CHAPTER 20

CHEMOPREVENTION OF COLORECTAL CANCER BY ANTI-INFLAMMATORY AGENTS

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In recent years, colorectal cancer has increasingly become a major cause of cancer mortality in advanced countries, including Japan. Therefore, elucidation of the mechanisms of colorectal carcinogenesis and the search for chemopreventive agents are important and urgent tasks. Since chronic inflammatory status and associated changes, such as elevation of production of cytokines and growth factors, appear to predispose to cancer development in any site of the body, the metabolic pathways that are switched on under such conditions might be good targets for chemopreventive agents.

In human colorectal cancer tissue, overexpression of enzymes associated with inflammation, such as inducible cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) have been reported (1,2). Thus, it is suggested that their reaction products, prostaglandin E_2 (PGE_2) and nitric oxide (NO), might contribute to the development of colorectal cancer. To date, several mechanisms involved in colorectal neoplasia have been clarified. K-*ras* mutations contribute to the induction of hyperplastic changes (3). Mutated K-*ras* activates the mitogen-activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI3K)/Akt pathways and results in cyclin D1 and COX-2 overexpression, which in turn may induce iNOS expression in the presence of inflammatory stimuli (4,5). Overexpressed COX-2 produces excess prostaglandins (PGs) and causes cell proliferation and inhibition of apoptosis, to some extent mediated by PGE_2 receptor subtypes EP1, EP2, and EP4 (6).

Adenomatous polyposis coli (APC) or β-*catenin* mutations appear to be involved in the generation of dysplastic lesions (3), stabilizing β-catenin protein in the cytoplasm and activating β-catenin/Tcf signaling to up-regulate target genes, such as cyclin D1 (7). β-Catenin alteration is suggested to be involved in increased expression of iNOS (4). NO produced by iNOS causes DNA damage and neovascularization, which promotes carcinogenesis. Moreover, NO itself could induce COX-2 expression (8).

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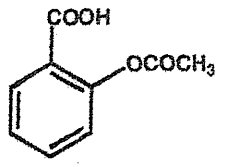
With elucidation of the mechanisms of colorectal carcinogenesis, a great deal of interest has been concentrated on anti-inflammatory agents, including nonsteroidal anti-inflammatory drugs (NSAIDs), which act by inhibiting COX enzymes. Screening of anti-inflammatory agents as potential colorectal cancer chemopreventive agents has been carried out using several *in vivo* animal models. The majority feature application of azoxymethane (AOM), a very potent carcinogen that induces colorectal cancers at high incidence in rats and mice. Short-term treatment with AOM results in the development of putative preneoplastic aberrant crypt foci (ACF). Such biomarker lesions are thought to be useful surrogates for tumors in assessing the effects of agents capable of preventing carcinogenesis in the colon (9,10). Furthermore, the *Apc* gene–deficient mouse, an animal model of human familial adenomatous polyposis (FAP) characterized by large numbers of intestinal polyps because of a truncation mutation in the *Apc*, is also a useful model to evaluate cancer chemopreventive agents. Indeed, many FAP model mice, such as Apc^{1309} (C57BL/6J$^{Apc/ApcD1309}$) (mutation at codon 1309; develop ca.35 polyps/mouse), Apc^{Min} (*Min*) (mutation at codon 850; develop ca.100 polyps/mouse), Apc^{D716} (mutation at codon 716; develop ca.300 polyps/mouse), and Apc^{1638} (mutation at codon 1638; develop ca.10 polyps/mouse) strains are now used world wide (11–15).

In this chapter we aim to provide a summary of this field of research with attention to possible mechanisms of action and potential application of anti-inflammatory agents for practical cancer prevention.

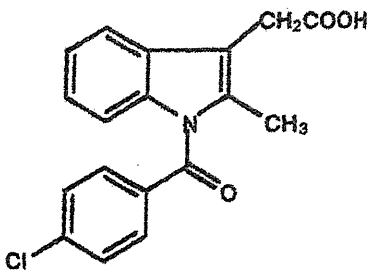
ANTI-INFLAMMATORY AGENTS TARGETING CYCLOOXYGENASE

A large number of epidemiological studies have indicated that NSAIDs can reduce the risk of colorectal cancer. For example, people who take aspirin regularly demonstrate at most a 40% reduction in the relative risk of colorectal cancer and associated mortality (16). Furthermore, celecoxib, a COX-2-selective inhibitor, and indomethacin and sulindac, conventional NSAIDs (Figure 20.1) that inhibit both COX-1 and COX-2, can actually cause regression of existing colorectal polyps in patients with FAP (17–19). Although there are several molecular mechanisms assumed to be involved in the reduction of colorectal cancer by NSAIDs, such as inactivation of Akt, activation of AMP-activated protein kinase (AMPK), and inhibition of transcription factor nuclear factor-κB (NF-κB), the most likely possibility is linked directly to their inhibition of COX (20–22).

