PGE Synthase (PGES)

PGES is a key enzyme involved in the biosynthesis of PGE_2 that acts downstream of COX. So far, at least three forms of PGES have been cloned and characterized. These are known as cytosolic PGES (cPGES), microsomal prostaglandin E synthase-1 (mPGES-1), and mPGES-2.

cPGES was originally identified as heat shock protein 90 (Hsp90)-associated protein p23 [29]. cPGES is constitutively expressed in the cytosol under basal conditions in a wide variety of cells and tissues. cPGES is functionally coupled with COX-1 and may contribute to the physiological production of PGE_2 for maintenance of homeostasis. Interestingly, mPGES-1 shows coordinated induction with COX-2 by various inflammatory stimuli in cancer cells [30], macrophages [31, 32], fibroblasts [33-35], osteoblasts [36], and chondrocytes [37]. mPGES-1 is preferentially linked with COX-2 rather than COX-1 [31]. Recent reports have demonstrated that mPGES-1 plays an important role in various pathophysiological events related to a wide variety of conditions and processes, including pain [38-40], fever [41-44], bone resorption [36], vascular angiogenesis [39], reproduction [45, 46], Alzheimer's disease [47], atherosclerosis [48, 49], and tumorigenesis [50].

In the RA synovium, mPGES-1 is localized in synovial lining cells, mononuclear and fibroblast-like cells from the sublining, infiltrating synovial macrophages, and vascular endothelial cells [37]. Moreover, mPGES-1 expression isselectively upregulated in the synoviumwhen RA is active, while its expression insynovial tissue is minimal when the disease is inactive [37, 51]. These findings suggest that induction of mPGES-1 in the synovial tissue of patients with active RA plays a major role in PGE_2-related joint inflammation.

Because mPGES-2 is constitutively expressed in various cells/tissues and is functionally coupled with both COX-1 and COX-2, it could have a role in PGE_2 production related to both homeostasis (with COX-1) and inflammation (with COX-2). However, mPGES-2 immunoreactivity has been detected in synovial lining cells and sublining cells, and it is similarly detected in both active and resting RA tissues [51]. Thus, mPGES-2 is probably associated with basal production of PGE_2 that is unrelated to inflammation.

PGE_2-Induced Upregulation of mPGES-1

Our previous study showed that NSAIDs, such as selective COX-2 inhibitors, decrease the expression of mPGES-1 by IL-1β-stimulated RA

synovial fibroblasts [52], with this inhibition being overcome by addition of PGE_2 itself. Various physiological effects of PGE_2 are mediated via the EP receptor, which has four subtypes (EP1, EP2, EP3, and EP4). Among these, the EP2 and EP4 receptors increase cyclic AMP via activation of adenylate cyclase. We detected EP2 and EP4 receptors in the synovial fibroblasts of RA patients. In addition to PGE_2, selective EP2 and EP4 receptor agonists increase mPGES-1 expression as does an activator of adenylate cyclase (forskolin), suggesting that PGE_2 enhances the expression of mPGES-1 in rheumatoid synovial fibroblasts by increasing cAMP through activation of the EP2 and EP4 receptors.

We also observed similar findings in chondrocytes. An outline of the positive feedback mechanism regulating mPGES-1 expression is shown in Figure 3. In conclusion, PGE_2 is a strong enhancer of the induction of mPGES-1 expression by IL-1β. Autoregulation of mPGES-1 expression by PGE_2 may play an important role in the vicious circle of inflammation associated with arthritis.

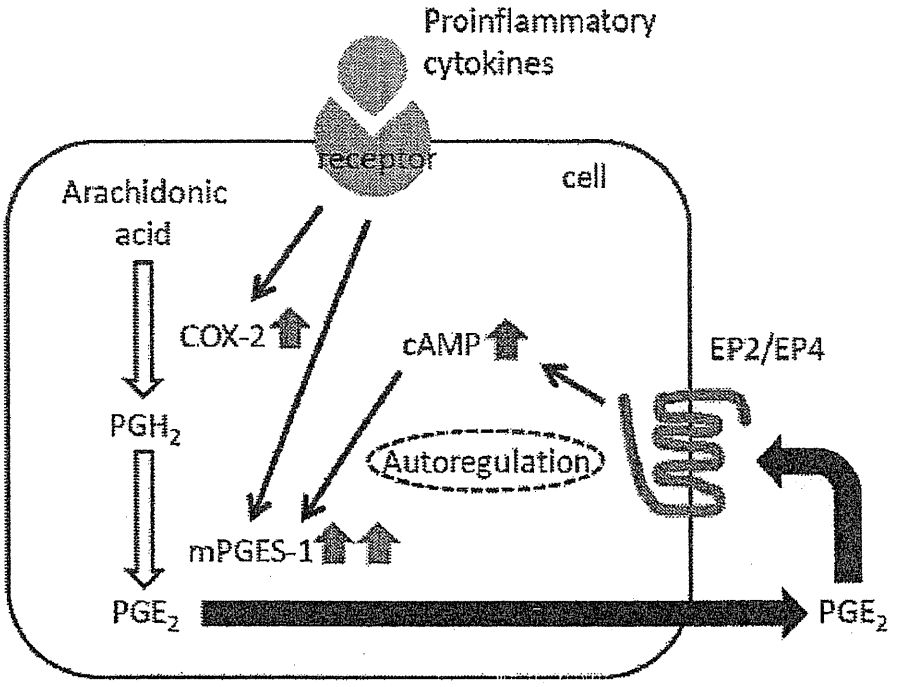

Figure 3. Pathway for positive regulation of mPGES-1 expression by PGE_2. COX-2 and mPGES-1 show coordinated induction in cultured synovial fibroblasts by stimulation with proinflammatory cytokines. At sites of inflammation, PGE_2 further enhances mPGES-1 expression associated with an increase of cyclic AMP (cAMP) *via* the EP2 and EP4 receptors.

Adiponectinand PGE_2 in RA

We found that the adipokine adiponectin increased COX-2 and mPGES-1 mRNA expression in synovial fibroblasts obtained from RA patients [53]. In contrast, leptin and resistin, even at levels up to 10-fold higher (100 ng/mL) than their serum concentrations, did not increase COX-2 and mPGES-1 mRNA expression by RA synovial fibroblasts (Figure 4).

To determine whether adiponectin increased the expression of PGESs and promoted to PGE_2 synthesis, we performed Western blotting analysis [53]. We found that adiponectin increased the expression of mPGES-1 protein in a concentration-dependent manner, whereas cPGES protein expression was unchanged.

Adiponectin enhanced PGE_2 production by RA synovial fibroblastsat concentrations found in human serum, while leptin and resistin had no significant effect on PGE_2 production (Figure 5). Small interfering RNA (siRNA) for the adiponectin receptor (AdipoR1 and AdipoR2) gene decreased adiponectin-induced PGE_2 production. Adiponectin-induced PGE_2 production was also significantly decreased by inhibitor of AMP-activated protein kinase (AMPK), suggesting thatwas at least partly induced by signal transduction via AdipoR1.

Yamauchi et al., [54] demonstrated that the peroxisome proliferator–activated receptorα (PPARα) signaling pathway exists downstream of AdipoR2. We found that an antagonist of the PPARα pathway reducesadiponectin-induced PGE_2 production by RA synovial fibroblasts.

NF-κB is known to play a central role in the regulation of inflammatory reactions in various cells [55]. Regarding PGE_2 production by RA synovial fibroblasts, NF-κB has an important role in the transcriptional regulation of COX-2 [56]. We demonstrated that adiponectin promotes NF-κB translocation in RA synovial fibroblasts, suggesting that adiponectin induces COX-2 expression in RA synovial fibroblasts via activation of NF-κB. Since the mPGES-1 promoter does not contain an NF-κB–responsive element, expression of mPGES-1 might be induced indirectly after activation of NF-κB [57], unlike COX-2. An increase of PGE_2 production due to COX-2 activation by adiponectin could lead to autocrine enhancement of mPGES-1 expression [52].

