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ORIGINAL ARTICLE

Oxaliplatin induces prostaglandin E2 release in vascular endothelial cells

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Abstract

Purpose Oxaliplatin (L-OHP) is known to induce adverse reactions at the injection site, including vascular pain, but the underlying mechanisms have not been clarified. Vascular pain during intravenous L-OHP administration can be inhibited by taking non-steroidal anti-inflammatory drugs (NSAIDs). In this study, we investigated the involvement of the arachidonic acid cascade and prostaglandin (PG) E_2 and 15d-PGJ_2 in vascular pain sensation during intravenous delivery of L-OHP.

Methods Cultured normal human umbilical cord vein endothelial cells (HUVECs) were treated with L-OHP or L-OHP + NSAID flurbiprofen for 2 h and analyzed for the release of PGE_2 and 15d-PGJ_2 into culture supernatant by ELISA.

Results The results showed that L-OHP significantly and dose-dependently increased PGE_2 secretion by HUVECs; however, flurbiprofen effectively prevented PGE_2 increase. On the other hand, cisplatin, another platinum anticancer drug, did not stimulate PGE_2 production. Other PGs, including 15d-PGJ_2, 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$, and PGD_2 were not increased by L-OHP or cisplatin. Protein expression analysis revealed that cyclooxygenase 1 and cytoplasmic PGE synthase involved in constitutive PG metabolism were expressed in HUVECs but not affected by L-OHP exposure.

Conclusions This study indicates that L-OHP treatment specifically upregulated PGE_2 secretion by vascular endothelial cells, which may contribute to vascular pain, and that NSAIDs can be used to inhibit PGE_2 release and attenuate L-OHP-induced hyperalgesia.

Keywords Oxaliplatin · Vascular pain · Prostaglandin E_2 · Non-steroidal anti-inflammatory drug · Cyclooxygenase · Prostaglandin E synthase

Introduction

Oxaliplatin (L-OHP), a platinum-based anticancer agent, is used for treatment of colorectal, pancreatic, and gastric cancers in Japan. A conventional chemotherapy regimen used as the first-line treatment for colorectal cancer is FOLFOX, which includes L-OHP and fluorouracil (5-FU) and requires a special device for continuous infusion of 5-FU through the central venous system. Relatively new chemotherapeutic regimens for colorectal cancer treatment, XELOX (L-OHP + capecitabine) and SOX (L-OHP + S1), show equivalent clinical effects to the conventional regimen in terms of progression-free survival and occurrence adverse events [1, 2]. The advantage is that the new regimens do not contain 5-FU requiring central venous delivery and are easier to administer. However, L-OHP is known to induce injection-site reactions such as vascular pain. Thus, a phase II clinical trial revealed that the XELOX regimen caused infusion-site reactions in 65% of patients [3], whereas there are no reports of vascular pain due to FOLFOX administered through the central venous access port.

Along with L-OHP, several other anticancer agents are known to cause injection-site reactions, including gemcitabine (GEM), epirubicine (EPI), vinorelbine (VNR), and dacarbazine (DTIC). A previous study suggested that the risk for vascular pain is higher for women and the elderly in case of GEM and for patients with high body mass index in case of VNR [4, 5]. For DTIC, the cause of vascular pain

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has been attributed to photodecomposition products [6] and for EPI, the incidence of vascular pain was shown to be higher after administration of liquid formulations compared to lyophilized formulations [7]. To prevent vascular pain, GEM was dissolved in 5% dextrose in water rather than in normal saline [8], the administration rate of VNR and EPI was shortened [9, 10], and the DTIC drip route was shielded from light [11]. It has also been reported that nonsteroidal anti-inflammatory drugs (NSAIDs) decreased vascular pain in patients treated with L-OHP [12] but not in those treated with GEM [4].

L-OHP induced injection-site reactions, including vascular pain, only if administered into brachial veins but not if delivered through the central vein [13], suggesting that the administration route is a factor. Peripheral vessels have a smaller diameter and slower blood flow than central veins, and consequently, retain L-OHP in high concentrations around the administration site for a longer time, which may increase the exposure of blood vessel cells to L-OHP. Indeed, in many cases the onset of vascular pain was described within 2 h after L-OHP administration, also suggesting a possibility that the pain can be attenuated by taking NSAIDs [12]. The mechanism underlying the analgesic effect of NSAIDs is the suppression of prostaglandins (PGs) production through inhibition of cyclooxygenase (COX) activity. Therefore, it can be hypothesized that L-OHP causes vascular pain by inducing the release of PGs from vascular endothelial cells.