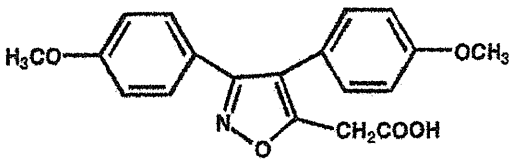
Prostanoid synthesis starts with release of arachidonic acid (AA) from cell membrane phospholipids, mediated primarily via the action of phospholipase A_2 (6). COX then catalyzes the conversion of AA to PGG_2 and under the influence of peroxidase activity of the enzyme, this is rapidly converted to PGH_2. There are two isoforms of COX: the constitutive enzyme, COX-1, present in many cells and tissues, and the inducible enzyme, COX-2, produced in response to growth factors, mitogens, and pro-inflammatory cytokines. PGH_2 is additionally isomerized to PGE_2, PGD_2, PGF_2, PGI_2, and thromboxane A_2 by their respective PG synthases (6). Nonenzymatic



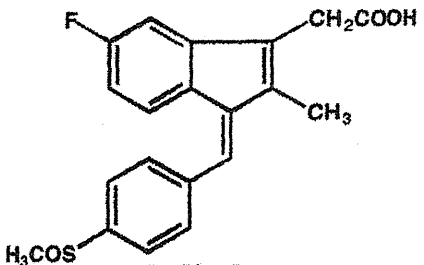
Aspirin



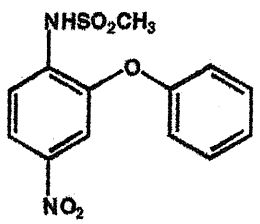
Indomethacin



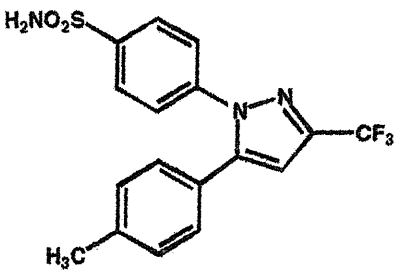
Mofezolac



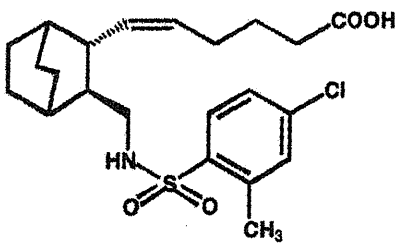
Sulindac



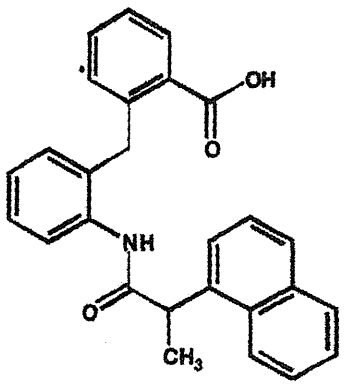
Nimesulide



Celecoxib



ONO-8711



ONO-AE2-227

FIGURE 20.1 Structures of COX-1 and COX-2 inhibitors.

dehydration of PGD_2 results in generation of PGJ_2, 12-PGJ_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15-Δ-PGJ_2).

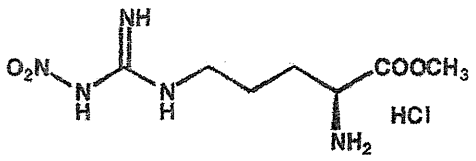
As PGE_2 synthesis is elevated in colon cancer, it is likely that PGE_2 would enhance carcinogenesis more than other prostanoids. In fact, in an AOM-induced colorectal carcinogenesis model in F344 rats, administration of PGE_2 enhanced colon carcinogenesis through induction of cell proliferation and reduction of apoptosis (23). It is conceivable that both COX isoforms may play important roles in colorectal carcinogenesis. Dietary administration of 1200 ppm mofezolac, [3,4-di(4-methoxyphenyl)-5-isoxazolyl acetic acid], a COX-1-selective inhibitor (Figure 20.1), was found to reduce the number of ACF per rat treated with AOM. Treatment with the same dose of mofezolac reduced the number of intestinal polyps in *APC*^{I309} mice to 59% of that in the untreated control mice (24). Dietary administration of 400 ppm nimesulide, a COX-2-selective inhibitor (Figure 20.1), was found to reduce the number of intestinal polyps in *Min* mice (25). Furthermore, homologous genetic disruption of either COX-1 or COX-2 markedly reduced polyp formation in *Min* mice (26).

There are four PGE_2 receptor subtypes, EP1 to 4, and assessment of mRNA expression has demonstrated up-regulation of EP1 and EP2 and down-regulation of EP3 in AOM-induced rat and mouse colon cancers. EP4 mRNA is consistently expressed in normal mucosa and tumors (27). Using PGE receptor subtype knockout mice, the roles of these receptors in colon carcinogenesis have been investigated (27–29). EP1 receptor selective antagonists, ONO-8711, {6-[(2*S*,3*S*)-3-(4-chloro-2-methylphenylsulfonylaminomethyl)-bicyclo[2.2.2]octan-2-yl]-5*Z*-hexenoic acid}, and the EP4 receptor–selective antagonist, ONO-AE2-227, 2-[2-{2-(1-naphthyl)propanoylamino}phenyl]methylbenzoic acid, inhibited development of AOM-induced ACF in male C57BL/6J mice (Figure 20.1). Moreover, when *Min* mice were given 500 ppm ONO-8711 in the diet, the number of intestinal polyps was reduced significantly, to 57% of that in the untreated control mice (27). Deficiencies of EP1 and EP4 also caused a decrease in ACF formation in the colons of mice treated with AOM (28,29). Sonoshita et al. reported that double knockout of *Apc* and *EP2* genes decreased intestinal polyp development (30).