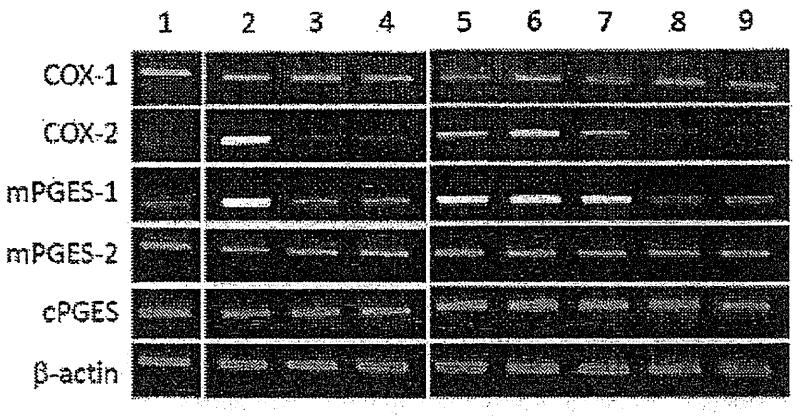

Figure 4. Effects of adipokines on COX and PGES mRNA expression. Synovial fibroblasts were incubated with IL-1β, adiponectin, leptin, or resistinat the indicated concentration for 18 h. Total RNA was isolated from cells and was subjected to the reverse transcription-polymerase chain reaction for each target enzyme and for β-actin. Lane 1, untreated; Lane 2, IL-1β (1 ng/mL); Lane 3, leptin (10 ng/mL); Lane 4, leptin (100 ng/mL); Lane 5, adiponectin (0.1 μg/mL); Lane 6, adiponectin (1 μg/mL); Lane 7, adiponectin (10 μg/mL); Lane 8, resistin (10 ng/mL); Lane 9, resistin (100 ng/mL).

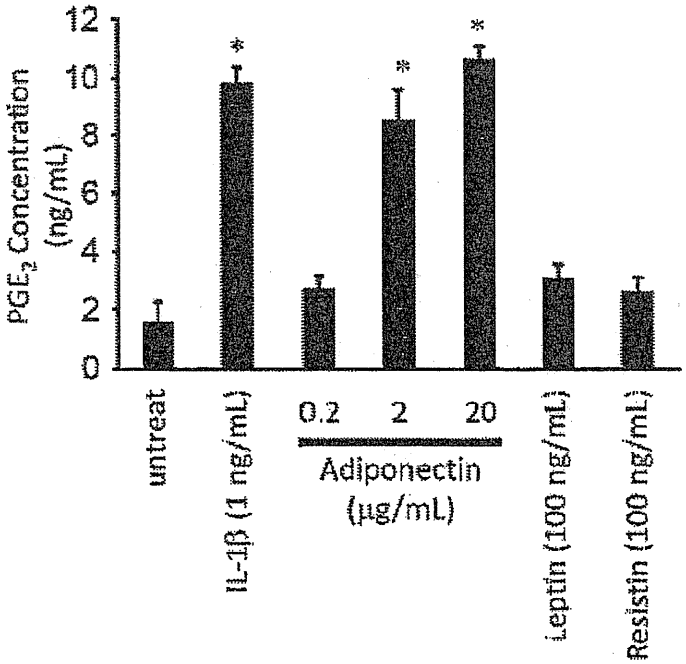

Figure 5. Prostaglandin E_2 production by RA synovial fibroblasts treated with adipokines. Synovial fibroblasts were incubated with IL-1β, adiponectin, leptin, or resistinat the indicated concentration for 18 h. After washing the cells, 3 μM arachidonic acidwas added for 30 min and then the PGE_2 concentration in the medium was measured by enzyme-linked immunosorbent assay. Bars show the mean and SD (n = 3). *=$p < 0.01$ versus untreat by Tukey's multiple comparison test.

Potential Role of AA metabolites in RA

Table1 shows various effects of PGE_2 that could be involved in joint inflammation and destruction. It can be suggested that reducing the bioactivity of PGE_2 is important to slow disease progression in RA.

We summarized the contribution of the PGE_2 biosynthesis pathway to arthritis in mice [58]. It was reported that mPGES-1-deficient mice showed marked reduction of inflammatory and histopahological changes such as pannus formation and joint erosion when used to create models of collagen-induced arthritis and collagen antibody-induced arthritis, which are animal models of inflammatory arthritis that resemble human RA [38, 39]. cPLA2-deficient mice also displayed considerable amelioration of both clinical and histological changes in these models [59-61].

These findings suggest that regulation of PGE_2 biosynthesis by both cytokines and adipokines may be a possible therapeutic target for RA.

REFERENCES

[1] Brennan, F.M. & McInnes, I.B. (2008). Evidence that cytokines play a role in rheumatoid arthritis. *J. Clin. Invest.*, 118, 3537-3545.

[2] Ahima, R.S. & Flier, J.S. (2000). Adipose tissue as an endocrine organ. *Trends Endocrinol. Metab.*, 11, 327-332.

[3] Meier, U. & Gressner, A.M. (2004). Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin. Chem.*, 50, 1511-1525.

[4] Tilg, H. & Moschen, A.R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.*, 6, 772-783.

[5] Oh, D.K;Ciaraldi, T; Henry, R.R. (2007). Adiponectin in health and disease. *Diabetes Obes. Metab.*, 9, 282-289.

[6] Yoshino, T; Kusunoki, N; Tanaka, N; Kaneko, K; Kusunoki, Y; Endo, H; Hasunuma, T; Kawai, S. (2011). Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. *Intern. Med.*, 50,269-275.

[7] Migita, K; Maeda, Y; Miyashita, T; Kimura, H; Nakamura, M; Ishibashi, H; Eguchi, K. (2006). The serum levels of resistin in rheumatoid arthritis patients. *Clin. Exp. Rheumatol.*, 24, 698-701.

[8] Senolt, L; Housa, D; Vernerová, Z; Jirásek, T; Svobodová, R;Veigl, D; Anderlová, K; Müller-Ladner, U; Pavelka, K; Haluzík, M. (2007). Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum. *Ann. Rheum. Dis.*,66, 458-463.

[9] Rho, Y. H; Solus, J; Sokka, T; Oeser, A; Chung, C. P; Gebretsadik, T; Shintani, A; Pincus, T; Stein, C.M. (2009). Adipocytokines are associated with radiographic joint damage in rheumatoid arthritis. *Arthritis Rheum*, 60, 1906-1914.

[10] Otero, M; Lago, R; Gomez, R; Lago, F; Dieguez, C; Gómez-Reino, J. J; Gualillo, O. (2006). Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis., *Ann. Rheum. Dis.*, 65, 1198-1201.

[11] Targońska-Stepniak, B; Majdan, M; Dryglewska, M. (2008). Leptin serum levels in rheumatoid arthritis patients: relation to disease duration and activity. *Rheumatol. Int.*, 28, 585-591.

[12] Gunaydin, R; Kaya, T; Atay, A; Olmez, N; Hur, A; Koseoglu, M. (2006). Serum leptin levels in rheumatoid arthritis and relationship with disease activity. *South Med. J.*, 99, 1078-1083.

[13] Härle, P; Sarzi-Puttini, P; Cutolo, M; Straub, R.H. (2006). No change of serum levels of leptin and adiponectin during anti-tumour necrosis factor antibody treatment with adalimumab in patients with rheumatoid arthritis. *Ann. Rheum. Dis.*, 65, 970-971.

[14] Senolt, L; Pavelka, K; Housa, D; Haluzík, (2006). M. Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. *Cytokine*, 35, 247-252.

[15] Popa, C; Netea, M. G; de Graaf, J; van den Hoogen, F. H; Radstake, T. R; Toenhake-Dijkstra, H; van Riel, P.L; van der Meer, J. W; Stalenhoef, A.F; Barrera, P. (2009). Circulating leptin and adiponectin concentrations during tumor necrosis factor blockade in patients with active rheumatoid arthritis. *J. Rheumatol.*, 36, 724-730.

[16] Harizi, H; Corcuff, J. B; Gualde, N. (2008). Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. *Trends in Molecular Medicine*, 14, 461-469.

[17] Kita, Y; Ohto, T; Uozumi, N; Shimizu, T. (2006). Biochemical properties and pathophysiological roles of cytosolic phospholipase A2s. *Biochim. Biophys. Acta.*, 1761, 1317-1322.

[18] Ghosh, M; Tucker, D.E; Burchett, S.A; Leslie, C.C. (2006). Properties of the Group IV phospholipase A2 family. *Prog. Lipid. Res.*, 45, 487-510.

[19] Tai, N; Kuwabara, K; Kobayashi, M; Yamada, K; Ono, T; Seno, K; Gahara, Y; Ishizaki, J; Hori, Y. (2010). Cytosolic phospholipase A2 alpha inhibitor, pyrroxyphene, displays anti-arthritic and anti-bone destructive action in a murine arthritis model. *Inflamm. Res.*, 59, 53-62.