PGs are inflammatory lipid mediators produced by various cells in many tissues, which are shown to be involved in inflammation [14], pain [14], bone metabolism [15], onset and progression of cancer [16], and gastric mucosal protection [17]. Different PGs such as PGE_2, PGD_2, PGF_2, PGI_2, and thromboxane A_2 show distinct physiological activities through interaction with specific receptors. Among them, PGE_2 is known to enhance nociception in inflammatory disorders and heighten sensitivity to neuropathic pain in various diseases [18].

The biosynthesis of PGE_2 is performed in three steps by the following enzymes: phospholipase A_2, which liberates arachidonic acid from the cell membrane; COX, which converts arachidonic acid to PGH_2, a common precursor of physiologically active PGs; and PGE synthase (PGES), which isomerizes PGH_2 to PGE_2. There are two COX isoenzymes, COX-1 and COX-2, and three forms of PGES, cytoplasmic cPGES and membrane-bound mPGES-1 and mPGES-2 [18, 19], and it was reported that COX-2 and mPGES-1 could be related to inflammatory pain [20, 21]. However, the involvement of PGE_2-synthesizing enzymes in vascular pain is unclear. Previous studies showed that 15d-PGJ_2, a product of PGD_2 metabolism, is a ligand of transient receptor potential ankyrin 1 (TRPA1), which is a temperature-sensitive cation channel playing a role in acute peripheral neuropathy and cold hyperalgesia [22, 23], but its association with vascular pain is unknown.

In this study, we tested a hypothesis that vascular pain may be due to increased release of PGE_2 or 15d-PGJ_2 by vascular endothelial cells exposed to L-OHP. Cultured human umbilical vein endothelial cells (HUVECs) were treated with L-OHP alone or together with a NSAID and analyzed for the release of PGs into culture supernatant and expression of PGE-synthesizing enzymes.

Materials and methods

Cell culture

Normal HUVECs (Cell Applications Inc., San Diego, CA, USA) were maintained in MCDB 131 medium supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine (all from Thermo Fisher Scientific Inc., Waltham, MA, USA), 10% fetal bovine serum, and basic fibroblast growth factor (ReproCELL Inc., Tokyo, Japan). HUVECs were seeded (1.0×10^5 cells/mL) in flasks coated with type I collagen and cultured at 37 °C and 5% CO_2; cells from passages 4 to 15 were used for experiments.

Cell stimulation

Stock solutions of L-OHP (FUJIFILM Wako Pure Chemical Co., Tokyo, Japan), cisplatin (CDDP; FUJIFILM Wako Pure Chemical Co.), and flurbiprofen (Flu; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were prepared by dissolving the drugs in medium to 10 mM, 10 mM, and 20 mM, respectively.

In clinical practice, patients receiving XELOX and SOX regimens are administered L-OHP at 130 mg/m^2 in 500 mL of infusion solution. To determine cell treatment conditions corresponding to clinical situation, we considered the molecular weight of L-OHP (397), the average human body surface area (1.73 m^2), and its concentration in the infusion bag (1 mM). Accordingly, L-OHP was used at 1 mM, 100 µM, and 10 µM. HUVECs of 70–80% confluence were seeded on 12-well plates coated with type I collagen at a density of 1.0×10^5 cells/mL for 24 h and treated with L-OHP, or CDDP (1 mM, clinical concentration). In the NSAIDs group, flurbiprofen (20 µM, clinical concentration) was added 1 h before L-OHP (1 mM). In the vehicle group, the same volume of medium was added. After 2 h exposure, 200 µL of culture supernatant was collected and centrifuged; 150 µL was stored at − 80 °C until analysis of PGs concentrations and 50 µL at − 20 °C for western blotting analysis.

Elisa

Concentrations of PGs in culture supernatant were measured using appropriate ELISA kits (PGE_2, 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGD_2: Cayman Chemical Company, Ann Arbor, MI, USA, 15d-PGJ_2:Abcam, Cambridge, UK) and expressed as pg/mL.