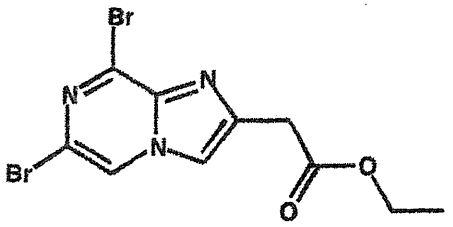
In contrast, deficiency of EP3 was found to enhance colon tumor formation after exposure to AOM (27). The available observations suggest that EP1, EP2, and EP4 are promotive in colon carcinogenesis, while EP3 could play suppressive roles, particularly in late stages. Of note, deficiencies of other specific membrane receptors—DP for PGD_2, FP for PGF_2, IP for PGI_2, and TP for thromboxane A_2—did not decrease ACF formation after AOM treatment (29).

ANTI-INFLAMMATORY AGENTS TARGETING iNOS

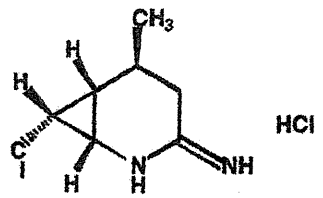
NO is an essential mediator of physiological processes in the digestive tract, maintaining the mucosa and regulating blood flow and peristalsis (31). However, overproduction of NO contributes to tissue damage, colon cancer cell growth, and DNA deamination (32,33). L-Arginine is converted to L-citrulline and NO by NOS, neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) NOS isoforms. Thus, L-arginine



L-NAME



SG-51



ONO-1714

FIGURE 20.2 Structures of NOS inhibitors.

analogs, *N*(ω)-nitro-L-arginine methyl ester (L-NAME), have attracted attention as possible inhibitors (Figure 20.2). iNOS expression is barely detectable in normal colon epithelial or stromal cells. However, it is found in lesions in which β-catenin alterations are observed, [i.e., human colon adenomas and adenocarcinomas (32,33)], and in AOM-induced rat colon dysplastic ACF and tumors (4).

Administration of a specific iNOS inhibitor, ONO-1714, {(1*S*,5*S*,6*R*,7*R*)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]heptane hydrochloride}(Figure 20.2), at doses of 10, 20, 50, and 100 ppm, reduced the number of AOM-induced ACFs in the F344 rats up to 53% of the untreated control value. Moreover, long-term treatment (32 weeks) revealed that 100 ppm ONO-1714 decreased the number of large colon tumors (>3 mm in diameter) (34). These results suggest that iNOS plays roles in both early and late stages of colon carcinogenesis.

In contrast to the normal mucosa, iNOS is also overexpressed in inflamed colonic mucosa, to almost the same extent as in colonic adenocarcinomas, in mice receiving AOM and/or dextran sodium sulfate (DSS) (35,36). An explanation of this expression by inflammatory stimuli could be obtained using IEC-6 rat intestinal epithelial cells transfected with K-*ras* mutant cDNA. In transfected IEC cells, induction of iNOS expression mediated by interleukin-1β (IL-1β) or lipopolysaccharide

was elevated markedly compared to the case with transfection of control vector or wild-type K-*ras* cDNA (5). These results suggest that activating mutations of K-*ras* caused by the carcinogen AOM are associated with up-regulation of iNOS expression in the presence of inflammatory stimuli. It has been reported that ONO-1714 effectively inhibited DSS-induced large bowel carcinogenesis in *Min* mice. Of interest in this context, the suppressive effects of ONO-1714 on the development of large bowel adenocarcinomas were closely correlated with the inhibition of serum triglyceride levels and the inhibition of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and IL-1β, and COX-2 mRNA levels (36).

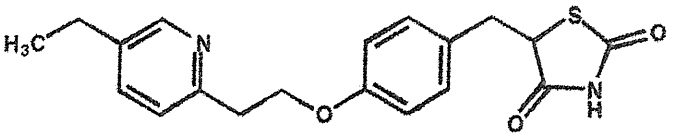
ANTI-INFLAMMATORY AGENTS TARGETING PPAR-γ

Peroxisome proliferator–activated receptor γ (PPAR-γ) is a member of the ligand-activated nuclear receptor superfamily, which plays key roles in fat metabolism. Moreover, PPAR-γ has been implicated in the pathophysiology of inflammation, obesity, and diabetes (37). Recently, it was shown that activation of PPAR-γ by 15-Δ-PGJ_2 or antidiabetic thiazolidinediones exerts antiproliferation, apoptosis, differentiation, and anti-inflammation effects in cancer cells (37). It has been reported that 15-Δ-PGJ_2 inhibits NF-κB-dependent gene expression either by functional inactivation of IKK, thereby preventing IκB degradation and nuclear entry of NF-κB, or via direct interference with binding of NF-κB to target DNA sequences.

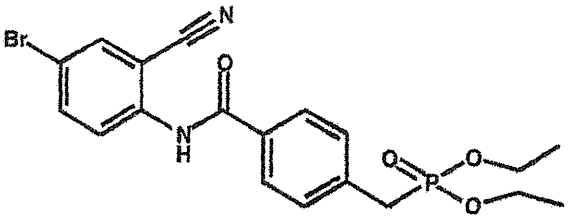
Administration of pioglitazone, {(±)-5-[4-[2-(5-ethyl-2-pyridyl)ethoxy] benzyl]thiazolidine-2,4-dione monohydrochloride}, a potent PPAR-γ ligand (Figure 20.3), at doses of 100 and 200 ppm in Apc^{1309} mice reduced the total numbers of polyps up to 67% of the value in the untreated control group (38). In *Min* mice, treatment with 100 to 1600 ppm for 14 weeks also showed a decrease of intestinal polyps upto 9% of the control number (39). Of note, there exists a PPRE in the promoter region of the *LPL* gene, and pioglitazone treatment induced LPL expression in the liver and intestinal epithelial cells in *Apc*-deficient mice.