[20] Xie, W. L; Chipman, J. G; Robertson, D. L; Erikson, R.L; Simmons, D.L. (1991). Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. USA*, 88, 2692-2696.

[21] Kujubu, D.A; Fletcher, B. S; Varnum, B.C; Lim, R. W; Herschman, H.R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/ cyclooxygenase homologue. *J. Biol. Chem.*, 266, 12866-12872.

[22] Vane, J. R. (1996). Botting RM. Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol.*, 25, 9–21.

[23] Vane, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.*, 231, 232-235.

[24] Siegle, I; Klein, T; Backman, J. T; Saal, J. G; Nüsing, R.M; Fritz, P. (1998). Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. *Arthritis Rheum.*, 41, 122-129.

[25] Crofford, L.J; Wilder, R. L; Ristimäki, A.P; Sano, H; Remmers, E.F; Epps, H. R; Hla, T. (1994), Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1 beta, phorbol ester, and corticosteroids. *J. Clin. Invest.*, 93, 1095-1101.

[26] Kalinski, P. (2012). Regulation of immune responses by prostaglandin E2. *J. Immunol.*, 188, 21-28.

[27] Egg, D; Günther, R; Herold, M; Kerschbaumer, F. (1980). Prostaglandins E2 and F2 alpha concentrations in the synovial fluid in rheumatoid and traumatic knee joint diseases. *Z Rheumatol.*, 39, 170-175.

[28] Kawai, S; Nishida, S; Kato, M; Furumaya, Y; Okamoto, R; Koshino, T; Mizushima, Y. (1998). Comparison of cyclooxygenase-1 and -2 inhibitory activities of various nonsteroidal anti-inflammatory drugs using human platelets and synovial cells. *Eur. J. Pharmacol.*, 347, 87-94.

[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.

[30] Jakobsson, P.J; Thoren, S; Morgenstern, R. (1999). Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc. Natl. Acad. Sci. USA,* 96, 7220-7225.

[31] Murakami, M; Naraba, H; Tanioka, T; Semmyo, N; Nakatani, Y; Kojima, F; Ikeda, T; Fueki, M; Ueno, A; Oh, S; Kudo, I. (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.

[32] Lazarus, M; Kubata, B. K; Eguchi, N; Fujitani, Y; Urade, Y; Hayaishi, O. (2002). Biochemical characterization of mouse microsomalprostaglandin E synthase-1 and its colocalization with cyclooxygenase-2 in peritoneal macrophages. *Arch. Biochem. Biophys.*, 397, 336-341.

[33] Kojima, F; Naraba, H; Sasaki, Y; Okamoto, R; Koshino, T; Kawai, S. (2002). Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. *J. Rheumatol.*, 29, 1836-1842.

[34] Stichtenoth, D. O; Thoren, S; Bian, H; Peters-Golden, M; Jakobsson, P. J; Crofford, L.J. (2001). Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J. Immunol.*, 167, 469-474.

[35] Han, R; Tsui, S; Smith, T.J. (2002). Up-regulation of prostaglandin E2 synthesis by interleukin-1beta in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E2 synthase expression. *J. Biol. Chem.*, 277, 16355-16364.

[36] Saegusa, M; Murakami, M; Nakatani, Y; Yamakawa, K; Katagiri, M; Matsuda, K; Nakamura, K; Kudo, I; Kawaguchi, H. (2003). Contribution of membrane-associated prostaglandin E2 synthase to bone resorption. *J. Cell Physiol.*, 197, 348-356.

[37] Kojima, F; Naraba, H; Miyamoto, S; Beppu, M; Aoki, H; Kawai, S. (2004). Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. *Arthritis Res. Ther.*, 6, R355-R365.

[38] Trebino, C. E; Stock, J. L; Gibbons, C. P; Naiman, B. M; Wachtmann, T. S; Umland, J. P; Pandher, K; Lapointe, J. M; Saha, S; Roach, M.L; Carter, D; Thomas, N. A; Durtschi, B. A; McNeish, J. D; Hambor, J. E; Jakobsson, P.J; Carty, T. J; Perez, J. R; Audoly, L. P. (2003). Impaired

inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc. Natl. Acad. Sci. USA*, 100, 9044-9049.

[39] Kamei, D; Yamakawa, K; Takegoshi, Y; Mikami-Nakanishi, M; Nakatani, Y; Oh-Ishi, S; Yasui, H; Azuma, Y; Hirasawa, N; Ohuchi, K; Kawaguchi, H; Ishikawa, Y; Ishii, T; Uematsu, S; Akira, S; Murakami, M; Kudo, I. (2004). Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. *J. Biol. Chem.*, 279, 33684-33695.

[40] Mabuchi, T; Kojima, H; Abe, T; Takagi, K; Sakurai, M; Ohmiya, Y; Uematsu, S; Akira, S; Watanabe, K; Ito, S. (2004). Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain. *Neuroreport*, 15, 1395-1398.

[41] Yamagata, K; Matsumura, K; Inoue, W; Shiraki, T; Suzuki, K; Yasuda, S; Sugiura, H; Cao, C; Watanabe, Y; Kobayashi, S. (2001). Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J. Neurosci.*, 21, 2669-2677.

[42] Ivanov, A. I; Pero, R.S; Scheck, A. C; Romanovsky, A.A. (2002). Prostaglandin E(2)-synthesizing enzymes in fever: differential transcriptional regulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 283, R1104-R1117.

[43] Engblom, D; Saha, S; Engström, L; Westman, M; Audoly, L. P; Jakobsson, P.J; Blomqvist, A. (2003). Microsomal prostaglandin E synthase-1 is the central switch during immune- induced pyresis. *Nat. Neurosci.*, 6, 1137-1138.

[44] Ivanov, A. I.& Romanovsky, A.A. (2004). Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front Biosci.*, 9, 1977-1993.

[45] Filion, F; Bouchard, N; Goff, A. K; Lussier, J. G; Sirois, J. (2001). Molecular cloning and induction of bovine prostaglandin E synthase by gonadotropins in ovarian follicles prior to ovulation in vivo. *J. Biol. Chem.*, 276, 34323-34330.

[46] Lazarus, M; Munday, C. J; Eguchi, N; Matsumoto, S; Killian, G.J; Kubata, B.K; Urade, Y. (2002). Immunohistochemical localization of microsomal PGE synthase-1 and cyclooxygenases in male mouse reproductive organs. *Endocrinology*, 143, 2410-2419.

[47] Satoh, K; Nagano, Y; Shimomura, C; Suzuki, N; Saeki, Y; Yokota, H. (2000). Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated rat astrocytes. *Neurosci. Lett.*, 283, 221-223.

[48] Cipollone F., Prontera C., Pini B. et al., Over expression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* (2001) 104 921–927.

[49] Cipollone, F; Prontera, C; Pini, B; Marini, M; Fazia, M; De Cesare, D; Iezzi, A; Ucchino, S; Boccoli, G; Saba, V; Chiarelli, F; Cuccurullo, F; Mezzetti, A. (2003). The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control. *Circulation,* 108, 1070-1077.

[50] Kamei, D; Murakami, M; Nakatani, Y; Ishikawa, Y; Ishii, T; Kudo, I. (2003). Potential role of microsomal prostaglandin E synthase-1 in tumorigenesis. *J. Biol. Chem.,* 278, 19396-19405.

[51] Murakami, M; Nakashima, K; Kamei, D; Masuda, S; Ishikawa, Y; Ishii, T; Ohmiya, Y; Watanabe, K; Kudo, I. (2003). Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. *J. Biol. Chem.,* 278, 37937-37947.

[52] Kojima, F; Naraba, H; Sasaki, Y; Beppu, M; Aoki, H; Kawai, S. (2003). Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. *Arthritis Rheum.,* 48, 2819-2828.

[53] Kusunoki, N; Kitahara, K; Kojima, F; Tanaka, N; Kaneko, K; Endo, H; Suguro, T; Kawai, S. (2010). Adiponectin stimulates prostaglandin E(2) production in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum.,* 62, 1641-1649.

[54] Yamauchi, T; Nio, Y; Maki, T; Kobayashi, M; Takazawa, TIwabu, M; Okada-Iwabu, M; Kawamoto, S; Kubota, N; Kubota, T; Ito, Y;Kamon, J; Tsuchida, A; Kumagai, K; Kozono, H; Hada, Y; Ogata, H; Tokuyama, K; Tsunoda, M; Ide, T; Murakami, K; Awazawa, M; Takamoto, I; Froguel, P; Hara, K;Tobe, K; Nagai, R; Ueki, K; Kadowaki, T. (2007). Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat. Med.,* 13, 332-339.