Western blotting

The expression of enzymes involved in PG biosynthesis was assessed by immunoblotting. Briefly, 15 µL of each sample, 5 µL of marker, and 10 µL of rat macrophage cells stimulated with LPS (positive control) were loaded into a Mini-PROTEAN TGX Gel (Bio-Rad Laboratories Inc., Hercules, CA, USA) and resolved by SDS-PAGE. Proteins were transferred to a Transblot Turbo PVDF membrane (Bio-Rad), which was blocked in 5% skim milk at 4 °C for 18 h with gentle shaking, washed twice with 0.05% Tween TBS (TBST), and incubated with primary antibodies diluted in 0.05% skim milk at 4 °C for 18 h with gentle shaking. The following primary rabbit antibodies were used: polyclonal anti-COX-1 and anti-COX-2 (1:1000; Cell Signaling Technology Japan, K.K., Tokyo, Japan), polyclonal anti-mPGES-1, anti-mPGES-2, and anti-cPGES (1:1000; Cayman Chemical Company), and monoclonal anti-β-actin (1:1000; Cell Signaling Technology Japan). After washing four times with TBST, the membranes were incubated with anti-rabbit HRP-conjugated IgG (1:1000; Cell Signaling Technology Japan) in 0.02% skim milk at room temperature for 60 min with shaking, washed four times with TBST, and incubated with ECL reagent (Bio-Rad) for 5 min. Signals were detected using Chemi Doc XRS (Bio-Rad).

Statistical analysis

Data on PG concentrations were expressed as the mean ± standard deviation of two independent experiments. Differences between groups were analyzed with independent *t* test using SPSS Statistics 24 (IBM Corp., Armonk, NY, USA) and a *p* value less than 0.05 was considered as statically significant.

Results

Increase of PGE_2 secretion by HUVECs treated with L-OHP

L-OHP treatment increased PGE_2 concentration in culture medium and the response was dose-dependent: from 56.9 pg/mL in the control (vehicle) group to 117.0 pg/mL, 225.9 pg/mL ($p < 0.01$), and 263.8 pg/mL ($p < 0.01$) in 10 µM, 100 µM, and 1 mM L-OHP groups, respectively (Fig. 1). However, the combined treatment with L-OHP and a NSAID (Flu, 20 µM) reversed the L-OHP-induced PGE_2 release by HUVECs to 40.0 pg/mL ($p < 0.01$). CDDP (1 mM) did not significantly increase PGE_2 concentration in culture supernatant (94.8 pg/mL).

Concentrations of other PGs: 6-keto $PGF_{1\alpha}$ (PGI_2 metabolite), $PGF_{2\alpha}$, PGD_2, and 15d-PGJ_2 (PGD_2 metabolite) were not significantly influenced by stimulation of HUVECs with either L-OHP or CDDP (Fig. 2).

Effect of L-OHP on the expression of PGE_2 synthesis-related enzymes in HUVECs

As PGE_2 production by HUVECs was affected by stimulation with L-OHP and L-OHP + Flu, we next analyzed the expression of enzymes involved in PGE_2 biosynthesis by western blotting. The results indicated that COX-1, mPGES-2, and cPGES were expressed in HUVECs but COX-2 and mPGES-1 were not. There was no change in the expression level of COX-1, mPGES-2, and cPGES after treatment of HUVECs with L-OHP or CDDP (Fig. 3).

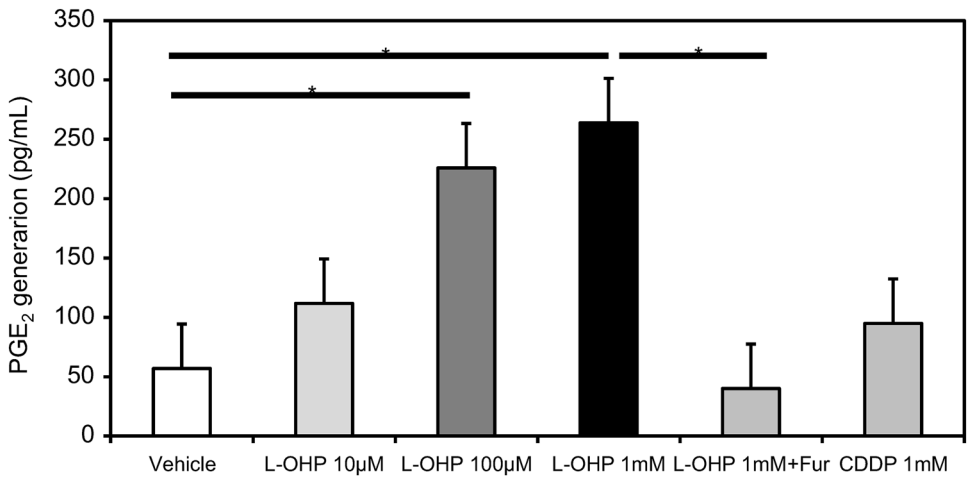

Fig. 1 PGE_2 concentrations in culture supernatant of HUVECs stimulated with the indicated drugs for 2 h. *$p < 0.01$ versus control (vehicle)

