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Involvement of hypothalamic cyclooxygenase-2, interleukin-1β and melanocortin in the development of docetaxel-induced anorexia in rats

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ABSTRACT

Docetaxel, a taxane derivative, is frequently used for the treatment of advanced breast cancer, non-small cell lung cancer, and metastatic prostate cancer. Clinical reports demonstrated that docetaxel-based chemotherapy often induces anorexia, but the etiology is not completely understood. To elucidate possible mechanisms, we investigated the involvement of central interleukin (IL)-1β, cyclooxygenase (COX)-2, and pro-opiomelanocortin (POMC) in the development of docetaxel-induced anorexia in rats.

Rats received docetaxel (10 mg/kg, i.p.) with or without pretreatment with selective COX-2 inhibitors, NS-398 (10 and 30 mg/kg, i.g.) or celecoxib (10 and 30 mg/kg, i.g.), and a non-selective COX inhibitor, indomethacin (10 mg/kg, i.g.), then food intake was monitored for 24 h after administration. We also examined expression of IL-1β, COX-2, and POMC mRNA in hypothalamus of docetaxel-treated rats and the effect of a COX-2 inhibitor on docetaxel-induced POMC mRNA expression.

Food consumption in rats was significantly decreased 24 h after administration of docetaxel and anorexia was partially reversed by all COX inhibitors. Administration of docetaxel increased IL-1β, COX-2, and POMC mRNA expression in the hypothalamus of rats. The time required to increase these gene expressions was comparable to the latency period of docetaxel-induced anorexia in rats. In addition, pretreatment with COX-2 inhibitors suppressed docetaxel-induced expression of POMC mRNA.

These results suggest that IL-1β and COX-2 mRNA expression and subsequent activation of POMC in the hypothalamus may contribute to the development of docetaxel-induced anorexia in rats.

1. Introduction

Most chemotherapeutic agents frequently induce therapy-related anorexia (Dreizen et al., 1990; Tohgo et al., 2002; Wood et al., 2006a). Insufficient control of this troublesome symptom reduces patients' quality of life (Edmonson et al., 1996; Okada et al., 1999) and leads to increased risk of malnutrition (Dreizen et al., 1990). Corticosteroids, such as dexamethasone, and progestational drugs, such as megestrol acetate, are known to lead to increased appetite and are used for the pharmacological treatment of anorexia in cancer patients (Behl and Jatoi, 2007). However, chronic administration of these drugs has the potential to induce severe adverse effects such as secondary adrenal insufficiency (Bulchandani et al., 2008; Han et al., 2012).

The mechanism of this appetite improvement may be related to inhibition of the production of inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (MacDonald et al., 2003), but there is little evidence that inflammatory mediators contribute to the development of chemotherapy-induced anorexia. Wood et al. (2006a,b) reported that many side effects induced by cancer chemotherapeutic agents such as anorexia, fatigue, and changes in sleep patterns are similar to symptoms associated with systemic inflammation, referred to as "sickness behavior", which is triggered by the production of inflammatory cytokine. Administration of cancer chemotherapeutic agents has been shown to increase production of inflammatory cytokines (Pusztai et al., 2004; Elsea et al., 2008). Therefore, inflammatory cytokines and subsequent signal transduction pathways are thought to be involved in the development of chemotherapy-induced anorexia.

Several studies using lipopolysaccharide (LPS), a potent endotoxin derived from the cell wall of gram-negative bacteria, have

Abbreviations: α-MSH, alpha-melanocyte-stimulating hormone; COX, cyclooxygenase; CRF, corticotropin releasing factor; IL-1β, interleukin-1β; PG, prostaglandin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GI, gastrointestinal; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NS-398, N-[2-cyclohexyloxy-4-nitrophenyl]-methanesulfonamide; POMC, pro-opiomelanocortin; RT-PCR, reverse transcription polymerase chain reaction.

