Mod Rheumatol (2008) 18:542–551
DOI 10.1007/s10165-008-0102-8

REVIEW ARTICLE

# Pro-apoptotic effect of nonsteroidal anti-inflammatory drugs on synovial fibroblasts

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Received: 24 March 2008 / Accepted: 12 June 2008 / Published online: 19 July 2008


**Abstract** Rheumatoid arthritis (RA) is a systemic inflammatory disease that mainly affects the articular synovial tissues. Although the etiology of RA has not yet been elucidated, physical and biochemical inhibition of synovial hyperplasia, which is the origin of articular destruction, may be an effective treatment for RA. Nonsteroidal anti-inflammatory drugs (NSAIDs) have long been used for the treatment of RA. The mechanism of action of NSAIDs generally involves the inhibition of cyclooxygenase (COX) at sites of inflammation. Thus, NSAIDs were not generally considered to have a so-called anti-rheumatic effect, including inhibition of progressive joint destruction and induction of remission. However, certain conventional NSAIDs and celecoxib, a selective COX-2 inhibitor, have been reported to inhibit synovial hyperplasia by inducing the apoptosis of human synovial fibroblasts. Therefore, it has been suggested that such NSAIDs may not only have an anti-inflammatory effect but also an anti-rheumatic effect. In this review, we summarize findings about the pro-apoptotic effect, in other words, anti-proliferative effect of NSAIDs on synovial fibroblasts from patients with RA.

**Keywords** Apoptosis · NSAIDs · Celecoxib · Synovial fibroblasts

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## Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that mainly affects the articular synovial tissues. It is thought that an autoimmune response to the synovium is induced in RA patients when genetic factors are combined with various environmental ones, resulting in the occurrence of chronic inflammation. However, the etiology of RA has not yet been elucidated.

Guidelines for the management of RA by American College of Rheumatology have been published [1] and were also prepared in 2004 by a research group of the Japanese Ministry of Health, Labour and Welfare [2]. These guidelines cover a wide variety of drugs, including the new biological preparations, as well as glucocorticoids, disease-modifying anti-rheumatic drugs (DMARDs), and nonsteroidal anti-inflammatory drugs (NSAIDs) that have long been used for the treatment of rheumatic diseases [3]. Although the role of NSAIDs in the treatment of RA has been decreasing, these are still convenient drugs to employ for their anti-inflammatory and analgesic effects.

The mechanism of action of NSAIDs generally involves the inhibition of cyclooxygenase (COX) at sites of inflammation. As a result, these drugs exhibit a therapeutic effect by inhibiting the production of inflammatory mediators known as prostaglandins (PGs), including $PGE_2$ and $PGI_2$. In the treatment of RA, NSAIDs are used as symptomatic therapy with analgesic and anti-inflammatory effects mediated via the inhibition of COX. However, other mechanisms of action of NSAIDs except for COX inhibition have also been discussed [3]. For instance, certain NSAIDs have been reported to inhibit inflammation by suppression of nuclear factor (NF)-$\kappa$B due to I$\kappa$B-kinase $\beta$ inhibition in mononuclear cells [4]. In this review, we summarize data about the

pro-apoptotic effect of NSAIDs on synovial fibroblasts of patients with RA.

## Synovial proliferation and apoptosis

Healthy synovial tissue is essential for normal joint function. In RA, hyperplasia of the synovium and formation of granulation tissue (pannus) occur together with the infiltration of inflammatory cells, such as T cells and macrophages (Fig. 1). Activated pannus may eventually cause the destruction of bone and cartilage via the release of various mediators, including inflammatory cytokines such as interleukin (IL)-1$\beta$ and tumor necrosis factor (TNF)-$\alpha$ [5]. Synovial hyperplasia may be due to an increase in the proliferation of cells composing the pannus. This has been suggested by detection of the increased expression of various markers of proliferation and growth factors, including platelet-derived growth factor, basic fibroblast growth factor, and transforming growth factor-$\beta$ [6]. Another reason for the formation of pannus might be a decrease of apoptosis [7]. Physical and biochemical inhibition of synovial hyperplasia, which is the initial factor leading to articular destruction, may be effective treatments for RA [8].

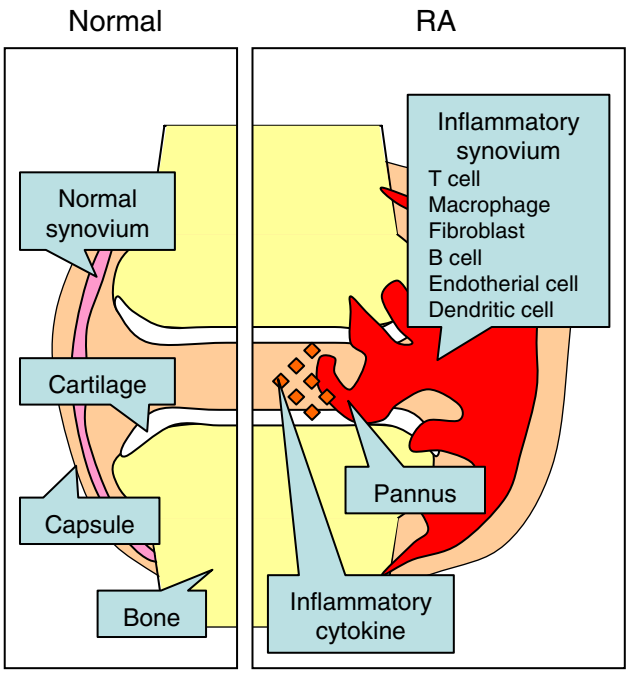



**Fig. 1** Synovial proliferation in rheumatoid arthritis (RA). In articular joint of RA, hyperplasia of the synovium and formation of granulation tissue (pannus), which were composed of inflammatory cells, such as T cells, macrophages, and B cells, secrete inflammatory mediators to articular cavity