NO-1886, 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (40), is an agent that can induce LPL agonist activity, but unlike bezafibrate and pioglitazone, does not possess PPAR agonistic activity (41) (Figure 20.3). Its administration at doses of 400 and 800 ppm also significantly decreased the total number of intestinal polyps to 48% and 42% of the untreated control value, respectively, in *Min* mice, along with a marked increase in *LPL* mRNA levels in the liver and small intestine (42).

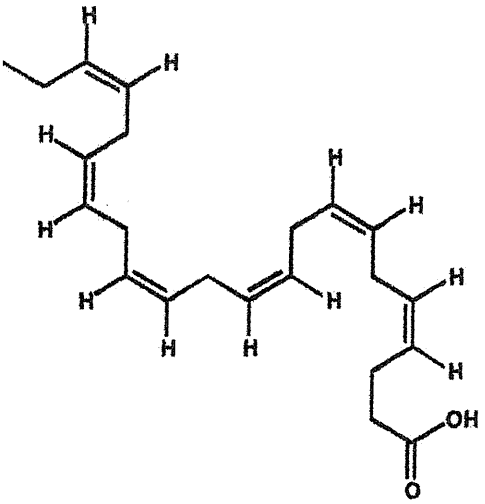
It is interesting that LPL is considered to possess both lipid-modifying and anti-inflammatory functions. It has been reported that LPL suppresses TNF-α- and interferon-γ-evoked inflammation-related gene expression in endothelial cells through inactivation of NF-κB (43). Experiments conducted to clarify the mechanism of NO-1886 influence on colorectal carcinogenesis revealed that the expression levels of TGF-α-induced COX-2 mRNA in human colon cancer cells DLD-1 were reduced. On the other hand, there was no obvious change in the mRNA levels for COX-1 and iNOS. The results were also confirmed by β-gal reporter gene assay in DLD-1 cells (42). Consistent with the *in vitro* data, administration of 400 and



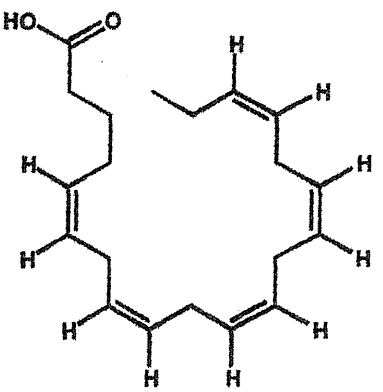
Pioglitazone



NO-1886



Docosahexaenoic acid



Eicosapentaenoic acid

FIGURE 20.3 Structures of lipid-lowering agents.

800 ppm NO-1886 reduced COX-2 mRNA levels in nonpolyp parts of the small intestine of *Min* mice (42).

OTHER CANDIDATES AS CANCER CHEMOPREVENTIVE AGENTS WITH ANTI-INFLAMMATORY POTENTIAL

Other agents from natural compounds that can suppress COX-2 and iNOS are flavonoids and phenolic antioxidants. Putative chemopreventive agents such as catechin, epicatechin, quercetin, kaempferol, genistein, resveratrol, and resorcinol, all having a common resorcin moiety, have been found to suppress COX-2 promoter activity effectively with and without TGF-α stimulation in DLD-1 cells (44,45)

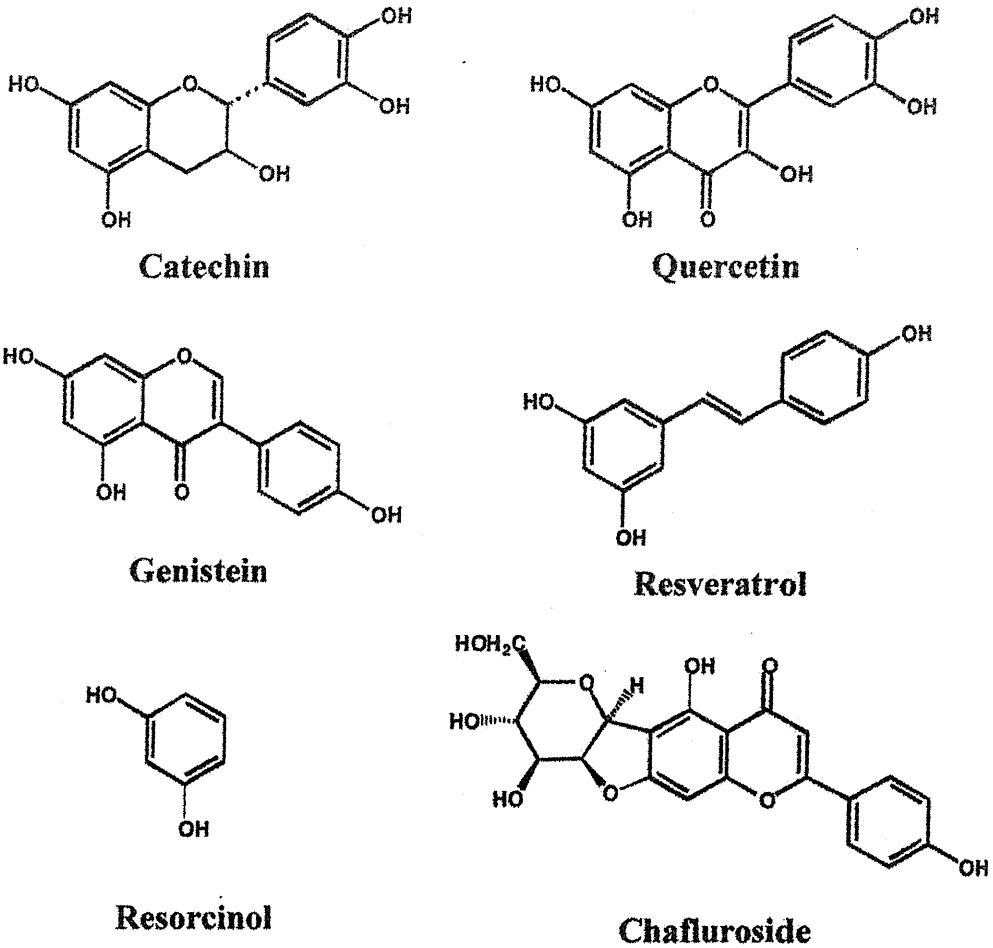
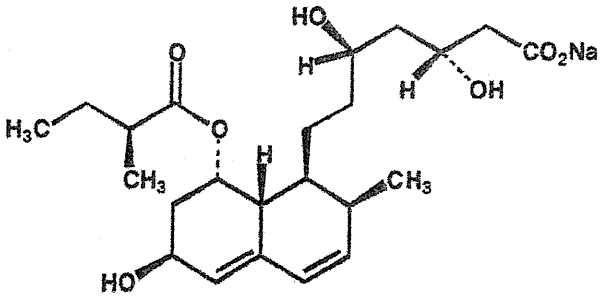