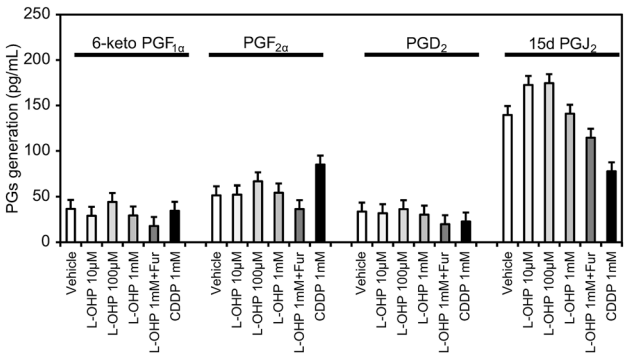

Fig. 2 PGs concentrations in culture supernatant of HUVECs after 2 h stimulation with the indicated drugs

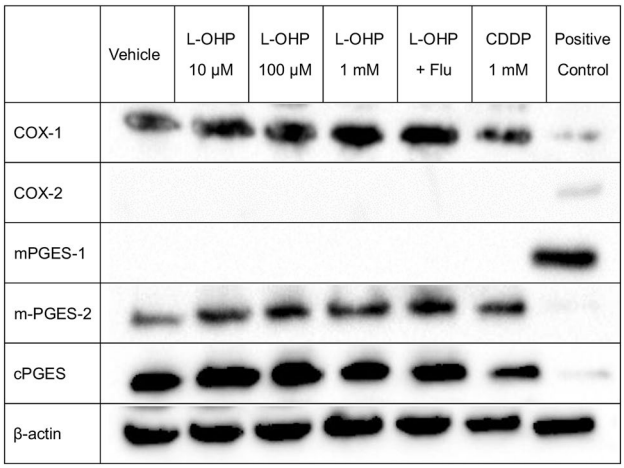

Fig. 3 Expression of enzymes involved in PGs biosynthesis. HUVECs were stimulated with the indicated drugs for 2 h and analyzed for protein expression by western blotting

Discussion

In this study, we examined whether PGs release by vascular endothelial cells could be the cause of pain after L-OHP injection into peripheral veins. The results revealed that HUVECs stimulated in vitro by L-OHP in clinically relevant concentrations released increased amounts of PGE_2 into culture supernatant in an L-OHP dose-dependent manner. At the same time, CDDP, another platinum-based anticancer drug which does not cause hyperalgesia as a side effect, did not stimulate PGE_2 secretion by HUVECs. Furthermore, our data indicate that the pretreatment with Flu as a representative NSAID prevented the increase of PGE2 production by HUVECs. However, L-OHP did not affect the release of other PGs or the expression of PGE_2-synthesizing enzymes COX-1, mPGES-2, and cPGES.

PGE_2 is considered to be a sensitizer of nociceptors in tissue [24]; furthermore, it was shown that PGE_2 can induce hyperalgesia directly [25] through receptor-mediated mechanisms [26]. Therefore, our present results suggest that L-OHP may cause vascular pain by increasing PGE_2 secretion by vascular endothelial cells and that Flu may attenuate this adverse effect by inhibiting PGE_2 release at the L-OHP injection site. This conclusion is supported by the data that NSAIDs tend to suppress vascular pain due to L-OHP injection [12]. We observed the increase in PGE_2 secretion within 2 h after L-OHP administration, which is consistent with the timing of vascular pain onset revealed in a previous study [12], suggesting that vascular pain can be reduced by NSAIDs. Furthermore, an in vivo study reported that premedication with indomethacin was effective in attenuating vascular pain due to propofol [27]. On the other hand, a retrospective study indicated that NSAIDs did not exert analgesic effects on vascular pain caused by GEM [4], suggesting that GEM-associated pain is mediated by a different mechanism.