Several molecules, which induce apoptosis in vitro have been reported as preventing agents in in vivo experimental models of RA. Peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) is an intranuclear transcription factor that promotes differentiation of adipocytes [9]. It also inhibits production of proinflammatory cytokines by macrophage [10]. On the other hand, Kawahito et al. [11] reported that articular destruction is significantly inhibited by administration of 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$), an endogenous ligand of PPAR$\gamma$, by induction of apoptosis on synovial fibroblasts in experimental arthritis of rats. Administration of an anti-Fas antibody that also induces apoptosis inhibits arthritis in mice [12, 13]. Stimulation of RA synovial fibroblasts by macrophage inhibitory factor (MIF) reduces apoptosis, while MIF knock-out mice have less severe arthritis due to increased apoptosis in the synovium [14]. Although these findings suggest the possibility of achieving an anti-rheumatic effect by inhibiting hyperplasia of the synovial tissues, there have been no clinical studies of pro-apoptotic agents such as PPAR$\gamma$ ligands and anti-Fas antibodies targeting this endpoint.

## Pro-apoptotic action of NSAIDs on synovial fibroblasts

### Conventional NSAIDs

First, we found that some of the conventional NSAIDs (inhibitors both of COX-1 and COX-2), indomethacin, diclofenac, oxaprozin, and zaltoprofen, all inhibited the proliferation of RA synovial fibroblasts by the induction of apoptosis, which was confirmed by detection of DNA fragmentation [15] (Fig. 2). On the other hand, ketoprofen, acetaminophen, and NS-398, a selective COX-2 inhibitor, did not induce apoptosis of RA synovial fibroblasts. Since PPAR$\gamma$ ligands such as 15d-$PGJ_2$ and troglitazone have a pro-apoptotic effect on synovial fibroblasts [11], we hypothesized that the mechanism of these pro-apoptotic NSAIDs was mediated by PPAR$\gamma$ activation.

Some conventional NSAIDs, such as ibuprofen, indomethacin, flufenamic acid, and fenoprofen, are known to cause the transcriptional activation of PPAR$\gamma$ in C3H10T1/2 mouse fibroblast cells [16]. In addition, activation of PPAR$\gamma$ was obtained by ibuprofen, indomethacin, and fenoprofen in human monocytes [10]. Kawahito et al. [11] reported that 15d-$PGJ_2$, an endogenous ligand of PPAR$\gamma$, ameliorate experimental arthritis of rats. Therefore, we investigated the effect of NSAIDs on PPAR$\gamma$ activity in RA synovial fibroblasts, one of the target cells in RA (Fig. 3). PPAR$\gamma$ activation was measured by luciferase reporter gene assay. Indomethacin, diclofenac, oxaprozin, and zaltoprofen induced PPAR$\gamma$ activation, while ketoprofen, acetaminophen, and NS-398,

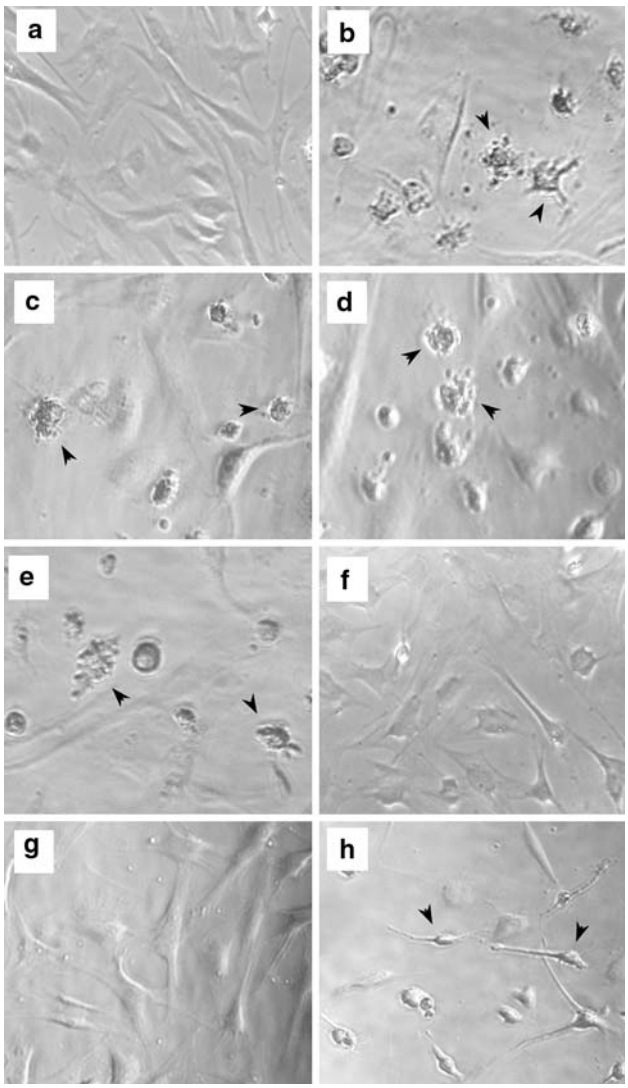



**Fig. 2** Morphology of NSAID-treated rheumatoid synovial fibroblasts. Rheumatoid synovial cells were untreated (**a**) or treated with 300 μM indometacin (**b**), 100 μM diclofenac (**c**), 300 μM oxaprozin (**d**), 300 μM zaltoprofen (**e**), 300 μM ketoprofen (**f**), 300 μM acetaminophen (**g**), or 300 μM NS-398 (**h**) for 24 h. Cell morphology was observed with a light microscope. *Arrows* indicate representative morphological changes of synovial fibroblasts (×60). Reprinted from Yamazaki et al. [15], with kind permission from American Society for Pharmacology and Experimental Therapeutics