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demonstrated that IL-1β synthesized in the hypothalamus plays a role in the development of sickness behavior (Bluthé et al., 1994; Dantzer et al., 1998). In response to IL-1β, vascular endothelial cells in the hypothalamus express cyclooxygenase (COX)-2 and subsequently synthesize and release prostaglandin (PG) E2 (Quan et al., 2003). PGE2 diffused into the brain parenchyma binds to its receptors, referred to E-prostanoid (EP) receptor: EP1, EP2, EP3, and EP4, and elicits inflammation-associated symptoms such as fever, pain, loss of appetite, and lethargy (Samad et al., 2001). Johnson et al. (2002) and Lugarini et al. (2002) have demonstrated that a selective inhibitor of COX-2 attenuated LPS-induced anorexia in rats. Ohinata et al. (2006) reported the anorexinergic effect of PGE2 is expressed via the EP4 receptor in the hypothalamus. Taken together, anorexia during systemic inflammation also seems to be mainly dependent on increases in IL-1β and COX-2 expressions in hypothalamus and signal transduction pathways mediating by COX-2-derived PGE2 and the EP4 receptor.

Pro-opiomelanocortin (POMC) and its derived peptides, in particular α-melanocyte-stimulating hormone (α-MSH), have also been known to contribute to the regulation of feeding, satiety, and energy homeostasis (Vergoni and Bertolini, 2000; Harrold and Williams, 2006). Scarlett et al. (2007) reported that IL-1β and PGE2 induce POMC mRNA expression, subsequently activating POMC neurons in the arcuate nucleus of the hypothalamus and eliciting anorexia. Asarian and Langhans (2010) also suggested that PGE2 induces the release of α-MSH from hypothalamus and activates the central melanocortin system. From the findings mentioned above, we hypothesized that the anorexic effects of chemotherapeutic agents may be mediated by expression of IL-1β and COX-2, and subsequent expression of POMC mRNA.

Docetaxel, classified as a taxane and a microtubule-stabilizing agent, is used worldwide for the treatment of breast, ovarian, and non-small cell lung cancers. Clinical investigations have reported that docetaxel frequently induces symptoms related to sickness behavior such as fever, fatigue, and anorexia (Dreyfuss et al., 1996; Pazdur et al., 1999). To elucidate the possible mechanisms of chemotherapy-induced anorexia, we chose docetaxel from the chemotherapeutic agents and examined the effects of COX inhibitors on docetaxel-induced anorexia in rats. We also investigated the expression of COX-2, IL-1β, and POMC mRNA in the hypothalamus of docetaxel-treated rats with or without a selective COX-2 inhibitor.

2. Materials and methods

2.1. Animals

Male Wistar/ST rats, weighting about 200 g at the beginning of the experiment, were obtained from Japan SLC (Hamamatsu, Shizuoka, Japan). They were singly housed in home cages in a room with a regular light/dark cycle (lights on 0600 h–1800 h) at a constant temperature (25 ± 1 °C) and humidity (50 ± 5%). Four rats were used in each experiment. All experiments were approved by the Animal Care Committee of the School of Allied Health Sciences, Faculty of Medicine, Osaka University, and were conducted in accordance with the Animal Experiment Guidelines of Osaka University.

2.2. Behavioral experiment

2.2.1. Docetaxel-induced anorexia in rats

To investigate the time-course of docetaxel-induced anorexia in rats, an automatic food intake monitoring system (FDM700SW, Melquest Ltd, Toyama, Japan) with α-cellulose bedding (ALPHA-dri®, Shepherd Specialty Papers, Chicago, IL, USA) was used. This apparatus consists of a home cage made of polyvinyl chloride (26 cm × 20 cm × 23 cm), a food container, and a controller equipped with a load cell (weight sensor). Rats were adapted to the experimental environment for 5 days and allowed free access to standard laboratory chow pellets (MF, Oriental Yeast, Osaka, Japan) and tapped water throughout the experimental period. On the day of the experiment, rats received an injection of docetaxel (5 and 10 mg/kg, intraperitoneally, (i.p.)) at 1800 h and their cumulative amount of food intake was monitored hourly to the nearest 0.01 g for 24 h. Data were stored and analyzed using a laptop PC. Control rats were treated with vehicle.

2.2.2. Effect of COX inhibitors on docetaxel-induced anorexia

Rats were housed in individual hand-made acrylic cages (23 cm × 23 cm × 20 cm) and food pellets were provided in a stainless steel container placed in the respective cages. On the day of the experiment, indomethacin (1, 10 and 30 mg/kg), rats intragastrically (i.g.) received NS-398 (1, 10 and 30 mg/kg), or celecoxib (1, 10 and 30 mg/kg) at 1730 h and i.p. injection of docetaxel at 1800 h. Control animals were treated with an i.g. vehicle. Their food intake was measured for 24 h following administration of drugs. Spilled food was collected and weighed to calculate actual consumption.