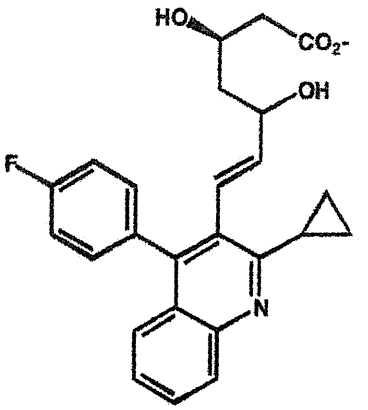
FIGURE 20.4 Structures of natural phenolic antioxidants.

(Figure 20.4). Moreover, ferulic acid derivatives can suppress COX-2 promoter activity, and butyrate reduces iNOS promoter activity (46,47). A new flavone derivative, chafuroside, (2*R*,3*S*,4*S*,4*aS*,11*bS*)-3,4,11-trihydroxy-2-(hydroxymethyl)-8-(4-hydroxyphenyl)-3,4,4*a*,11*b*-tetrahydro-2*H*,10*H*-pyrano[2′,3′:4,5]furo[3,2-*g*] chromen-10-one, has been isolated as a strong anti-inflammatory compound from oolong tea leaves and found to exert strong suppressive effects on intestinal tumorigenesis (48) (Figure 20.4). Administration of 10 ppm chafuroside reduced AOM-induced ACF formation and total numbers of polyps in the *Min* mice to 56% of the untreated control value.

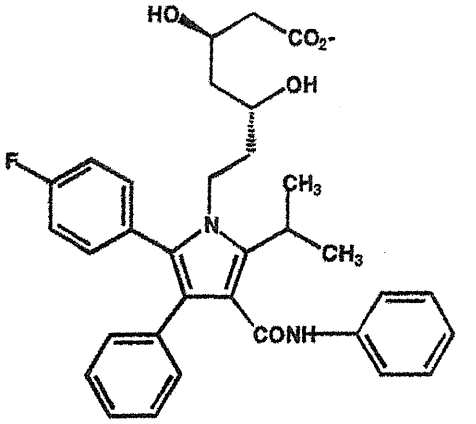
A major component of fish oil, docosahexaenoic acid (DHA) lowered the numbers of moderately differentiated adenocarcinomas developing in the middle and distal colon after AOM treatment in F344 rats compared to untreated controls, with significant reductions in levels of PGE_2 and AA in the blood plasma (49) (Figure 20.3). DHA also exerts inhibitory effects on intestinal polyp development in $Apc^{\Delta 716}$ mice (50).



Pravastatin



Pitavastatin



Atorvastatin

FIGURE 20.5 Structures of statins.

Synthetic agents that can suppress COX-2 and iNOS include statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, which are commonly used for the treatment of hypercholesterolemia. Pravastatin and atorvastatin have been reported to suppress 1,2-dimethylhydrazine (DMH) or AOM-induced colon cancer development in mice and rats, respectively (51,52). In addition, atorvastatin at a dose of 100 ppm reduced the incidence of small intestinal polyps in *Min* mice to 70% of the value in untreated control mice (53). Furthermore, 10 ppm pitavastatin, (+)-monocalcium bis{(3*R*,5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate}, a novel lipophilic statin, decreased the incidence of colon adenomas or adenocarcinomas induced by AOM/DSS treatment in ICR mice to about 78% of that in the untreated control group (54) (Figure 20.5). In addition to the main function of statins, which is inhibition of the synthesis of mevalonate, they also suppress inflammation. Treatment of *Min* mice with pitavastatin at dose of 40 ppm decreased the total number of polyps to 65.8% of the untreated control value (55). Serum levels of total cholesterol and triglycerides were slightly reduced, and those of IL-6, leptin, and monocyte chemoattractant protein-1 (MCP-1) were decreased. mRNA expression levels of COX-2, IL-6, iNOS, MCP-1, and

plasminogen activator inhibitor-1 (Pai-1) were reduced significantly in intestinal nonpolyp parts by pitavastatin treatment (55).