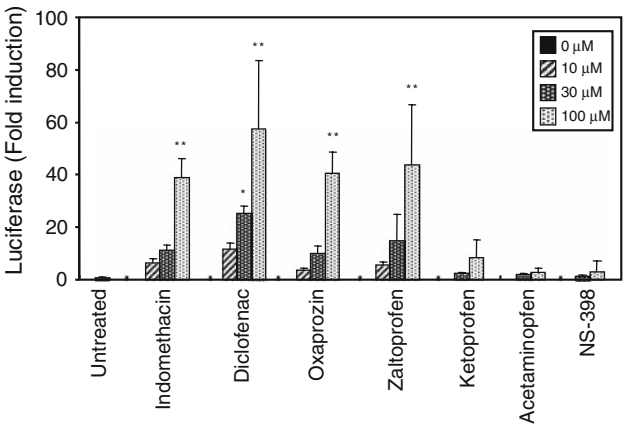



**Fig. 3** Effect of NSAIDs on activation of peroxisome proliferator-activated receptor γ (PPARγ) in rheumatoid synovial cells. Rheumatoid synovial cells were cotransfected with a PPAR response element-driven luciferase reporter plasmid, PPARγ expression plasmid, and internal control plasmid. The transfected cells were treated with NSAIDs for 18 h. The fold-induction of luciferase activity is relative to untreated control cells. Data are the mean ± SD for triplicate cultures. Results are representative of three independent experiments. $*P < 0.05$ and $**P < 0.001$ versus untreated control cells. Reprinted from Yamazaki et al. [15], with kind permission from American Society for Pharmacology and Experimental Therapeutics

which do not induce apoptosis of RA synovial fibroblasts, did not promote PPARγ activation. Furthermore, the ability of NSAIDs and PPARγ ligands to stimulate the activation of PPARγ correlated with their ability to decrease cell viability and ability to induce DNA fragmentation in synovial fibroblasts.

Then we studied sodium salicylate and aspirin, which are the historical NSAIDs, to assess their apoptosis-inducing effect on RA synovial fibroblasts [17]. At relatively higher concentrations comparable to those that cause COX inhibition, sodium salicylate and aspirin induced apoptosis of these cells. It was also suggested that these drugs induced apoptosis via a mechanism independent of COX inhibition. Sodium salicylate and aspirin are known as potent inhibitors of the transcription factor NF-κB [18], and it has been shown that inhibition of the NF-κB pathway by pyrrolidinedithiocarbamate or N-acetylcysteine is linked to the induction of apoptosis in a variety of cells [19–21]. However, our additional study revealed that these inhibitors of NF-κB did not cause apoptosis of RA synovial fibroblasts [17]. Moreover, salicylates did not promote the activation of PPARγ in our experiments, so the mechanism of their pro-apoptotic effects on RA synovial fibroblasts is still unknown.

## Celecoxib

COX-2 is an isozyme of COX that is markedly induced by inflammatory stimuli and is considered to be closely related to the process of inflammation. Celecoxib, which selectively inhibits COX-2, was developed by investigating the 3D structure of COX-2. In addition to inhibition of COX-2, celecoxib has been reported to inhibit the proliferation of various cancer cells, mainly by inducing apoptosis [22]. We investigated the effect of selective COX-2 inhibitors on apoptosis in RA synovial fibroblasts [23] (Fig. 4). Among six selective COX-2 inhibitors (celecoxib, etodolac, meloxicam, nimesulide, NS-398, and rofecoxib), only celecoxib induced the apoptosis of RA synovial fibroblasts, whereas the other COX-2 inhibitors did not. This indicated