[55] Barnes, P.J. & Karin, M. (1997). Nuclear factor-kappa B: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.,* 336, 1066-1071.

[56] Crofford, L.J; Tan, B; McCarthy, C. J; Hla, T. (1997). Involvement of nuclear factor kappa B in the regulation of cyclooxygenase-2 expression by interleukin-1 in rheumatoid synoviocytes. *Arthritis Rheum.,* 40, 226-236.

[57] Fahmi, H. (2004). mPGES-1 as a novel target for arthritis. *Curr. Opin. Rheumatol.*,16, 623-627.

[58] Kojima, F; Kato, S; Kawai, S. (2005). Prostaglandin E synthase in the pathophysiology of arthritis. *Fundam. Clin. Pharmacol.*, 19, 255-261.

[59] Hegen, M; Sun, L; Uozumi, N; Kume, K; Goad, M.E; Nickerson-Nutter, C.L; Shimizu, T; Clark, J.D. (2003). Cytosolic phospholipase A2alpha-deficient mice are resistant to collagen-induced arthritis. *J. Exp. Med.*, 197, 1297-302.

[60] Myers, L.K; Kang, A. H; Postlethwaite, A. E; Rosloniec, E. F; Morham, S. G; Shlopov, B. V; Goorha, S; Ballou, L.R. (2000). The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum.*,43, 2687-2693.

[61] McCoy, J.M; Wicks, J. R; Audoly, L.P. (2002). The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. *J. Clin. Invest.*, 110, 651-658.

[62] Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M. Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol. Pain*. 2005 Jan 17;1:3.

[63] Villarreal CF, Sachs D, Cunha FQ, Parada CA, Ferreira SH. The role of Na(V)1.8 sodium channel in the maintenance of chronic inflammatory hypernociception. *Neurosci. Lett.* 2005 Sep 30;386(2):72-7.

[64] Amaya F, Wang H, Costigan M, Allchorne AJ, Hatcher JP, Egerton J, Stean T, Morisset V, Grose D, Gunthorpe MJ, Chessell IP, Tate S, Green PJ, Woolf CJ. The voltage-gated sodium channel Na(v)1.9 is an effector of peripheral inflammatory pain hypersensitivity. *J. Neurosci.* 2006 Dec 13;26(50):12852-60.

[65] Wang C, Gu Y, Li GW, Huang LY. A critical role of the cAMP sensor Epac in switching protein kinase signalling in prostaglandin E2-induced potentiation of P2X3 receptor currents in inflamed rats. *J. Physiol.* 2007 Oct 1;584(Pt 1):191-203.

[66] Nishihara I, Minami T, Watanabe Y, Ito S, Hayaishi O. Prostaglandin E2 stimulates glutamate release from synaptosomes of rat spinal cord. *Neurosci. Lett.* 1995 Aug 18;196(1-2):57-60.

[67] Nicol GD, Klingberg DK, Vasko MR. Prostaglandin E2 increases calcium conductance and stimulates release of substance P in avian sensory neurons. *J. Neurosci.* 1992 May;12(5):1917-27.

[68] Kohno T, Wang H, Amaya F, Brenner GJ, Cheng JK, Ji RR, Woolf CJ. Bradykinin enhances AMPA and NMDA receptor activity in spinal cord

dorsal horn neurons by activating multiple kinases to produce pain hypersensitivity. *J. Neurosci.* 2008 Apr 23;28(17):4533-40.

[69] Harvey RJ, Depner UB, Wässle H, Ahmadi S, Heindl C, Reinold H, Smart TG, Harvey K, Schütz B, Abo-Salem OM, Zimmer A, Poisbeau P, Welzl H, Wolfer DP, Betz H, Zeilhofer HU, Müller U. GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science.* 2004 May 7;304(5672):884-7.

[70] Morimoto K, Shirata N, Taketomi Y, Tsuchiya S, Segi-Nishida E, Inazumi T, Kabashima K, Tanaka S, Murakami M, Narumiya S, Sugimoto Y. Prostaglandin E2-EP3 Signaling Induces Inflammatory Swelling by Mast Cell Activation. *J. Immunol.* 2013 Dec 16. [Epub ahead of print]

[71] Foudi N, Gomez I, Benyahia C, Longrois D, Norel X. Prostaglandin E2 receptor subtypes in human blood and vascular cells. *Eur. J. Pharmacol.* 2012 Nov 15;695(1-3):1-6.

[72] Suzawa T, Miyaura C, Inada M, Maruyama T, Sugimoto Y, Ushikubi F, Ichikawa A, Narumiya S, Suda T. The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective *EPs*. *Endocrinology.* 2000 Apr;141(4):1554-9.

[73] Li X, Okada Y, Pilbeam CC, Lorenzo JA, Kennedy CR, Breyer RM, Raisz LG. Knockout of the murine prostaglandin EP2 receptor impairs osteoclastogenesis in vitro. *Endocrinology.* 2000 Jun;141(6):2054-61.

[74] Li X, Pilbeam CC, Pan L, Breyer RM, Raisz LG. Effects of prostaglandin E2 on gene expression in primary osteoblastic cells from prostaglandin receptor knockout mice. *Bone.* 2002 Apr;30(4):567-73.

[75] Li X, Tomita M, Pilbeam CC, Breyer RM, Raisz LG. Prostaglandin receptor EP2 mediates PGE2 stimulated hypercalcemia in mice in vivo. Prostaglandins *Other Lipid Mediat.* 2002 Apr;67(3-4):173-80.

[76] Moreno-Rubio J, Herrero-Beaumont G, Tardio L, Alvarez-Soria MA, Largo R. Non steroidalanti inflammatory drugs and prostaglandin E(2) modulate the synthesis of osteoprotegerin and RANKL in the cartilage of patients with severe knee osteoarthritis. *Arthritis Rheum.* 2010 Feb;62(2):478-88.

[77] Brändström H, Jonsson KB, Ohlsson C, Vidal O, Ljunghall S, Ljunggren O. Regulation of osteoprotegerin mRNA levels by prostaglandin E2 in human bone marrow stroma cells. *Biochem. Biophys. Res. Commun.* 1998 Jun 18;247(2):338-41.

[78] Suda K, Udagawa N, Sato N, Takami M, Itoh K, Woo JT, Takahashi N, Nagai K. Suppression of osteoprotegerin expression by prostaglandin E2 is crucially involved in lipopolysaccharide-induced osteoclast formation. *J. Immunol.* 2004 Feb 15;172(4):2504-10.

[79] Notoya K, Jovanovic DV, Reboul P, Martel-Pelletier J, Mineau F, Pelletier JP. The induction of cell death in human osteoarthritis chondrocytes by nitric oxide is related to the production of prostaglandin E2 via the induction of cyclooxygenase-2. *J. Immunol.* 2000 Sep 15;165(6):3402-10.

[80] Miyaura C, Inada M, Suzawa T, Sugimoto Y, Ushikubi F, Ichikawa A, Narumiya S, Suda T. Impaired bone resorption to prostaglandin E2 in prostaglandin E receptor EP4-knockout mice. *J. Biol. Chem.* 2000 Jun 30;275(26):19819-23.

[81] Kim CH, Park YG, Noh SH, Kim YK. PGE2 induces the gene expression of bone matrix metalloproteinase-1 in mouse osteoblasts by cAMP-PKA signaling pathway. *Int. J. Biochem. Cell Biol.* 2005 Feb;37(2):375-85.

[82] Attur M, Al-Mussawir HE, Patel J, Kitay A, Dave M, Palmer G, Pillinger MH, Abramson SB. Prostaglandin E2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor. *J. Immunol.* 2008 Oct 1;181(7):5082-8.

[83] Miles RR, Sluka JP, Halladay DL, Santerre RF, Hale LV, Bloem L, Thirunavukkarasu K, Galvin RJ, Hock JM, Onyia JE. ADAMTS-1: A cellular disintegrin and metalloprotease with thrombospondin motifs is a target for parathyroid hormone in bone. *Endocrinology.* 2000 Dec;141(12):4533-42.

