄ receptors contribute to 5-HT release from the small intestine even though 5-HT₄ receptor agonists and 5-HT₄ receptor antagonists can not be administered to humans after pretreatment with tetrodotoxin. It was assumed that 5-HT₄ receptor agonists indirectly produced an increase in 5-HT release from the EC cells. A small dose of tropisetron (ICS 205-930) induces 5-HT₃ receptor antagonistic action whereas a large dose produces a 5-HT₄ receptor antagonistic effect; tropisetron inhibited vomiting. The fact that tropisetron inhibited copper sulfate-induced emesis suggests that copper sulfate-induced emesis is mediated by the 5-HT₄ receptors.

5-HT_{1A} receptor: It has been reported that selective 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT) (Lucot and Crampton, 1989; Wolff and Leander, 1994) and flesinoxan inhibited the emesis induced by xylazine, motion sickness and cisplatin. Xylazine causes emesis via CTZ excitation. Motion sickness occurs due to the impairment of the vestibular system. It has been reported that 5-HT_{1A} receptor agonists produce emesis by interfering with the integrated emetic reflex. It was also assumed that 5-HT_{1A} receptor agonists possess an inhibitory action for 5-HT release at serotonergic nerve endings.

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2. Heteroreceptors

A variety of heteroreceptors on the EC cells modulate 5-HT release. Excitation of nicotinic and muscarinic receptors (Schwörer *et al.*, 1987) and β -adrenoceptors (Racke *et al.*, 1988) facilitates 5-HT release. Excitation of α -adrenoceptors and GABA receptors (Schwörer *et al.*, 1989) inhibits 5-HT release.

VI. CONCLUSION

Cisplatin-induced 5-HT elevation in the intestine stimulates the afferent vagal nerve fibers. This afferent vagal nerve stimulation may induce abnormal discharge which may, in turn, directly excite the vomiting center in the medulla oblongata. Excitation of this vomiting center may evoke the vomiting reflex. The fact that abdominal vagotomy inhibited 85% of the cisplatin-induced emesis indicates that abdominal vagal afferent nerves seem to be the major pathway for cisplatin-induced emesis.

The initial trigger of this pathway may be due to 5-HT release from the EC cells. The 5-HT release from these cells is regulated by a polymodal mechanism and is calcium dependent. A selective 5-HT₃ receptor agonist, 2-methyl 5-HT, produced a dose-dependent 5-HT release. The 5-HT₃ agonist-induced 5-HT release was prevented by 5-HT₃ antagonists; but other kinds of 5-HT₃ antagonists did not prevent the 5-HT release induced by 5-HT₃ agonists. A selective 5-HT₄ agonist, 5-methoxytryptamine, produced 5-HT release dose-dependently; it was assumed that this release occurs indirectly via the cholinergic interneurons. 5-HT_{1A} agonist possesses a broad spectrum of antiemetic action; one such mechanism appears to be associated with 5-HT release. Not only autoreceptors, but also many heteroreceptors on the EC cells might be associated with 5-HT release from the EC cells.

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