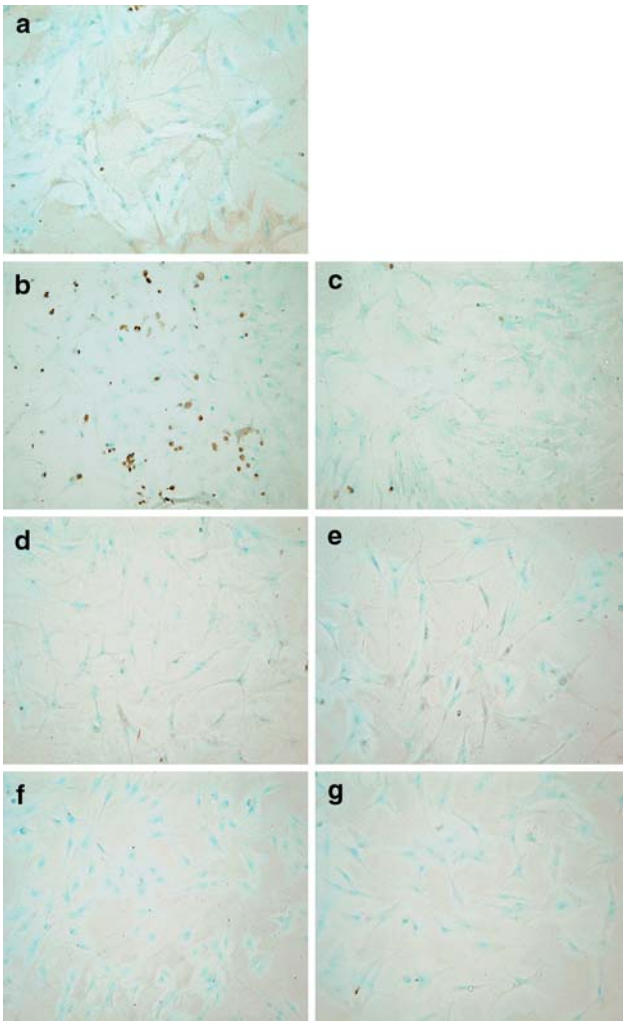



**Fig. 4** Detection of apoptosis in RA synovial fibroblasts, by the TUNEL assay. Cells were incubated for 24 h. **a** Without any COX-2 inhibitors or with **b** celecoxib (40 μM), **c** etodolac (100 μM), **d** meloxicam (100 μM), **e** nimesulide (100 μM), **f** NS-398 (100 μM), or **g** rofecoxib (100 μM). Apoptotic cells exhibiting TUNEL staining are brown; normal cells counterstained with methyl green are blue (×200). Reprinted from Kusunoki et al. [23], with kind permission from John Wiley & Sons, Inc.

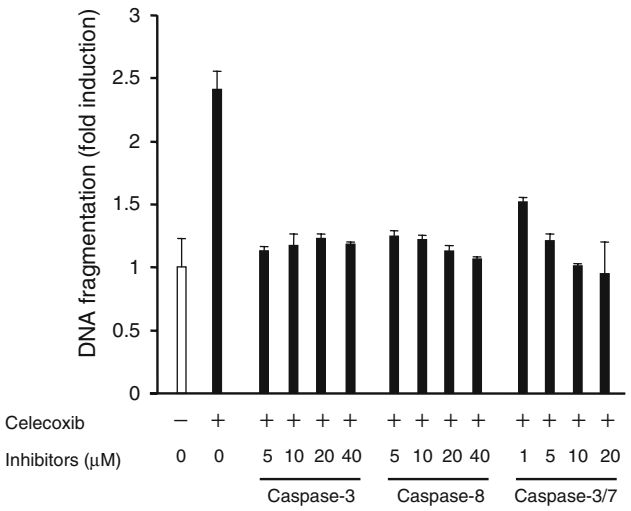



**Fig. 5** Effect of caspase inhibitors on celecoxib-induced DNA fragmentation in RA synovial fibroblasts. Cells were incubated with celecoxib (40 μM) and with caspase inhibitors [Z-DEVD-FMK (a caspase-3 inhibitor), Z-IETD-FMK (a caspase-8 inhibitor), and Z-VAD-FMK (a caspase-3/7 inhibitor)] for 24 h at the indicated concentrations, after which DNA fragments in the cytoplasm were measured by enzyme immunoassay. The fold-induction of DNA fragmentation is shown relative to the control value (untreated cells). Representative results from two independent experiments are shown; values are the mean and SD from triplicate cultures. Reprinted from Kusunoki et al. [23], with kind permission from John Wiley & Sons, Inc.

that the pro-apoptotic effect of celecoxib on RA synovial fibroblasts was independent of COX-2 inhibition. This pro-apoptotic effect was suppressed by caspase inhibitors (Fig. 5). In addition, celecoxib did not cause transcriptional activation of PPAR$\gamma$ in RA synovial fibroblasts.

Epidemiological studies have shown that chronic intake of aspirin is associated with a reduction in the incidence of colorectal cancer [24]. NSAIDs have also been shown to exert a pro-apoptotic effect on various cell lines, particularly colon cancer cells [25]. We previously investigated the pro-apoptotic effect of six selective COX-2 inhibitors indicated above on human colorectal cancer cells, and found that only celecoxib induced apoptosis again, which was induced via a mechanism that was unrelated to COX inhibition [26]. We found that celecoxib reduced the phosphorylated Akt, an anti-apoptotic molecule, in colon cancer cell lines [26]. Several NSAIDs such as indomethacin [27], dicrofenac [28], salicylic acid [29], etodolac [30], nimesulide [31], and NS-398 [32] inhibited Akt activation in vitro experiments using cancer cell lines. Celecoxib alters intracellular calcium by inhibiting $Ca^{2+}$ ATPases in the endoplasmic reticulum [33], and blocks TNF-induced activation of NF-$\kappa$B [34]. However, these intracellular changes induced by NSAIDs were not consistent among several different cell types. The mechanisms of pro-apoptotic effects of NSAIDs on cancer cells as well as synovial fibroblasts are still remained to be studied.

## TT101, a new derivative of celecoxib

Although celecoxib suppressed the proliferation of RA synovial fibroblasts and induced apoptosis at the optimal concentrations were higher (10–40 μM) compared with those for COX-2 inhibition (0.01–10 μM) [23]. The mean maximum plasma concentration of celecoxib in healthy volunteers was reported to be 1.4, 2.5, and 7.7 μM after single doses of 100, 400, and 800 mg, respectively [35], showing that insufficient concentrations for pro-apoptotic effect on RA synovial tissue. Therefore, we tried to develop potent inducer of apoptosis by modification of the