2.2.3. Effect of twice administration of COX inhibitor on docetaxel-induced anorexia

Another group of rats were i.g. administered celecoxib at a dose of 30 mg/kg 30 min before and 6 h after administration of docetaxel at a dose of 10 mg/kg. Their food intake was monitored for 24 h using the automatic food intake monitoring system.

2.3. RT-PCR experiment

2.3.1. Effects of docetaxel on the expression of IL-1β, COX-2, and POMC mRNA in hypothalamus of rats

Eight and 18 h after administration of docetaxel or the vehicle, rats were deeply anesthetized by inhalation of seveflurane (Sevofrane®, Maruishi Pharmceutical Co., Ltd., Osaka, Japan). Brains were removed and the whole hypothalamus was dissected from the surrounding tissues. Total RNA was extracted using an RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). RNA was converted into a first-stranded cDNA synthesis kit (SuperScript III First-Strand Synthesis System for reverse transcription (RT); Invitrogen, Carlsbad, CA, USA), which was then used as a template for the reverse transcription polymerase chain reaction (RT-PCR) with IL-1β, COX-2, POMC, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) specific primers using a Takara PCR Thermal Cycler. (IL-1β sense: 5′-TGA TGT TCC CAT TAG ACA GC-3′; IL-1β antisense: 5′-GAG GTG CTG ATT ACC AGT T-3′, COX-2 sense: 5′-TGA TGA CTG CCC AAC TCC CAT G-3′; COX-2 antisense 5′-AAT GTT GAA GGT GTC CGG CAG C-3′; POMC sense: 5′-GAG TTC AAG AGG GAG CTG GA-3′; POMC antisense: 5′-CTT CTC GGA GGT CAT GAA GC-3′ and GAPDH sense: 5′-GAA CGG GAA GCT CAC TGG CAT GGC-3′; GAPDH antisense: 5′-TGA GGT CCA CCA CCC TGT TGC TG-3′). Temperature conditions for RT-PCR were as follows: Step 1: 94 °C for 30 s, Step 2: 94 °C for 60 s; 58 °C for 30 s, and 72 °C for 60 s (IL-1β, COX-2, and POMC: 32 cycles; GAPDH: 28 cycles). PCR products (IL-1β: 378 bp, COX-2: 230 bp, POMC: 158 bp, and GAPDH: 610 bp) were separated on 1.8% agarose gels (Nacalai Tesque, Kyoto, Japan) and stained with a 1/10,000 dilution of SYBR Safe (Invitrogen). Gels were captured with E-graph (AE-900, ATTO, Tokyo) and band densities were analyzed for quantification using the ATTO CS Analyzer ver. 3.0.

2.3.2. Effects of a COX inhibitor on docetaxel-induced expression of POMC mRNA

The method was almost identical to that of the detection of docetaxel-induced POMC mRNA experiment, except that celecoxib (30 mg/kg) was i.g. injected 30 min before only or 30 min before and 6 h after docetaxel administration. Control animals received i.g. vehicle.

2.4. Statistical analysis

Data are expressed as mean values ± S.E.M. Differences in means were analyzed using a non-parametric Mann–Whitney *U* test or Kruskal–Wallis test, and a one-way analysis of the variance (ANOVA), followed by post hoc Dunnett multiple comparison tests, were performed where appropriate. A *P* value of less than 0.05 was considered significant.

2.5. Drugs

Docetaxel (Taxotere® inj. Sanofi-Aventis, Tokyo, Japan) was dissolved in 6.5% (v/v) ethanol (Sigma–Aldrich Japan, Tokyo, Japan). Indomethacin (Sigma–Aldrich, St. Louis, MO, USA), NS-398 (Wako Pure Chemical, Osaka, Japan), and celecoxib (LKT Laboratories, St. Paul, MN, USA) were suspended in an aqueous 0.5% carboxy methyl cellulose (Sigma–Aldrich Japan). All drugs were prepared immediately before injection.

3. Results

3.1. Docetaxel-induced anorexia in rats

Prior to the experiment, all rats ate approximately 20 g of food pellets. As shown in Fig. 1(A) and (B), vehicle and 5 mg/kg of docetaxel had no effect on food intake. However, 10 mg/kg of docetaxel inhibited feeding behavior 8 h following administration and induced no lethality during the observation period. Food intake