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Evaluation of two Japanese regulatory actions using medical information databases: a 'Dear Doctor' letter to restrict oseltamivir use in teenagers, and label change caution against co-administration of omeprazole with clopidogrel

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Keywords: **clopidogrel, medical information database, omeprazole, oseltamivir, pharmacovigilance, regulatory action**

SUMMARY

What is known and objective: **The implementation of appropriate epidemiological methodology using medical information databases (MIDs) to evaluate the effects of regulatory actions has been highly anticipated. To assess scientific methods for active pharmacovigilance using MIDs, we conducted a quantitative assessment of the impact of two regulatory actions by the Japanese government: (i) restriction of use of oseltamivir in teenagers in March 2007 and (ii) caution against the co-administration of omeprazole (OPZ) with clopidogrel (CPG) in April 2010.**

Methods: **Data were obtained from four hub hospitals in Japan. We measured the seasonal proportion of patients prescribed oseltamivir to those prescribed neuraminidase inhibitors for the 2002/2003 to 2010/2011 seasons. The monthly proportion of patients co-administered OPZ and CPG (OPZ+CPG) to those prescribed CPG was measured from May 2009 to April 2011. We evaluated the changes observed with implementation of the regulatory actions. To estimate the impact of the actions, we conducted segmented regression analysis using interrupted time series data. The impact was assessed by two parameter estimates of the regression model: the change in level for short-term effects and change in trend for long-term effects.**

Results and discussion: **The use of oseltamivir in the target 10–19 years age group showed a significant and large decline (63·16%) immediately after the intervention ($P = 0{\cdot}0008$). No change was observed in OPZ+CPG, although there was a relative inhibitory trend for OPZ+CPG compared with co-administration of lansoprazole or rabeprazole with CPG as the control group. When restricted to new users of CPG, the stratified results were consistent with the overall results.**

What is new and conclusion: **The current analysis demonstrates the effectiveness of two regulatory actions. The results of the current study indicate that MID research can contribute to assessing and improving pharmacovigilance activities.**

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WHAT IS KNOWN AND OBJECTIVE

Drug regulatory authorities regularly furnish healthcare providers with drug information and conduct regulatory actions to protect patients from avoidable risks when an adverse drug event (ADE) or other drug-related safety concern is identified.[1] A revision to drug labelling is one of the most common regulatory mechanisms for disseminating updated safety information. The *Dear Doctor* letter (also called a *yellow letter*), in the U.S. and Japan, and the direct healthcare professional communication, in the EU, are written communications for highlighting urgent or serious problems.[2] However, these safety warnings are known to have weaknesses.[3,4] To minimize risk, it is important that both the short- and long-term effects of regulatory actions are measured and assessed in a timely and appropriate manner. If a regulatory action is not effective, additional safety measures should be considered.

Oseltamivir is a neuraminidase inhibitor shown to be effective in the treatment and prophylaxis of influenza A and B viral infections.[5,6] Its use has spread worldwide from its successful launch in 1999.[7] Seventy-five per cent of the total production of oseltamivir was used in Japan through March 2007.[8] However, a number of case reports of delirium and abnormal behaviour after treatment with oseltamivir, especially in teenagers, led to public concern about its safety.[9] In March 2007, the Japanese Ministry of Health, Labour, and Welfare (MHLW) ordered the holder of marketing approval to issue a *Dear Doctor* letter, essentially restricting the use of oseltamivir in children and adolescents aged 10–19 years.[10]

Clopidogrel (CPG) is an antiplatelet drug used for the prevention of recurrent ischaemic cerebrovascular disease and the treatment for ischaemic heart disease after percutaneous coronary intervention.[11] CPG is a pro-drug requiring activation by cytochrome P450 enzymes, including CYP 2C19.[12,13] Proton pump inhibitors (PPIs) are occasionally co-administered to reduce the risk of gastrointestinal bleeding associated with CPG.[14] Because certain PPIs are considered to have inhibitory effects on CYP2C19,[15,16] the U.S. Food and Drug Administration (FDA)[17] and the European Medicines Agency[18] have warned against the co-administration of omeprazole (OPZ) or esomeprazole with CPG, to prevent cardiovascular events caused by loss of

effectiveness of CPG. However, this issue remains controversial,[19–23] and there are some differences in the intensity of regulatory actions among regulatory authorities. In Japan, the MHLW did not issue the highest level warning, similar to the FDA black-box warning. Instead, they cautioned against the concurrent use of OPZ and CPG (OPZ+CPG) in April 2010 through changes to the conventional label. Esomeprazole was not approved by the MHLW at the time. This caution was not applied to the co-administration of CPG with lansoprazole or rabeprazole (LPZ/RPZ+CPG) because of the difference in the inhibitory effects on CYP2C19.

Recently, there has been considerable anticipation of the effective use of medical information databases (MIDs) for pharmacovigilance. To this end, many countries have directed large efforts towards establishing nationwide MID systems and the scientific methodologies to exploit them for active drug surveillance. In May 2008, the FDA announced the Sentinel Initiative with a Sentinel System for performing active nationwide surveillance using the electronic health data of a minimum of 100 million people.[24,25] In Japan, the MHLW launched its Sentinel Project in Japan (J-Sentinel) in 2010 for more accurate and comprehensive benefit-risk assessment.[26] An extensive MID covering 10 million individuals by 2015 is to be established under its J-sentinel plan.

To achieve the aims of the J-Sentinel project, we initiated collaborative research with four hub hospitals in Japan with the goal of establishing appropriate epidemiological methodology for pharmacovigilance using MIDs. In our research project, we used the four MIDs to conduct a quantitative assessment of the direct impact of the two regulatory actions on the prescribing of the target drugs in a timely and effective manner.

METHODS

Data source and study organization

We used MIDs of the University of Tokyo Hospital (Tokyo, Japan), Hamamatsu University Hospital (Shizuoka, Japan),[27] Kagawa University Hospital (Kagawa, Japan) and Kyushu University Hospital (Fukuoka, Japan), which covered nearly one million individuals. The average numbers of outpatients and inpatients per day in the four hospitals were more than 7200 and 3100, respectively. In each hospital, the number of target patients per defined period was counted using their local MID, and the summary data without personally identifiable information were submitted to the National Institute of Health Sciences (Tokyo, Japan). The summary data from the four hospitals were combined and used for the current analysis. The study was approved by the ethics committees of the Hamamatsu University Hospital and the National Institute of Health Sciences together with other pharmacoepidemiological studies. For the other three university hospitals, such approval was not necessary according to current ethical regulations in Japan.

Study design for oseltamivir

The outcome and intervention were defined as the proportion of patients prescribed oseltamivir relative to those prescribed neuraminidase inhibitors, and the *Dear Doctor* letter for oseltamivir issued on 20 March 2007, respectively. We used the number of patients taking any neuraminidase inhibitors as the denominator assuming that these patients in our four hospitals were representative of influenza cases in Japan (Figure S1). Period from the beginning of April of one year to the end of March of the following year was defined as a season, and the number of patients in each season was measured during the observation period from the 2002/2003 season to the 2010/2011 season. The seasons of 2007/2008 and later were assigned to the post-intervention period. Patients were classified into two different age groups: 10–19 years as the target group and 20 years or over as the control group. Children aged 0–9 years were not included in the study, because oseltamivir in dry syrup formulation was used preferentially for this age group, regardless of the regulatory action. The neuraminidase inhibitors examined in this study included oseltamivir, zanamivir, peramivir and laninamivir. As peramivir and laninamivir were approved in Japan in January 2010 and September 2010, respectively, the patient number for these two prescriptions was counted over two seasons (2009/2010 to 2010/2011) and one season (2010/2011), respectively. Oseltamivir and zanamivir were approved in December 2000 and December 1999 in Japan, respectively, and were in clinical use before the observation period.

Study design for OPZ+CPG

The outcome and intervention were defined as the proportion of patients prescribed OPZ+CPG relative to those prescribed CPG, and the label revision issued on 27 April 2010, respectively. The number of patients prescribed CPG per month was counted during the study period from the beginning of May 2009 to the end of April 2011. The period from beginning of May 2010 and later was assigned to the post-intervention period. PPIs examined in this study included oral OPZ, lansoprazole and rabeprazole, but PPI-antibiotic combination products for the treatment of *Helicobacter pylori* only were excluded because of the different indications. A prescription including one of the PPIs and CPG within the same day was defined as co-administration. Patients were classified into two different treatment groups: OPZ+CPG as the target group and LPZ/RPZ+CPG as the control group. *New users* were defined as patients who had not received CPG within six months prior to the month in which CPG was prescribed.