**Table 1** Properties of bioactivities of several NSAIDs

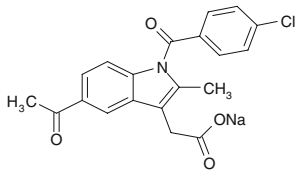
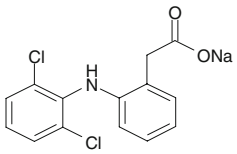
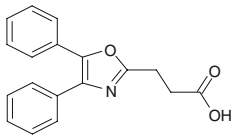
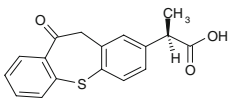
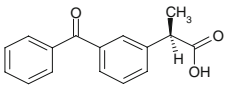
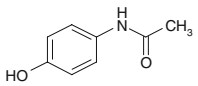
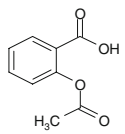
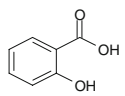
| NSAIDs | Structure | Pro-apoptotic effect on RSF | Activation of PPARγ | Inhibitory effect of Akt activation | COX-1 $IC_{50}$ (μM) | COX-2 $IC_{50}$ (μM) | COX-1/ COX-2 ratio |
|---|---|---|---|---|---|---|---|
| Indomethacin |  | ++ [15] | + [15] | + [27] | 0.013 [15] | 0.044 [15] | 0.30 |
| Diclofenac |  | ++ [15] | + [15] | + [28] | 0.076 [44] | 0.026 [44] | 2.92 |
| Oxaprozin |  | ++ [15] | + [15] | NT | 2.2 [15] | 36 [15] | 0.061 |
| Zaltoprofen |  | ++ [15] | + [15] | NT | 1.3 [15] | 0.34 [15] | 3.82 |
| Ketoprofen |  | − [15] | − [15] | − [38] | 0.11 [15] | 0.88 [15] | 0.13 |
| Acetaminofen |  | − [15] | − [15] | − [39] | 42 [15] | 11 [15] | 3.81 |
| Aspirin |  | ++ [17] | − [17] | − [38] | 1.7 [45] | 7.5 [45] | 0.23 |
| Salicilic acid |  | ++ [17] | − [17] | + [29] | 4,956 [45] | 34,440 [45] | 0.14 |

**Table 1** continued

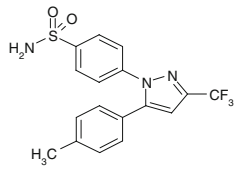
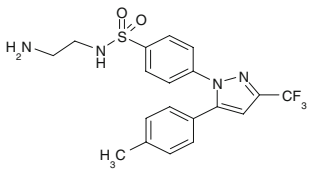
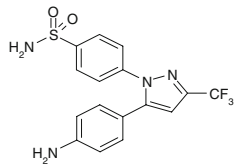
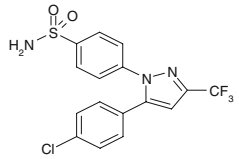
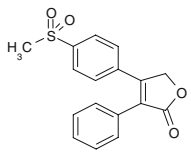
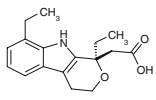
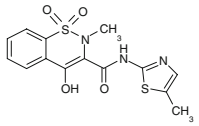
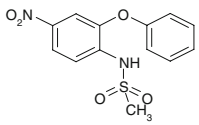
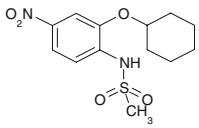
| NSAIDs | Structure | Pro-apoptotic effect on RSF | Activation of PPARγ | Inhibitory effect of Akt activation | COX-1 $IC_{50}$ (μM) | COX-2 $IC_{50}$ (μM) | COX-1/ COX-2 ratio |
|---|---|---|---|---|---|---|---|
| Celecoxib |  | ++ [23, 36] | − [23] | + [26] | 82 [44] | 0.0032 [36] | 25,625 |
| TT101 |  | +++ [36] | NT | − [36] | NT | 0.31 [36] | NT |
| TT201 |  | + [36] | NT | − [36] | NT | 0.13 [36] | NT |
| SC236 |  | ++ [36] | + [40] | + [41] | NT | 0.0071 [36] | NT |
| Rofecoxib |  | − [23] | NT | NT | >100 [44] | 0.048 [36] | >2,083 |
| Etodolac |  | − [23] | NT | + [30] | >100 [44] | 53 [44] | >1.89 |
| Meloxicam |  | − [23] | − [42] | NT | 37 [44] | 6.1 [44] | 6.07 |

**Table 1** continued

| NSAIDs | Structure | Pro-apoptotic effect on RSF | Activation of PPARγ | Inhibitory effect of Akt activation | COX-1 $IC_{50}$ (μM) | COX-2 $IC_{50}$ (μM) | COX-1/ COX-2 ratio |
|---|---|---|---|---|---|---|---|
| Nimesulide |  | − [23] | − [43] | + [31] | 10 [45] | 1.9 [45] | 5.26 |
| NS-398 |  | − [15, 23, 36] | − [15, 23] | + [32] | 125 [44] | 0.012 [36] | 10,416 |