In the arachidonic acid cascade [28] (Fig. 4), cPGES may be functionally associated with COX-1 in immediate PGE_2 production and mPGES-1 with COX-2 in

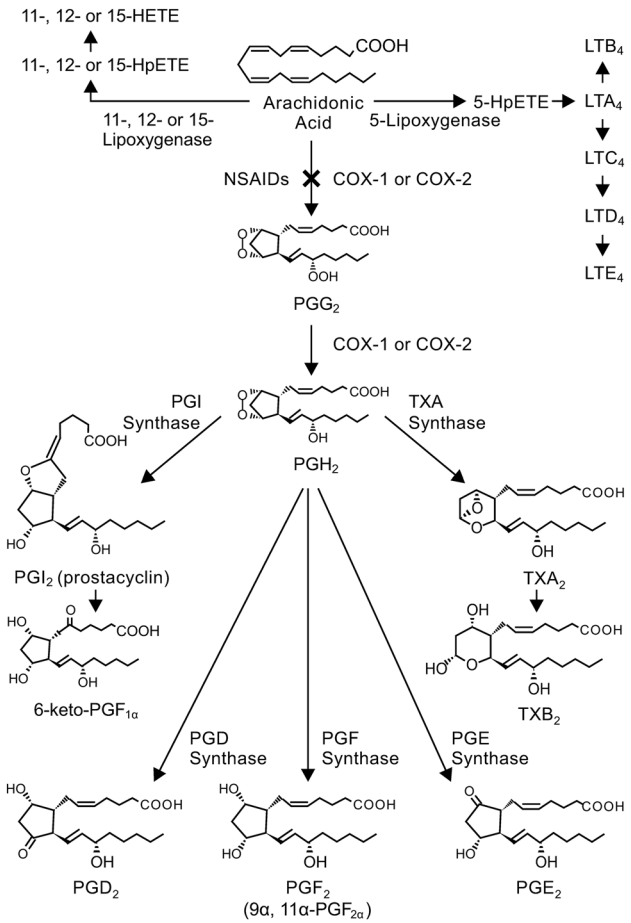

Fig. 4 The arachidonic acid cascade [28]

inflammation-induced PGE_2 release [29]. Our western blotting analysis indicates that COX-1 and cPGES are expressed in HUVECs, whereas COX-2 and mPGES-1 are not, suggesting that increased PGE_2 secretion may be an immediate response mediated by the constitutive activity of COX-1 and cPGES.

It has also been suggested that L-OHP causes perceptual abnormalities such as acute peripheral neuropathy via TRPA1 channel [22], which has been reported as a ligand of 15d-PGJ_2 [23]. However, as we did not observe any changes in 15d-PGJ_2 levels, the association of 15d-PGJ_2 with L-OHP-induced vascular pain was not revealed.

Anticancer drugs are toxic to normal cells. Therefore, we performed preliminary experiments to examine L-OHP cytotoxicity to HUVECs after 2 h exposure (duration of clinical administration), which did not reveal a significant decrease in cell viability. However, L-OHP as a platinum-based anticancer agent inhibits DNA synthesis and causes cell death by promoting DNA crosslinking, which may not be detected after short exposure (2 h).

Several limitations of this study should be mentioned. First, we analyzed only PGs as pain-associated factors, whereas there are a number of other molecules, including bradykinin, ATP, serotonin, cytokine, neurotrophin, and nitric oxide, which may cause hyperalgesia. Second, we used vascular endothelial cells, which are one of the cell types constituting blood vessels, whereas fibroblasts and smooth muscle cells were not evaluated; furthermore, we did not consider the effects of L-OHP on blood cells. Finally, the research was conducted in vitro and the data should be validated in animal models and human studies.