Statistical analysis

To estimate the effects of the two regulatory interventions, we conducted segmented regression analysis using interrupted time series data.[28] The effect was assessed using two parameters, level (β_2) and trend (β_3), according to the following linear regression model:

$$Y_t = \beta_0 + \beta_1 \times \text{time}_t + \beta_2 \times \text{intervention}_t + \beta_3 \times \text{time after intervention}_t + e_t$$

where Y_t is the proportion of patients prescribed the target drug in regularly spaced intervals at time *t*; *time* is a continuous variable indicating time from the start of the observation period; *intervention* is a binary variable for pre ($\text{intervention}_t = 0$)- or post-intervention ($\text{intervention}_t = 1$); *time after intervention* is a continuous variable indicating time from the intervention; β_2 represents the change in the level at the intervention, from the last point before intervention to the first point after intervention, indicating the short-term effect; β_3 represents the trend change in the slope in the post-intervention period compared with that in the preintervention period, indicating the long-term effect.

Ordinary least squares regression analysis assumes that the error terms associated with each observation are independent.[28,29]

As autocorrelation was detected in some linear regression models, the autoregressive error model using the Yule-Walker estimation method for first-order autoregression was applied as appropriate.[30] After adjusting for autocorrelation, the Durbin–Watson statistics for the final model indicated no autocorrelation. Values of $P < 0.05$ (two-sided) were considered statistically significant. All statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Results of action against oseltamivir use

Mean seasonal numbers of the patients prescribed neuraminidase inhibitors prior to and after the regulatory action in the four hospitals are shown in Table 1. There was no significant difference in the total number of patients prescribed neuraminidase inhibitors between pre- and post-intervention periods (686 vs. 647, $P = 0.858$, Student's *t*-test), although the numbers varied from year to year due to variability in the prevalence of influenza.

The time series plots and results of the segmented regression analysis of mean seasonal proportions of patients prescribed oseltamivir to the total number of patients prescribed any neuraminidase inhibitor between different age groups using data from the four hospitals are shown in Fig. 1 and Table 2, respectively. There were significant reductions in prescription levels after the intervention (β_2): 63·16% for ages 10 to 19 years ($P = 0.0008$) and 16·50% for ages ≥20 years ($P = 0.0354$), respectively. The trends (β_3) for ages 10–19 years and ages ≥20 years were −1·37 ($P = 0.6948$) and 2·47 ($P = 0.2380$), respectively, indicating an insignificant 1·37% decrease per season for the target group, and an insignificant 2·47% increase by per season for the control group compared with the baseline trends.

Results of action against OPZ+CPG

The average monthly numbers of patients prescribed the study drugs prior to and after the regulatory action in the four hospitals are summarized in Table 3. After the regulatory action, the mean number of individuals prescribed PPIs, CPG and co-administration increased significantly and to the same extent, 9·7% (8 070 vs. 8 856, $P = 0.0017$; Mann–Whitney *U*-test), 13·2% (903 vs. 1 022, $P = 0.0020$) and 13·2% (342 vs. 387, $P = 0.0029$), respectively.

The time series plots for mean proportions of the numbers of OPZ+CPG and LPZ/RPZ+CPG prescribed to patients prescribed CPG per month in the four hospitals are shown in Fig. 2, and the results of the segmented regression analysis are summarized in Table 4. The number of individuals prescribed OPZ+CPG did not

Table 1. Mean seasonal numbers of patients prescribed neuraminidase inhibitors prior to and after the regulatory action (20 March 2007) in four hospitals, by age group

Drug group	Age group / Period	Total (≥ 10)		10–19		≥20	
		Pre[a]	Post[b]	Pre[a]	Post[b]	Pre[a]	Post[b]
Total		686(201)	647(411)	109(50)	145(161)	576(189)	502(252)
Oseltamivir		641(185)	470(312)	106(49)	40(54)	535(175)	430(259)
Zanamivir		44(23)	159(120)	3(4)	100(111)	41(24)	59(35)
Peramivir[c]		–	4(4)	–	0·5(0·7)	–	4(4)
Laninamivir[d]		–	65	–	20	–	45

[a]From 2002/2003 to 2006/2007 season (5 seasons).
[b]From 2007/2008 to 2010/2011 season (4 seasons).
[c]Counted for 2009/2010 and 2010/2011 seasons (2 seasons) after approval of peramivir in January 2010.
[d]Counted for 2010/2011 season after approval of laninamivir in September 2010. Data in parentheses indicate SD.

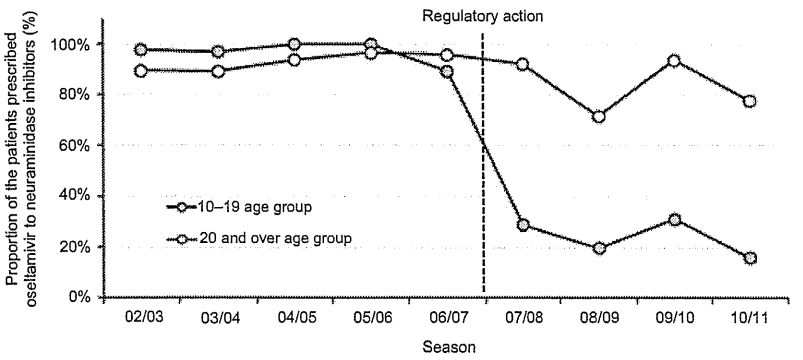

Fig. 1. Time series plots of seasonal change in the proportion of patients prescribed oseltamivir to neuraminidase inhibitors, by age group.

Table 2. Interrupted time series regression analysis of seasonal change in the proportion of the patients prescribed oseltamivir to neuraminidase inhibitors, by age group[a]

Group	Parameter	Coefficient	Standard error	*P*-value
10–19	Level change after action β_2	−63·16	8·69	0·0008
	Trend change after action β_3	−1·37	3·28	0·6948
≥20	Level change after action β_2	−16·50	5·28	0·0354
	Trend change after action β_3	2·47	1·78	0·2380

[a]Each model is adjusted for first-order autocorrelation as appropriate.

change significantly in level (P = 0·9250) or trend (P = 0·8040). In contrast, a slight but significant change in trend (a 0·60% increase per month compared with the preintervention trend, P = 0·0017) was observed in the LPZ/RPZ+CPG group.

For higher sensitivity in the detection of the intervention effect, we conducted a subanalysis of new users of CPG. Figure 3 shows the time series plots of mean proportions for OPZ+CPG and LPZ/RPZ+CPG among new CPG users. OPZ+CPG prescriptions did not show any change in level (P = 0·2814) or trend (P = 0·9945) in the new-user study. Prescriptions for LPZ/RPZ+CPG did not show any significant decline in level (4·99%, P = 0·1183), but there was an evident increase in trend (2·06% per month, P = 0·0001).

DISCUSSION

The interrupted time series is the strongest, quasi-experimental design for the evaluation of the longitudinal effects of an intervention that occurs at a particular time-point.[28,31–33] In addition, this design is recommended as a method with strong internal validity in a FDA-sponsored Mini-Sentinel project, the aim of which is to facilitate the Sentinel Initiative and to identify appropriate designs for evaluating FDA regulatory actions.[34] The interrupted time series design also allows analysis of aggregate data without identifiable personal information, which is suitable for a multicenter study. The analysis has the advantage of presenting the results in a graphical and intuitive way. Therefore, we adopted the interrupted time series analysis to evaluate the impact of regulatory actions in this study.

The regulatory action on oseltamivir had immediate effects, not only for the 10–19 years target age group, but also for the ≥20 years age group in our study. However, a steep decline was more prominent in the target group (63·16%) than in the control

Table 3. Mean monthly numbers of patients prescribed proton pump inhibitors, clopidogrel, or both prior to and after the regulatory action (27 April 2010) in four hospitals

		CPG total		PPIs total		OPZ subgroup		LPZ/RPZ subgroup	
Group	Period	Pre[a]	Post[b]	Pre[a]	Post[b]	Pre[a]	Post[b]	Pre[a]	Post[b]
PPIs				8 070(487)	8 856(454)	1 701(81)	1 708(54)	6 369(418)	7 147(432)
				Co-administration of PPI with CPG					
CPG		903(78)	1 022(52)	342(31)	387(36)	62(6)	51(7)	281(31)	335(38)

PPIs, proton pump inhibitors; OPZ, omeprazole; LPZ/RPZ, lansoprazole or rabeprazole; CPG, clopidogrel.
[a]From May 2009 to April 2010 (12 months).
[b]From May 2010 to April 2011 (12 months). Data in parentheses indicate SD.