*NT* not tested

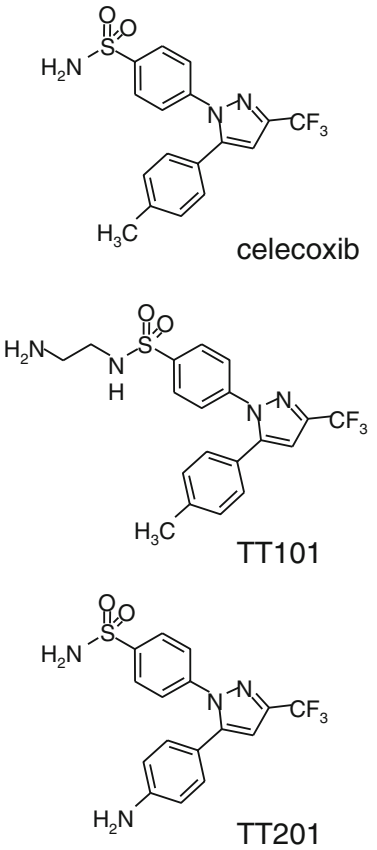



**Fig. 6** Chemical structure of celecoxib and its derivatives

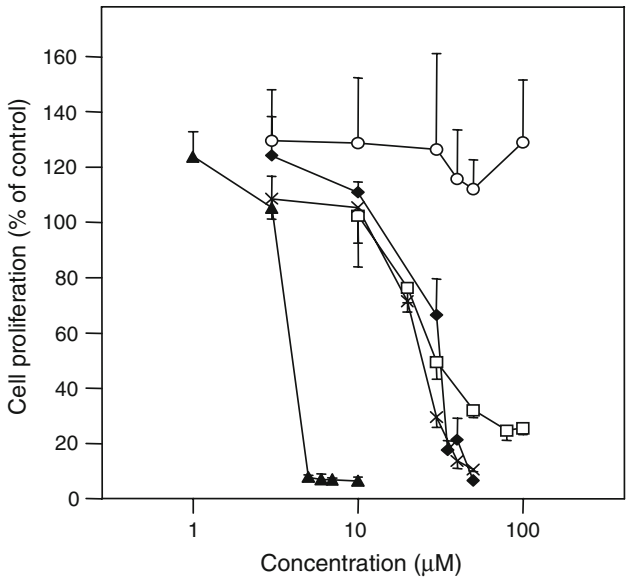



**Fig. 7** Effect of the each drug on the proliferation of synovial fibroblasts obtained from patients with RA. Cells were incubated with celecoxib (*closed diamonds*), TT101 (*closed triangles*), TT201 (*open squares*), SC-236 (*crosses*), or rofecoxib (*open circles*) for 24 h. Then proliferative activity was estimated from the nuclear incorporation of BrdU and was expressed as a percentage of the control value (untreated cells). Data are the mean ± SD for triplicate cultures, and representative results from three independent experiments are shown. Reprinted from Kusunoki et al. [36], with kind permission from American Society for Pharmacology and Experimental Therapeutics

structure of celecoxib. We synthesized two celecoxib derivatives (TT101 and TT201) and analyzed their pro-apoptotic effect on RA synovial fibroblasts [36].

We summarized properties of several NSAIDs from the view point of bioactivities and structures (Table 1). The sulfonamide group of celecoxib was changed to an N-(2-aminoethyl)-sulfonamide group when developing TT101, whereas the tolyl group in the terminal aromatic ring of celecoxib was changed to an aminophenyl group to create TT201 (Fig. 6). Interestingly, TT101 was more potent with respect to suppression of hyperplasia (Fig. 7) and induction of apoptosis (Fig. 8) in RA synovial fibroblasts when compared to celecoxib. NSAIDs without sulfonamide group,

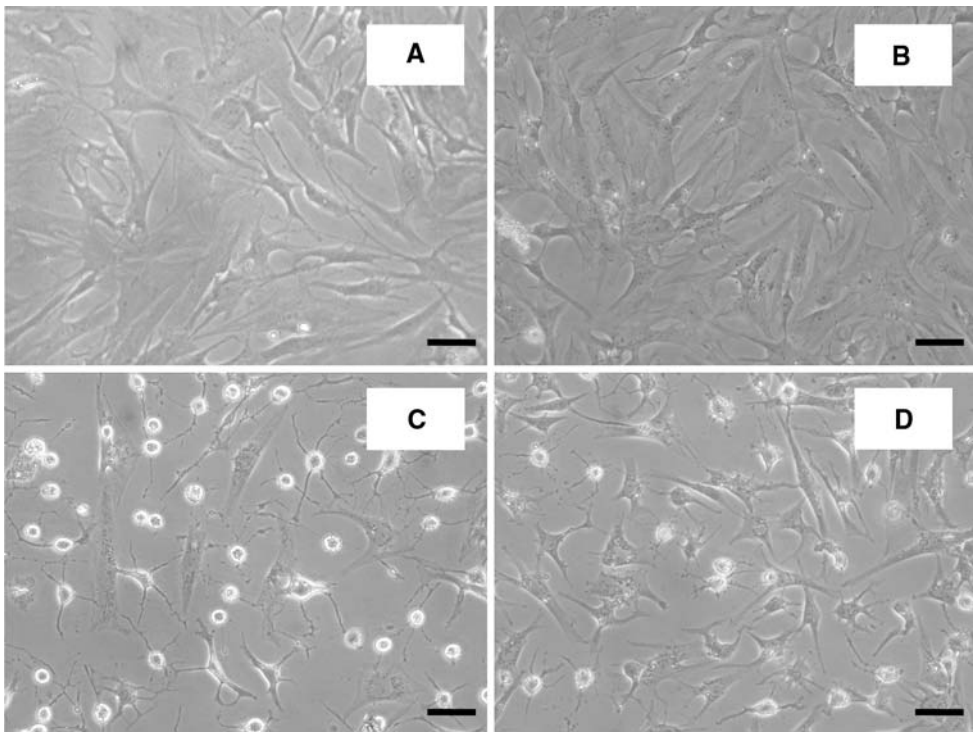



**Fig. 8** Morphological changes of the synovial fibroblasts from RA patients (**a** and **c**) or osteoarthritis patients (**b** and **d**) as observed by light microscopy. Cells were incubated for 24 h without (**a** and **b**) or with (**c** and **d**) TT101 at a concentration of 7 μM. *Bar* 60 μm. Reprinted from Kusunoki et al. [36], with kind permission from American Society for Pharmacology and Experimental Therapeutics