Nevertheless, our results suggest that PGE_2 secreted by vein endothelial cells exposed to L-OHP during intravenous administration may be a cause of vascular pain, which cancer patients suffer from during treatment and which sometimes makes doctors change the treatment regimen. Therefore, it is necessary to develop measures preventing medication-related pain in order to apply the best possible anti-cancer therapy. This study indicates that NSAIDs may be effective in this respect, which warrants further clinical research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals The article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Cassidy J, Clarke S, Díaz-Rubio E et al (2008) Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. J Clin Oncol 26:2006–2012
2. Yamada Y, Takahari D, Matsumoto H et al (2013) Leucovorin, fluorouracil, and oxaliplatin plus bevacizumab versus S-1 and oxaliplatin plus bevacizumab in patients with metastatic colorectal cancer (SOFT): an open-label, non-inferiority, randomised phase 3 trial. Lancet Oncol 14:1278–1286
3. Fuse N, Bando H, Chin K et al (2017) Adjuvant capecitabine plus oxaliplatin after D2 gastrectomy in Japanese patients with gastric cancer: a phase II study. Gastric Cancer 20:332–340
4. Suga Y, Sakaguchi Y, Ishizaki J et al (2012) Investigation for risk factor and preventive effect of NSAIDs, opioid on gemcitabine-induced vascular pain. Jpn J Pharm Heal Care Sci 38:177–183
5. Yoh K, Niho S, Goto K (2004) High body mass index correlates with increased risk of venous irritation by vinorelbine infusion. Jpn J Clin Oncol 34:206–209
6. Kawahara M, Ishida T, Emoto C et al (2001) Determination of a pain substance produced by the photodegradation of dacarbazine. Jpn J Clin Pharmacol Ther 32:15–22
7. Suga Y, Kumazaki M, Nishigami J et al (2009) Improvement of epirubicin-induced phlebitis to switch from liquid preparation to lyophilized formulation. Gan Kagaku Ryoho 36:93–96
8. Hino M, Haruta K, Yoshimura T et al (2008) Evaluation and measurement of vascular pain developing during administration of gemcitabine hydrochloride. J Jpn Soc Hosp Pharm 44:801–803
9. Yoh K, Niho S, Goto K et al (2007) Randomized trial of drip infusion versus bolus injection of vinorelbine for the control of local venous toxicity. Lung Cancer 55:337–341
10. Onogawa M, Ogawa K, Miyamura M (2010) Improvement of injection procedure for epirubicin hydrochloride to reduce venous pain. Jpn J Clin Pharmacol Ther 36:680–683
11. Morio K, Tsugane M, Okamoto Y et al (2013) Effectiveness of a newly designed shield to prevent the photodegradation of dacarbazine. Jpn J Clin Pharmacol Ther 39:381–387
12. Handa S, Kuroiwa R, Miyano M et al (2016) Assessment of injection site reactions for peripheral intravenous oxaliplatin infusion and potential remedies. Gan Kagaku Ryoho 43:985–988
13. Lapeyre-Prost A, Hug de Larauze MH, Chibaudel B et al (2016) Feasibility of capecitabine and oxaliplatin combination chemotherapy without central venous access device in patients with stage III colorectal cancer. Clin Colorectal Cancer 15:250–256
14. Ricciotti E, FitzGerald GA (2011) Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31:986–1000
15. Kawaguchi H, Pilbeam CC, Harrison JR et al (1995) The role of prostaglandins in the regulation of bone metabolism. Clin Orthop Relat Res 313:36–46
16. Wang D, Dubois RN (2006) Prostaglandins and cancer. Gut 55:115–122
17. Wallace JL (2008) Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? Physiol Rev 88:1547–1565
18. Murakami M, Naraba H, Tanioka T et al (2000) Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. J Biol Chem 275:32783–32792

19. Murakami M, Nakashima K, Kamei D et al (2003) Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J Biol Chem 278:37937–37947
20. Ballou LR, Botting RM, Goorha S, Zhang J, Vane JR (2000) Nociception in cyclooxygenase isozyme-deficient mice. Proc Natl Acad Sci USA 97:10272–10276
21. Kamei D, Yamakawa K, Takegoshi Y et al (2004) Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J Biol Chem 279:33684–33695
22. Zhao M, Isami K, Nakamura S, Shirakawa H, Nakagawa T, Kaneko S (2012) Acute cold hypersensitivity characteristically induced by oxaliplatin is caused by the enhanced responsiveness of TRPA1 in mice. Mol Pain 8:55
23. Taylor-Clark TE, Undem BJ, Macglashan DW, Ghatta S, Carr MJ, McAlexander MA (2008) Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). Mol Pharmacol 73:274–281
24. Ferreira SH, Moncada S, Vane JR (1997) Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. Br J Pharmacol 120(1973):401–412 **(discussion 399–400)**
25. Taiwo YO, Levine JD (1989) Prostaglandin effects after elimination of indirect hyperalgesic mechanisms in the skin of the rat. Brain Res 492:397–399
26. Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. Physiol Rev 79:1193–1226
27. Ando R, Watanabe C (2005) Characteristics of propofol-evoked vascular pain in anaesthetized rats. Br J Anaesth 95:384–392
28. Simmons DL, Botting RM, Hla T (2004) Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev 56:387–437
29. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I (2000) Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem 275:32775–32782