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Cancer-induced anorexia in tumor-bearing mice is dependent on cyclooxygenase-1

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ABSTRACT

It is well-established that prostaglandins (PGs) affect tumorigenesis, and evidence indicates that PGs also are important for the reduced food intake and body weight loss, the anorexia–cachexia syndrome, in malignant cancer. However, the identity of the PGs and the PG producing cyclooxygenase (COX) species responsible for cancer anorexia–cachexia is unknown. Here, we addressed this issue by transplanting mice with a tumor that elicits anorexia. Meal pattern analysis revealed that the anorexia in the tumor-bearing mice was due to decreased meal frequency. Treatment with a non-selective COX inhibitor attenuated the anorexia, and also tumor growth. When given at manifest anorexia, non-selective COX-inhibitors restored appetite and prevented body weight loss without affecting tumor size. Despite COX-2 induction in the cerebral blood vessels of tumor-bearing mice, a selective COX-2 inhibitor had no effect on the anorexia, whereas selective COX-1 inhibition delayed its onset. Tumor growth was associated with robust increase of PGE_2 levels in plasma – a response blocked both by non-selective COX-inhibition and by selective COX-1 inhibition, but not by COX-2 inhibition. However, there was no increase in PGE_2-levels in the cerebrospinal fluid. Neutralization of plasma PGE_2 with specific antibodies did not ameliorate the anorexia, and genetic deletion of microsomal PGE synthase-1 (mPGES-1) affected neither anorexia nor tumor growth. Furthermore, tumor-bearing mice lacking EP_4 receptors selectively in the nervous system developed anorexia. These observations suggest that COX-enzymes, most likely COX-1, are involved in cancer-elicited anorexia and weight loss, but that these phenomena occur independently of host mPGES-1, PGE_2 and neuronal EP_4 signaling.

1. Introduction

Cachexia, characterized by metabolic derangements leading to weight loss, is a severe problem in several chronic diseases, particularly malignant cancer (Fearon et al., 2011; Tisdale, 2010). Cachexia restricts the patients' tolerance and response to treatment, causes suffering and impaired quality of life, and reduces life expectancy. Cachexia differs from starvation in which weight loss induced by caloric restriction normally is followed by compensatory re-feeding mechanisms. In cancer anorexia, the expected counter-regulatory feeding response to reduced body mass is absent, indicating an uncoupling of food intake from energy expenditure (Bosaeus et al., 2001). Indeed, the weight loss in cachexia is paradoxically associated with anorexia (persistent satiety or undesire to eat) leading to reduced food intake. Since feeding and body weight are subject to close regulation by brain homeostatic and hedonic systems, cancer-induced anorexia is likely due to a complex interplay of mediators acting on the brain, but our understanding of the pathophysiology underlying reduced appetite in cancer is incomplete. However, inflammatory signaling is a central theme in cancer anorexia (Gelin et al., 1991; Ruud et al., 2010). In particular, inflammatory-induced prostaglandins have been proposed as key mediators (Cahlin et al., 2000b; Sandstrom et al., 1990). Among the prostaglandins, prostaglandin E_2 (PGE_2) is of special interest, since inhibition or deletion of the inducible cyclooxygenase (COX-2) or the inducible terminal PGE_2 isomerase, microsomal prostaglandin E synthase-1 (mPGES-1), largely prevents the anorexia during acute inflammation (Elander et al., 2007; Lugarini et al., 2002; Pecchi et al., 2006). Furthermore, induced synthesis of COX-2 and PGE_2 are critical in formation of many malignant tumors, foremost colorectal cancer (Brown and DuBois, 2005). Thus, epidemiological studies have revealed that administration of traditional non-steroidal anti-inflammatory drugs, as well as selective COX-2 inhibitors, is associated with decreased incidence of colorectal cancer (Steinbach et al., 2000), and selective inhibition of mPGES-1 with the supposedly added safety over COX and COX-2 inhibitors has emerged as an attractive approach for treating malignancies (Murakami and Kudo, 2006).

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Here we hypothesized that the PGE_2 synthesizing pathway also plays a role for the reduced food intake and body weight loss in cancer disease. We tested this hypothesis by examining the role of COX-1, COX-2, mPGES-1, and PGE_2 and its EP_4 receptor for the development of cancer-induced anorexia and weight loss in a mouse tumor model. In this model mice are inoculated with a methylcholanthrene-induced sarcoma MCG 101 (Lundholm et al., 1978), a non-metastasizing, undifferentiated epithelial-like solid tumor that produces PGE_2 in a cyclooxygenase dependent manner, both *in vitro* and *in vivo*, leading to elevated plasma concentrations of PGE_2 (Lonnroth et al., 1995). This tumor model has been used extensively in the Lundholm laboratory for the study of cancer cachexia. When implanted subcutaneously the tumors grow locally with a reproducible growth pattern. The animals die because of cancer cachexia 12–15 days after tumor implantation (Lundholm et al., 1978). We recently showed that mice lacking MyD88, an adaptor protein critical for Toll-like and IL-1 receptor family signaling, were protected against MCG 101-induced anorexia (Ruud et al., 2010), demonstrating the importance of inflammatory signaling for the reduced food intake in this model.