such as indomethacin, diclofenac, oxaprozin, zaltoprofen, also induced apoptosis in RA synovial fibroblasts. However, they activated PPAR$\gamma$ in the cells, while celecoxib did not. A pro-apoptotic effect of TT201 was weaker than that of celecoxib. Therefore, conformations of TT101 and celecoxib except sulfonamide group are possibly important to maintain pro-apoptotic effect. We also measured the COX-2 inhibitory effect of these compounds in RA synovial fibroblasts and found that the order of potency for the COX-2 inhibition by these drugs was celecoxib > TT201 > TT101 [36]. The potent pro-apoptotic effect of TT101 was also observed in colon cancer cell lines [37]. Although the mechanism of action of TT101 remains unclear, it may have potential as a novel anti-proliferation drug for rheumatoid synovial fibroblasts and colon cancer cells.

## Conclusion

Pro-apoptotic effects of NSAIDs including conventional NSAIDs, celecoxib and a derivative of celecoxib were reviewed. Although additional studies are needed, these results suggest that the induction of apoptosis caused by some NSAIDs may help to prevent the degradation of articular cartilage in RA after the inhibition of synovial hyperplasia and pannus formation.

**Acknowledgments** This work was supported in part by research grants from The Japanese Ministries of Education, Culture, Sports, Science and Technology (no. 17590477 to S.K.) and Health, Labour and Welfare (no. H17-Im-008 to S.K.), and by a Research Promotion grant from Toho University Graduate School of Medicine (no. 07–04 to S.K.). We thank Dr. Hiroshi Handa (Tokyo Institute of Technology, Yokohama, Japan) for providing TT101 and TT201. We also thank Dr. Fumiaki Kojima (University of Kentucky, Lexington, KY, USA) for suggestions about the methodology of the experiments, as well as Ms. Sonoko Sakurai for secretarial assistance.

**Competing interests** S.K. has served as consultants to and/or received honoraria from Pfizer Japan (Tokyo), the manufacture relatives of celecoxib, and Astellas Pharma (Tokyo), the selling company of celecoxib. N.K. and S.K. hold a patent for TT101 and TT201. R.Y. has no competing interests.

## References

1. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 update. Arthritis Rheum. 2002;46:328–46.
2. A research group of the Japanese Ministry of Health, Labour and Welfare. Guidelines for treatment of rheumatoid arthritis (in Japanese). In: Ochi T, Yamamoto K, Ryu J, editors. Diagnostic manual and guideline for the treatment of rheumatoid arthritis based on EBM. Tokyo: Japan Rheumatism Foundation; 2004. p. 51–104.
3. Kawai S. Current drug therapy for rheumatoid arthritis. J Orthop Sci. 2003;8:259–63.
4. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature. 1998;396:77–80.
5. Goldring SR. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheumatology (Oxford). 2003;42(Suppl 2):ii11–6.