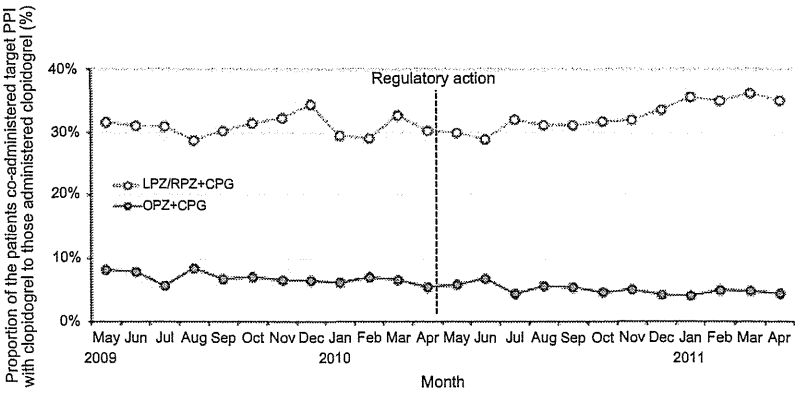

Fig. 2. Time series plots of monthly change in the proportion of patients co-administered proton pump inhibitors with clopidogrel to those administered clopidogrel, by treatment group.

Table 4. Interrupted time series regression analysis of monthly change in the proportion of patients co-administered proton pump inhibitors with clopidogrel, by treatment group[a]

Group	Parameter	Coefficient	Standard error	*P*-value
OPZ+CPG				
Total				
	Level change after action β_2	−0·06	0·58	0·9250
	Trend change after action β_3	0·02	0·08	0·8040
New-user subgroup[b]				
	Level change after action β_2	−1·90	1·71	0·2814
	Trend change after action β_3	0·001	0·25	0·9945
LPZ/RPZ+CPG				
Total				
	Level change after action β_2	−2·33	1·14	0·0550
	Trend change after action β_3	0·60	0·16	0·0017
New-user subgroup[b]				
	Level change after action β_2	−4·99	3·06	0·1183
	Trend change after action β_3	2·06	0·44	0·0001

OPZ+CPG, co-administration of omeprazole with clopidogrel; LPZ/RPZ+CPG, co-administration of lansoprazole or rabeprazole with clopidogrel.

[a]Each model is adjusted for first-order autocorrelation as appropriate.

[b]Patients who had not received clopidogrel within six months prior to the month in which clopidogrel was prescribed.

group (16·50%). The change in the control group was assumed to be due to external influences such as media publicity. The lower proportion of oseltamivir users after the intervention in the target group remained until the end of the follow-up period, suggesting a prolonged inhibitory effect for the regulatory action. Although a small but significant number of teenage patients were still prescribed oseltamivir after the action, this was considered reasonable because the restriction was not applied to patients at high risk of influenza[10] Therefore, the current results indicate that the regulatory action against oseltamivir use was effective both in the short-term and long-term.

To evaluate the generalizability of the current result to a larger population, our data were compared with those of two other Japanese studies, the MIHARI project conducted by the Pharmaceuticals and Medical Devices Agency, and Urushihara's report.[35,36] A comparison of the three data sets showed that our data were graphically consistent with both the MIHARI and Urushihara's results (Figure S2). In addition, a comparison of data from each hospital in this study revealed that the influence of the two regulatory actions was essentially identical among the four hospitals (data not shown). All our hospitals are equipped with a drug information (DI) room, the installation of which is encouraged by the MHLW to disseminate drug safety information. We estimated based on the MHLW documents[37] that approximately 65% of all hospitals in Japan already had a DI room in 2010, and therefore, our study hospitals were not considered to be special cases in terms of their response capability to regulatory actions. These findings indicate our four-hospital database is representative of the general population in Japan and is appropriate for use in a preliminary survey for pharmacovigilance purposes.

Regarding regulatory action against the use of OPZ+CPG, the target group showed no significant changes in prescription status after the regulatory action, but the control LPZ/RPZ+CPG group had a slight increase in trend. A comparison of the two groups indicates that this regulatory action might have a relatively small inhibitory effect in the target group.

To further examine the impact of this regulatory action, the data were stratified by selecting new users (i.e. patients without a medical history of CPG in the preceding six months) because continuous users of CPG were likely to continue receiving the same medication regardless of the regulatory action unless they experienced some problem. The results of the new-user study showed a significant increase in LPZ/RPZ+CPG prescriptions, which was consistent with the results from the complete data set. The difference between the two PPI groups, that is, no long-term change in OPZ+CPG prescriptions and gradual increase of LPZ/RPZ+CPG prescriptions, may reflect the response to the label change, suggesting that the intention of the regulatory action (label addition in drug-interaction section) is not communicated

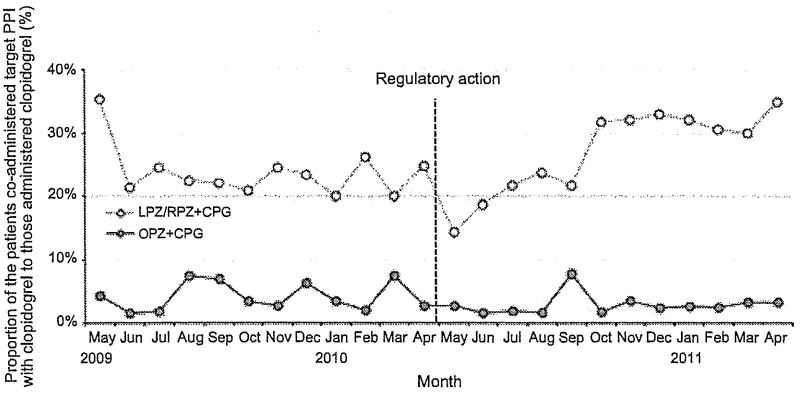

Fig. 3. Time series plots of monthly change in the proportion of patients co-administered proton pump inhibitors with clopidogrel to those administered clopidogrel only in the clopidogrel new-user population, by treatment group.

immediately, but leading to slower translation to the clinical setting. In addition, stratification by new users would be useful for eliminating bias due to the possible hangover effects in continuous users.

Drug regulatory authorities implement different levels of safety measures depending on the type of adverse reactions involved. In this study, we analysed the impact of two different levels of regulatory action, the *Dear Doctor* letter advisory for oseltamivir and a label change for OPZ+CPG. It was difficult to judge, based on our results, whether the *Dear Doctor* letter was more effective than the conventional label change. Moreover, the difference in time units of the two regulatory actions should be taken into account, especially when comparing their short-term effects. Additional evidence from future studies will be required to more accurately assess the impact of these regulatory actions. In particular, a comparative analysis of the impact of different levels of regulatory action for the same or related drugs would be beneficial.

Limitations

There are some limitations to this study. First, when using interrupted time series analysis to investigate the use of medications, it is generally recommended that 12 data points be used before and after the intervention.[28] However, in the current analysis of oseltamivir, fewer data points were available because data were collected only once annually to incorporate the influenza epidemic season. In addition, the impact of other sources, for example, media publicity and other regulatory notices, could not be distinguished from the effect of the regulatory action because of the annual intervals. Thus, this design may be better suited for the analysis of regulatory actions whose outcomes can be measured over shorter time intervals, for example monthly or weekly. Second, we examined a limited data set from only four hospitals. Although this analysis suggests generalizability of the method, a further large-scale study will be required to provide a more accurate and reliable assessment of the methodology for evaluating the impact of regulatory action.

WHAT IS NEW AND CONCLUSION

We have used analysis of interrupted time series with segmented regression of data from hospital MIDs to investigate the safe use of drugs and demonstrated the effectiveness of regulatory actions on the use of oseltamivir and OPZ+CPG. The results of the current study indicate that MID research can contribute to assessing and improving pharmacovigilance activities.

CONFLICT OF INTEREST

No conflict of interests have been declared.

SOURCE OF FUNDING

This study was supported by the Program for the Promotion of Studies in Health Science of the Ministry of Health, Labour and Welfare (Tokyo, Japan). The research was conducted independently of the funding organization.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Seasonal changes in average number of patients prescribed any neuraminidase inhibitors in our four hospitals and infected with Flu per sentinel in Japan.