An epidemiological study demonstrated that chronic use of statins for more than five years significantly reduced the risk of colorectal cancer by 47% (56). On the other hand, other epidemiological studies using first-generation statins did not provide fully consistent results (57,58). Discrepancies could be explained by suboptimal administration of the drugs and their characteristics. Atorvastatin and pitavastatin, third-generation statins, possess strong serum lipid-lowering and anti-inflammatory potential. Further epidemiological data using third-generation statins are desired to evaluate chemopreventive effects against colorectal cancer.

FUTURE PROSPECTS

Combination Effects

In the future, use of anti-inflammatory drugs with other chemopreventive agents might find clinical application. Methods targeting several molecules could allow reduction of the dosage of the individual agents, resulting in lowered side effects. Moreover, targeting several molecules could provide synergistic effects. The combinations aspirin + α-difluoromethylornithine, the selective iNOS inhibitor L-*N*(6)-(1-iminoethyl)lysine tetrazole amide (SC-51) + the selective COX-2 inhibitor celecoxib, atorvastatin + sulindac, and atorvastatin + naproxen (59–61) have already been shown to reduce AOM-induced colon ACF/cancer formation (Figures 20.1 and 20.5). Moreover, the combinations ONO-8711 + ONO-AE2-227 and mofezolac + nimesulide were found to reduce intestinal polyp formation in *Apc*-deficient mice (62,63) (Figure 20.1).

Targeting Obesity

Recently, there is increasing consensus that obesity should be thought of as a pre-inflammatory condition. Because hypertrophy of fatty tissue results in an increase and changed profiles of adipocytokines, low-grade inflammation is evoked. It has become clear that factors such as insulin resistance, dyslipidemia, and subsequent adipocytokine imbalance could be involved in the promotion of colorectal carcinogenesis. Animal experiments have shown that some adipocytokines may play important roles not only in progression to malignancy, but also in very early stages of colorectal cancer development.

Obese mice such as the KK-A^y strain were revealed to be highly susceptible to induction of colon ACF and development of colorectal carcinomas by treatment with AOM (64). In addition to severe hyperinsulinemia and hypertriglyceridemia, the KK-A^y mouse exhibits abdominal obesity and resulting elevation of serum adipocytokines/cytokines, such as IL-6, leptin, and Pai-1, compared with values for lean C57BL/6J mice. In the visceral fat tissue, significant overexpression of pro-inflammatory cytokine mRNAs, such as for IL-6, leptin, MCP-1, Pai-1, and TNF-α was confirmed; in contrast, that for adiponectin was decreased. Thus, consequent

adipocytokine imbalance is suggested to contribute to the promotion of colon carcinogenesis.

As correction of this balance must be considered as a mean of cancer prevention, it might be important to develop selective adipocytokine-regulated drugs and search for agents from drugs with few side effects. Administration of a Pai-1 inhibitor, SK-216, at 25- to 100-ppm doses in *Min* mice, characterized by high levels of serum Pai-1, was found to reduce serum Pai-1 and hepatic Pai-1 mRNA levels, and decreased total numbers of intestinal polyps significantly, up to 56% of the untreated group value (65). Thus, adipocytokines such as adiponectin and Pai-1 are considered to be key molecular targets for cancer chemopreventive approaches.

Clinical Trials

Among candidate substances, sulindac has been studied frequently in the clinical setting (66–68), being reported to reduce the number and size of colorectal adenomas in a double-blind randomized trial (66). However, it has been also reported that sulindac may cause serious side-effects at a dose of more than 100 mg/day, at least in Japanese FAP patients (67). COX-2 selective inhibitors, including celecoxib and rofecoxib, were hoped to be ideal cancer chemopreventive agents, because they cause little damage to the gastric mucosa (19, 69), but recent studies have revealed that they may cause cardiotoxicity (70,71). Another trial using natural fish oil, omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) (Figure 20.3), showed promising reduction in rectal polyp number and size in FAP patients (72).

Meanwhile, aspirin, one of the conventional NSAIDs, is again attracting attention as a chemopreventive agent. Widespread and long-term use of aspirin for cardiovascular disease prevention allowed the accumulation of abundant evidence regarding its safety profile, and the dual benefit for patients with significant risk factors for both cardiovascular disease and colorectal cancer is an obvious advantage.

The CAPP2 randomized trial in 1000 Lynch syndrome gene carriers found almost a 60% reduction in new cancer development at about five years after randomization (73). In this experiment aspirin (600 mg/day) was used for a minimum of two years (73). This finding suggests that follow-up for several years after randomized trials is necessary to evaluate the effects of aspirin, and potentially also for other colon cancer chemopreventive agents. It is clearly desirable that more data be accumulated, specifically for Asian populations, to better assess chemoprevention of colon cancer by aspirin in the future.

REFERENCES

1. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–1188.
2. Ambs S, Merriam WG, Bennett WP, Felley-Bosco E, Ogunfusika MO, Oser SM, et al. Frequent nitric oxide synthase-2 expression in human colon adenomas: implication for tumor angiogenesis and colon cancer progression. Cancer Res 1998;58:334–341.