6. Karouzakis E, Neidhart M, Gay RE, Gay S. Molecular and cellular basis of rheumatoid joint destruction. Immunol Lett. 2006;106:8–13.
7. Baier A, Meineckel I, Gay S, Pap T. Apoptosis in rheumatoid arthritis. Curr Opin Rheumatol. 2003;15:274–9.
8. Ospelt C, Neidhart M, Gay RE, Gay S. Synovial activation in rheumatoid arthritis. Front Biosci. 2004;9:2323–34.
9. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell. 1995;83: 803–12.
10. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature. 1998;391:82–6.
11. Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue K, et al. 15-deoxy-delta(12, 14)-PGJ(2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest. 2000;106:189–97.
12. Fujisawa K, Asahara H, Okamoto K, Aono H, Hasunuma T, Kobata T, et al. Therapeutic effect of the anti-Fas antibody on arthritis in HTLV-1 tax transgenic mice. J Clin Invest. 1996; 98:271–8.
13. Peng SL. Fas (CD95)-related apoptosis and rheumatoid arthritis. Rheumatology (Oxford). 2006;45:26–30.
14. Lacey D, Sampey A, Mitchell R, Bucala R, Santos L, Leech M, et al. Control of fibroblast-like synoviocyte proliferation by macrophage migration inhibitory factor. Arthritis Rheum. 2003;48:103–9.
15. Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S, Kawai S. Nonsteroidal anti-inflammatory drugs induce apoptosis in association with activation of peroxisome proliferator-activated receptor gamma in rheumatoid synovial cells. J Pharmacol Exp Ther. 2002;302:18–25.
16. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem. 1997;272:3406–10.
17. Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S, Kawai S. Aspirin and sodium salicylate inhibit proliferation and induce apoptosis in rheumatoid synovial cells. J Pharm Pharmacol. 2002;54:1675–9.
18. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science. 1994;265:956–9.
19. Tsai JC, Jain M, Hsieh CM, Lee WS, Yoshizumi M, Patterson C, et al. Induction of apoptosis by pyrrolidinedithiocarbamate and N-acetylcysteine in vascular smooth muscle cells. J Biol Chem. 1996;271:3667–70.
20. Wu M, Lee H, Bellas RE, Schauer SL, Arsura M, Katz D, et al. Inhibition of NF-kappa B/Rel induces apoptosis of murine B cells. EMBO J. 1996;15:4682–90.
21. Ozaki K, Takeda H, Iwahashi H, Kitano S, Hanazawa S. NF-kappa B inhibitors stimulate apoptosis of rabbit mature osteoclasts and inhibit bone resorption by these cells. FEBS Lett. 1997;410:297–300.
22. Kismet K, Akay MT, Abbasoglu O, Ercan A. Celecoxib: a potent cyclooxygenase-2 inhibitor in cancer prevention. Cancer Detect Prev. 2004;28:127–42.
23. Kusunoki N, Yamazaki R, Kawai S. Induction of apoptosis in rheumatoid synovial fibroblasts by celecoxib, but not by other selective cyclooxygenase 2 inhibitors. Arthritis Rheum. 2002;46: 3159–67.
24. Thun MJ, Namboodiri MM, Heath CW Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med. 1991;325:1593–6.
25. de Groot DJ, de Vries EG, Groen HJ, de Jong S. Non-steroidal anti-inflammatory drugs to potentiate chemotherapy effects: from lab to clinic. Crit Rev Oncol Hematol. 2007;61:52–69.
26. Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S, Kawai S. Selective cyclooxygenase-2 inhibitors show a differential ability to inhibit proliferation and induce apoptosis of colon adenocarcinoma cells. FEBS Lett. 2002;531:278–84.
27. Chun KS, Akunda JK, Langenbach R. Cyclooxygenase-2 inhibits UVB-induced apoptosis in mouse skin by activating the prostaglandin E2 receptors, EP2 and EP4. Cancer Res. 2007;67:2015–21.
28. Inoue A, Muranaka S, Fujita H, Kanno T, Tamai H, Utsumi K. Molecular mechanism of diclofenac-induced apoptosis of promyelocytic leukemia: dependency on reactive oxygen species, Akt, Bid, cytochrome and caspase pathway. Free Radic Biol Med. 2004;37:1290–9.
29. Derouet M, Thomas L, Moulding DA, Akgul C, Cross A, Moots RJ, et al. Sodium salicylate promotes neutrophil apoptosis by stimulating caspase-dependent turnover of Mcl-1. J Immunol. 2006;176:957–65.
30. Cheng J, Imanishi H, Liu W, Nakamura H, Morisaki T, Higashino K, et al. Involvement of cell cycle regulatory proteins and MAP kinase signaling pathway in growth inhibition and cell cycle arrest by a selective cyclooxygenase 2 inhibitor, etodolac, in human hepatocellular carcinoma cell lines. Cancer Sci. 2004;95:666–73.
31. Baoping Y, Guoyong H, Jieping Y, Zongxue R, Hesheng L. Cyclooxygenase-2 inhibitor nimesulide suppresses telomerase activity by blocking Akt/PKB activation in gastric cancer cell line. Dig Dis Sci. 2004;49:948–53.
32. Honjo S, Osaki M, Ardyanto TD, Hiramatsu T, Maeta N, Ito H. COX-2 inhibitor, NS398, enhances Fas-mediated apoptosis via modulation of the PTEN-Akt pathway in human gastric carcinoma cell lines. DNA Cell Biol. 2005;24:141–7.
33. Johnson AJ, Hsu AL, Lin HP, Song X, Chen CS. The cyclooxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum $Ca^{2+}$-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks. Biochem J. 2002;366:831–7.
34. Shishodia S, Koul D, Aggarwal BB. Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-kappa B activation through inhibition of activation of I kappa B alpha kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis. J Immunol. 2004;173:2011–22.
35. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA. 1999;96: 272–7.
36. Kusunoki N, Ito T, Sakurai N, Suguro T, Handa H, Kawai S. A novel celecoxib derivative potently induces apoptosis of human synovial fibroblasts. J Pharmacol Exp Ther. 2005;314:796–803.
37. Kusunoki N, Ito T, Sakurai N, Handa H, Kawai S. A celecoxib derivative potently inhibits proliferation of colon adenocarcinoma cells by induction of apoptosis. Anticancer Res. 2006;26:3229–36.
38. Lee HC, Park IC, Park MJ, An S, Woo SH, Jin HO, et al. Sulindac and its metabolites inhibit invasion of glioblastoma cells via down-regulation of Akt/PKB and MMP-2. J Cell Biochem. 2005;94:597–610.
39. Bae MA, Pie JE, Song BJ. Acetaminophen induces apoptosis of C6 glioma cells by activating the c-Jun NH(2)-terminal protein kinase-related cell death pathway. Mol Pharmacol. 2001;60: 847–56.
40. Planagumà A, Clària J, Miquel R, López-Parra M, Titos E, Masferrer JL, et al. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPARgamma activation. FASEB J. 2005;19:1120–2.

41. Lee A, Frischer J, Serur A, Huang J, Bae JO, Kornfield ZN, et al. Inhibition of cyclooxygenase-2 disrupts tumor vascular mural cell recruitment and survival signaling. Cancer Res. 2006;66:4378–84.
42. Taketa K, Matsumura T, Yano M, Ishii N, Senokuchi T, Motoshima H, et al. Oxidized low density lipoprotein activates peroxisome proliferator-activated receptor-{alpha} (PPAR{alpha}) and PPAR{gamma} through MAPK-dependent COX-2 expression in macrophages. J Biol Chem. 2008;283:9852–62.
43. Hollingshead HE, Borland MG, Billin AN, Willson TM, Gonzalez FJ, Peters JM. Ligand activation of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) and inhibition of cyclooxygenase 2 (COX2) attenuate colon carcinogenesis through independent signaling mechanisms. Carcinogenesis. 2008;29:169–76.
44. Kato M, Nishida S, Kitasato H, Sakata N, Kawai S. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes. J Pharm Pharmacol. 2001;53:1679–85.
45. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci USA. 1999;96:7563–8.