2. Materials and methods

2.1. Animals, tumor or sham implantations, and food intake monitoring

Adult and age-matched female wild-type mice (Taconic, Ejby, Denmark; Scanbur, Stockholm, Sweden; or Jackson, Sulzfeld, Germany), and genetically modified mice and their littermates [*Ptges*$^{-/-}$ (mPGES-1 deficient; Trebino et al., 2003), *Ptgs2*$^{-/-}$ (COX-2 knock-out; on a mixed B6;129P2 background; from Taconic), $EP4^{flox/flox}$ (Schneider et al., 2004), *Nestin*-Cre deleter (Tronche et al., 1999)], all on a C57BL/6 background if not otherwise stated, were used. Mice lacking the EP_4 receptor in neural cells ($EP4^{\Delta Nestin\text{-}Cre}$) and their control littermates ($EP4^{flox/flox}$) were obtained from crossings of $EP4^{Nestin\text{-}Cre/flox/+}$ and $EP4^{flox/flox}$ mice. Two fragments (3–4 mm^3) of the methylcholanthrene-induced sarcoma MCG 101 (Lundholm et al., 1978) were placed subcutaneously under gas anesthesia (4% isoflurane; Abbott Laboratories, Abbott Park, IL) on both sides of the dorsal midline using a tuohy needle. Control mice were handled in the same way but injections were done without tumor fragments (sham). Food intake was monitored in two ways: (1) The animals were housed in cages carrying raised bottom wire grids (Tecniplast, Varese, Italy) in a temperature-controlled (~20 ± 1.5 °C) environment and on a regular 12 h daylight schedule (lights on and off at 07/19 h), with free access to standard rodent laboratory pelleted chow (CRM-E, Special Diets Services, Witham, Essex, UK) and tap water. Food intake was registered on a daily basis with the measurements carried out during the light phase. Food spillage was corrected for, by collecting the gnaw waste, which was weighed; (2) food intake was recorded continuously via an automated feeding monitoring equipment (AccuScan Instruments, Columbus, OH) that allowed uninterrupted, undisturbed recordings of individual meals for each animal, as described elsewhere (Elander et al., 2007). In this setting, powdered food was used to limit spillage and hoarding of the diet. Feeding activity was retrieved every ~1.5 s and processed using DietDat and DietMax software (AccuScan Instruments). A meal was defined as whenever at least 0.01 g of food was removed from the food tray and considered terminated if the food was left undisturbed for ⩾5 min. Using this set-up, the daily food intake monitored amounts to ~95% of the total food intake recorded via manual readings (Elander et al., 2007).

The endpoint of the tumor-implantation experiments was for animal ethics reasons set to 10 days post implantation. However, when data permitted, i.e. a clear anorexic response was seen irrespective of treatment for two consecutive days, the experiment was terminated earlier, to minimize animal suffering.

All experiments were approved by the Animal Ethics Committee in Linköping. All reagents were from Sigma–Aldrich (St. Louis, MO) if not otherwise specified.

2.2. Drugs

Indomethacin [(Alpharma-Isis GmbH & Co KG, Langenfeld, Germany); 1 μg/g body weight; (Axelsson et al., 2005)], Liometacen® (Indomethacin Meglumine; Chiesi Farmaceutici S.P.A., Parma, Italy; 1 μg/g body weight), sodium naproxen (100–150 μg/g body weight), or parecoxib (Dynastat®, Pfizer, Sandwich, Kent, UK; 100 μg/g body weight; dose titrated here to completely obliterate the febrile response to lipopolysaccharide (LPS)) were provided via the drinking water. The concentration of the drugs in the water was calculated with account taken for the reduced water consumption that results from reduced food intake. The COX-1 inhibitor SC560 (5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole; Cayman Chemical, Ann Arbor, MI) was administered in specially prepared food pellets (AIN-76A; Research Diets Inc., Brunswick, NJ) containing 225 mg SC560 per kg food. At a daily food intake of 4 g, the ingested dose (30 mg/kg body weight) has been shown to completely inhibit the formation of TXB_2 [the stable metabolite of COX-1 dependent TXA_2 formation (Smith et al., 1998)] and to prevent the rapid increase in plasma corticosterone induced by LPS, that is dependent on COX-1 (Elander et al., 2010), without interfering with COX-2 dependent mechanisms (Smith et al., 1998).

2.3. RNA isolation, and cDNA synthesis and quantitative real-time PCR

Following asphyxiation in carbon dioxide, mice were rinsed from blood by transcardial perfusion with sterile saline. A block of the hypothalamus was dissected, immediately snap-frozen in liquid nitrogen, and kept at −80 °C. RNA was extracted, quantified, and transcribed as previously described (Wang et al., 2005b). Real-time PCR reactions were performed as singleplex on 10 ng of cDNA in duplicate for every sample in a 96-well format on the ABI Fast 7500 Real-Time PCR instrument using TaqMan® probes, primers, and the Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Assays were: *Ptgs1* (Mm00477214_m1), *Ptgs2* (Mm00478374_m1), and *Ptger4* (Mm00436053_m1). *Actb* (Mm00607939_s1) served as endogenous control. Assays for terminal prostanoids enzymes are given in Table 1. Relative gene expression changes in tumor-bearing mice were calculated using the $2^{-\Delta\Delta CT}$ method and expressed as fold change compared with sham-implanted mice.

2.4. Immunohistochemistry

COX-2 was detected with a polyclonal affinity-purified IgG antibody raised in goat against a synthetic peptide consisting of amino acids 586–604 of mouse COX-2 (sc-1747, lot # E2506; Santa Cruz Biotechnology, Santa Cruz, CA) as previously described (Ruud and Blomqvist, 2007). The specificity of the antibody was tested by staining brain sections from wild-type and COX-2 null mice that had been subjected to an i.p. injection of LPS.

2.5. Body temperature recordings

Core body temperature was measured telemetrically, in individually caged mice, and at near-thermoneutral conditions (28–30 °C). One week prior to recordings, a miniature transmitter (Data Sciences International, St. Paul, MN) was implanted in the