Figure S2 Time series plots of seasonal change in the proportion of patients prescribed oseltamivir to neuraminidase inhibitors, by age groups compared to other Japanese reported studies.

REFERENCES

1. Giezen TJ, Mantel-Teeuwisse AK, Straus SM, Schellekens H, Leufkens HG, Egberts AC. Safety-related regulatory actions for biologicals approved in the United States and the European Union. *JAMA*, 2008;**300**:1887–1896.
2. Piening S, Haaijer-Ruskamp FM, de Vries JT, van der Elst ME, de Graeff PA, Straus SM, Mol PG. Impact of safety-related regulatory action on clinical practice: a systematic review. *Drug Saf*, 2012;**35**:373–385.
3. Smalley W, Shatin D, Wysowski DK *et al.* Contraindicated use of cisapride: impact of food and drug administration regulatory action. *JAMA*, 2000;**284**:3036–3039.
4. Woosley RL. Drug labeling revisions-guaranteed to fail? *JAMA*, 2000;**284**:3047–3049.
5. Ward P, Small I, Smith J, Suter P, Dutkowski R. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J Antimicrob Chemother*, 2005;**55** (Suppl 1):i5–i21.
6. Jefferson T, Demicheli V, Rivetti D, Jones M, Di Pietrantonj C, Rivetti A. Antivirals for influenza in healthy adults: systematic review. *Lancet*, 2006;**367**:303–313.
7. Jefferson T, Jones M, Doshi P, Del Mar C. Possible harms of oseltamivir–a call for urgent action. *Lancet*, 2009;**374**:1312–1313.
8. Satoh K, Nonaka R, Ogata A, Nakae D, Uehara S. Effects of oseltamivir phosphate (Tamiflu) and its metabolite (GS4071) on monoamine neurotransmission in the rat brain. *Biol Pharm Bull*, 2007;**30**: 1816–1818.
9. Yorifuji T, Suzuki E, Tsuda T. Oseltamivir and abnormal behaviors: true or not? *Epidemiology*, 2009;**20**:619–621.
10. Ministry of Health, Labour and Welfare of Japan. Abnormal behaviors after receiving oseltamivir. 2007. Available at: http://www.mhlw.go.jp/houdou/2007/03/h0320-1.html (accessed 17 December 2013). [In Japanese]
11. Moliterno DJ, Steinhubl SR. Clopidogrel for percutaneous coronary revascularization: time for more pretreatment, retreatment, or both? *JAMA*, 2005;**294**:1271–1273.
12. Hulot JS, Bura A, Villard E *et al.* Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood*, 2006;**108**:2244–2247.
13. Trenk D, Hochholzer W, Fromm MF *et al.* Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol*, 2008;**51**:1925–1934.
14. Bhatt DL, Scheiman J, Abraham NS *et al.* ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American college of cardiology foundation task force on clinical expert consensus documents. *Circulation*, 2008;**118**: 1894–1909.
15. Gilard M, Arnaud B, Le Gal G, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated

to aspirin. *J Thromb Haemost*, 2006;**4**:2508–2509.
16. Gilard M, Arnaud B, Cornily JC *et al.* Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: the randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol*, 2008;**51**: 256–260.
17. US Food and Drug Administration. Information for Healthcare Professionals: Update to the labeling of Clopidogrel Bisulfate (marketed as Plavix) to alert healthcare professionals about a drug interaction with omeprazole (marketed as Prilosec and Prilosec OTC). 2009. Available at: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/DrugSafetyInformationforHeathcareProfessionals/ucm190787.htm (accessed 17 December 2013).
18. European Medicines Agency. Interaction between clopidogrel and proton-pump inhibitors. 2010. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2010/03/WC500076346.pdf (accessed 17 December 2013).
19. Frelinger AL 3rd, Lee RD, Mulford DJ *et al.* A randomized, 2-period, crossover design study to assess the effects of dexlansoprazole, lansoprazole, esomeprazole, and omeprazole on the steady-state pharmacokinetics and pharmacodynamics of clopidogrel in healthy volunteers. *J Am Coll Cardiol*, 2012; **59**:1304–1311.
20. Nakayama A, Morita H, Ando J, Fujita H, Ohtsu H, Nagai R. Adverse cardiovascular outcomes associated with concurrent use of clopidogrel or ticlopidine and proton-pump inhibitors in patients undergoing percutaneous coronary intervention. *Heart Vessels*, 2013;**28**:292–300.
21. Kwok CS, Loke YK. Effects of proton pump inhibitors on platelet function in patients receiving clopidogrel: a systematic review. *Drug Saf*, 2012;**35**:127–139.
22. Kwok CS, Jeevanantham V, Dawn B, Loke YK. No consistent evidence of differential cardiovascular risk amongst proton-pump inhibitors when used with clopidogrel: meta-analysis. *Int J Cardiol*, 2013;**167**:965–974.
23. Holmes MV, Perel P, Shah T, Hingorani AD, Casas JP. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA*, 2011;**306**:2704–2714.
24. Behrman RE, Benner JS, Brown JS, McClellan M, Woodcock J, Platt R. Developing the sentinel system–a national resource for evidence development. *N Engl J Med*, 2011;**364**:498–499.
25. Platt R, Carnahan RM, Brown JS *et al.* The U.S. food and drug administration's mini-sentinel program: status and direction. *Pharmacoepidemiol Drug Saf*, 2012;**21**(Suppl 1):1–8.
26. Ministry of Health, Labour and Welfare of Japan. Sentinel Project in Japan. 2010. Available at: http://www.mhlw.go.jp/stf/shingi/2r9852000000mlub-att/2r9852000000mlwj.pdf (accessed 17 December 2013). [In Japanese]
27. Kimura M, Tani S, Watanabe H *et al.* High speed clinical data retrieval system with event time sequence feature: with 10 years of clinical data of Hamamatsu University Hospital CPOE. *Methods Inf Med*, 2008;**47**:560–568.
28. Wagner AK, Soumerai SB, Zhang F, Ross-Degnan D. Segmented regression analysis of interrupted time series studies in medication use research. *J Clin Pharm Ther*, 2002;**27**:299–309.
29. Morgan OW, Griffiths C, Majeed A. Interrupted time-series analysis of regulations to reduce paracetamol (acetaminophen) poisoning. *PLoS Med*, 2007;**4**:e105.
30. SAS Institute Inc. SAS/ETS 9.3 User's Guide. 2011.
31. Stewart KA, Natzke BM, Williams T, Granger E, Casscells SW, Croghan TW. Temporal trends in anti-diabetes drug use in TRICARE following safety warnings in 2007 about rosiglitazone. *Pharmacoepidemiol Drug Saf*, 2009;**18**:1048–1052.
32. Kales HC, Zivin K, Kim HM *et al.* Trends in antipsychotic use in dementia 1999–2007. *Arch Gen Psychiatry*, 2011;**68**:190–197.
33. Serumaga B, Ross-Degnan D, Avery AJ, Elliott RA, Majumdar SR, Zhang F, Soumerai SB. Effect of pay for performance on the management and outcomes of hypertension in the United Kingdom: interrupted time series study. *BMJ*, 2011;**342**:d108.
34. Briesacher BA, Soumerai SB, Fang Z, Toh D, Andrade S, Wagner JL, Gurwitz JH. Methods to Evaluate the Impact of FDA Regulatory Actions. Mini-Sentinel Coordinating Center. 2013. Available at: http://www.mini-sentinel.org/work_products/Statistical_Methods/Mini-Sentinel_Methods_Impact-of FDA-Regulatory-Actions-White-Paper.pdf (accessed 17 December 2013).
35. Pharmaceuticals and Medical Devices Agency. Pilot study report on trends in use and impact of safety measurement using health insurance claim data 2011. Available at: http://www.info.pmda.go.jp/kyoten_iyaku/file/e_rece-report1106_001.pdf (accessed 17 December 2013). [In Japanese]
36. Urushihara H, Doi Y, Arai M *et al.* Oseltamivir prescription and regulatory actions vis-à-vis abnormal behavior risk in Japan: drug utilization study using a nationwide pharmacy database. *PLoS ONE*, 2011;**6**: e28483.
37. Ministry of Health, Labour and Welfare of Japan. Document for Central Social Insurance Medical Council. 2011. Available at: http://www.mhlw.go.jp/stf/shingi/2r9852000001qd1o-att/2r9852000001qdig.pdf (accessed 17 December 2013). [In Japanese]