3. Jen J, Powell SM, Papadopoulos N, Smith KJ, Hamilton SR, Vogelstein B, Kinzler KW. Molecular determinants of dysplasia in colorectal lesions. Cancer Res 1994;54:5523–5526.
4. Takahashi M, Wakabayashi K. Gene mutations and altered gene expression in azoxymethane-induced colon carcinogenesis in rodents. Cancer Sci 2004;95:475–480.
5. Takahashi M, Mutoh M, Shoji Y, Kamanaka K, Naka M, Maruyama T, et al. Transfection of K-rasAsp12 cDNA markedly elevates IL-1β and lipopolysaccharide-mediated inducible nitric oxide synthase expression in rat intestinal epithelial cells. Oncogene 2003;22:7667–7676.
6. Mutoh M, Takahashi M, Wakabayashi K. Roles of prostanoids in colon carcinogenesis and their potential targeting for cancer chemoprevention. Curr Pharm Des 2006;12:2375–2382.
7. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004;10:789–799.
8. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci USA 1993;90:7240–7244.
9. Boone CW, Steele VE, Kelloff GJ. Screening for chemopreventive (anticarcinogenic) compounds in rodents. Mutat Res 1992;267:251–255.
10. Olivo S, Wargovich MJ. Inhibition of aberrant crypt foci by chemopreventiveagents. *In Vivo* 1998;12:159–166.
11. Quesada CF, Kimata H, Mori M, Nishimura M, Tsuneyoshi T, Baba S. Piroxicam and acarbose as chemopreventive agents for spontaneous intestinal adenomas in APC gene 1309 knockout mice. Jpn J Cancer Res 1998;89:392–396.
12. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 1990;247:322–324.
13. Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. Proc Natl Acad Sci USA 1995;92:4482–4486.
14. Fodde R, Edelmann W, Yang K, van Leeuwen C, Carlson C, Renault B, et al. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci USA 1994;91:8969–8973.
15. McCart AE, Vickaryous NK, Silver A. Apc mice: models, modifiers and mutants. Pathol Res Pract 2008;204:479–490.
16. Thun MJ, Namboodiri MM, Heath CW Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325:1593–1596.
17. Giardiello FM, Yang VW, Hylind LM, Krush AJ, Petersen GM, Trimbath JD, et al. Primary chemoprevention of familial adenomatous polyposis with sulindac. N Engl J Med 2002;346:1054–1059.
18. Akasu T, Yokoyama T, Sugihara K, Fujita S, Moriya Y, Kakizoe T. Peroral sustained-release indomethacin treatment for rectal adenomas in familial adenomatous polyposis: a pilot study. Hepatogastroenterology 2002;49:1259–1261.
19. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–1952.
20. Kulp SK, Yang YT, Hung CC, Chen KF, Lai JP, Tseng PH, et al. 3-Phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. Cancer Res 2004;64:1444–1451.
21. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, et al. The ancient drug salicylate directly activates AMP-activated protein kinase. Science 2012;336:918–922.
22. Loveridge CJ, MacDonald AD, Thoms HC, Dunlop MG, Stark LA. The proapoptotic effects of sulindac, sulindac sulfone and indomethacin are mediated by nucleolar translocation of the RelA(p65) subunit of NF-kappaB. Oncogene 2008;27:2648–2655.
23. Kawamori T, Uchiya N, Sugimura T, Wakabayashi K. Enhancement of coloncarcinogenesis by prostaglandin E_2 administration. Carcinogenesis 2003;24:985–990.
24. Kitamura T, Kawamori T, Uchiya N, Itoh M, Noda T, Matsuura M, et al. Inhibitory effects of mofezolac, a cyclooxygenase-1 selective inhibitor, on intestinal carcinogenesis. Carcinogenesis 2002;23:1463–1466.
25. Nakatsugi S, Fukutake M, Takahashi M, Fukuda K, Isoi T, Taniguchi Y, et al. Suppression of intestinal polyp development by nimesulide, a selective cyclooxygenase-2 inhibitor, in Min mice. Jpn J Cancer Res 1997;88:1117–1120.

26. Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, et al. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res 2000;60:4705–4708.
27. Shoji Y, Takahashi M, Kitamura T, Watanabe K, Kawamori T, Maruyama T, et al. Downregulation of prostaglandin E receptor subtype EP3 during colon cancer development. Gut 2004;53: 1151–1158.
28. Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, et al. Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. Cancer Res 1999;59:5093–5096.
29. Mutoh M, Watanabe K, Kitamura T, Shoji Y, Takahashi M, Kawamori T, et al. Involvement of prostaglandin E receptor subtype EP4 in colon carcinogenesis. Cancer Res 2002;62:28–32.
30. Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in ApcΔ716 knockout mice. Nat Med 2001;7:1048–1051.
31. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology 2004;113:427–437.
32. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–285.
33. Hofseth LJ, Hussain SP, Wogan GN, Harris CC. Nitric oxide in cancer and chemoprevention. Free Radic Biol Med 2003;34:955–968.
34. Takahashi M, Mutoh M, Shoji Y, Sato H, Kamanaka Y, Naka M, et al. Suppressive effect of an inducible nitric oxide inhibitor, ONO-1714, on AOM-induced rat colon carcinogenesis. Nitric Oxide 2006;14:130–136.
35. Kohno H, Suzuki R, Sugie S, Tanaka T. β-Catenin mutations in a mouse model of inflammation-related colon carcinogenesis induced by 1,2-dimethylhydrazine and dextran sodium sulfate. Cancer Sci 2005;96:69–76.
36. Tanaka T, Kohno H, Suzuki R, Hata K, Sugie S, Niho N, et al. Dextran sodium sulfate strongly promotes colorectal carcinogenesis in *Apc*$^{Min/+}$ mice: inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. Int J Cancer 2006;118:25–34.
37. Na HK, Surh YJ. Peroxisome proliferator–activated receptor gamma (PPARgamma) ligands as bifunctional regulators of cell proliferation. Biochem Pharmacol 2003;66:1381–1391.
38. Niho N, Takahashi M, Kitamura T, Shoji Y, Itoh M, Noda T, et al. Concomitant suppression of hyperlipidemia and intestinal polyp formation in Apc-deficient mice by peroxisome proliferator-activated receptor ligands. Cancer Res 2003;63:6090–6095.
39. Niho N, Takahashi M, Shoji Y, Takeuchi Y, Matsubara S, Sugimura T, et al. Dose-dependent suppression of hyperlipidemia and intestinal polyp formation in Min mice by pioglitazone, a PPAR gamma ligand. Cancer Sci 2003;94:960–964.
40. Tsutsumi K, Inoue Y, Shima A, Iwasaki K, Kawamura M, Murase T. The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high-density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. J Clin Invest 1993;92:411–417.
41. Doi M, Kondo Y, Tsutsumi K. Lipoprotein lipase activator NO-1886 (ibrolipim) accelerates the mRNA expression of fatty acid oxidation–related enzymes in rat liver. Metabolism 2003;52:1547–1550.
42. Niho N, Mutoh M, Takahashi M, Tsutsumi K, Sugimura T, Wakabayashi K. Concurrent suppression of hyperlipidemia and intestinal polyp formation by NO-1886, increasing lipoprotein lipase activity in Min mice. Proc Natl Acad Sci USA 2005;102:2970–2974.
43. Kota RS, Ramana CV, Tenorio FA, Enelow RI, Rutledge JC. Differential effects of lipoprotein lipase on tumor necrosis factor-alpha and interferon-gamma-mediated gene expression in human endothelial cells. J Biol Chem 2005;280:31076–31084.
44. Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, Sugimura T, et al. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. Carcinogenesis 2000;21:959–963.
45. Mutoh M, Takahashi M, Fukuda K, Komatsu H, Enya T, Matsushima-Hibiya Y, et al. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure–activity relationship. Jpn J Cancer Res 2000;9:686–691.
46. Hosoda A, Ozaki Y, Kashiwada A, Mutoh M, Wakabayashi K, Mizuno K, et al. Syntheses of ferulic acid derivatives and their suppressive effects on cyclooxygenase-2 promoter activity. Bioorg Med Chem 2002;10:1189–1196.

47. Sasahara Y, Mutoh M, Takahashi M, Fukuda K, Tanaka N, Sugimura T, et al. Suppression of promoter-dependent transcriptional activity of inducible nitric oxide synthase by sodium butyrate in colon cancer cells. Cancer Lett 2002;177:155–161.
48. Niho N, Mutoh M, Sakano K, Takahashi M, Hirano S, Nukaya H, et al. Inhibition of intestinal carcinogenesis by a new flavone derivative, chafuroside, in oolong tea. Cancer Sci 2006;97:248–251.
49. Takahashi M, Fukutake M, Isoi T, Fukuda K, Sato H, Yazawa K, et al. Suppression of azoxymethane-induced rat colon carcinoma development by a fish oil component, docosahexaenoic acid (DHA). Carcinogenesis 1997;18:1337–1342.
50. Oshima M, Takahashi M, Oshima H, Tsutsumi M, Yazawa K, et al. Effects of docosahexaenoic acid (DHA) on intestinal polyp development in Apc delta 716 knockout mice. Carcinogenesis 1995;16:2605–2607.
51. Narisawa T, Fukaura Y, Tanida N, Hasebe M, Ito M, Aizawa R. Chemopreventive efficacy of low dose of pravastatin, an HMG-CoA reductase inhibitor, on 1,2-dimethylhydrazine-induced colon carcinogenesis in ICR mice. Tohoku J Exp Med 1996;180:131–138.
52. Reddy BS, Wang CX, Kong AN, Khor TO, Zheng X, Steele VE, et al. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F344 rats. Cancer Res 2006;66:4542–4546.
53. Swamy MV, Patlolla JMR, Steele VE, Kopelovich L, Reddy BS, Rao CV. Chemoprevention of familial adenomatous polyposis by low dose of atorvastatin and celecoxib given individually and in combination to APC^{min} mice. Cancer Res 2006;66:7370–7377.
54. Yasui Y, Suzuki R, Miyamoto S, Tsukamoto T, Sugie S, Kohno H, et al. A lipophilic statin, pitavastatin, suppresses inflammation-associated mouse colon carcinogenesis. Int J Cancer 2007;121: 2331–2339.
55. Teraoka N, Mutoh M, Takasu S, Ueno T, Yamamoto M, Sugimura T, et al. Inhibition of intestinal polyp formation by pitavastatin, a HMG-CoA reductase inhibitor. Cancer Prev Res (Phila) 2011;4: 445–453.
56. Poynter JN, Gruber SB, Higgins RD, Almog R, Bonner JD, Rennert HS, et al. Statins and the risk of colorectal cancer. N Engl J Med 2005;352:2184–2192.
57. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The antihypertensive and lipid-lowering treatment to prevent heart attack trial: major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care—the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT-LLT). JAMA 2002;288:2998–3007.
58. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS-Air Force/Texas coronary atherosclerosis prevention study. JAMA 1998;279:1615–1622.
59. Li H, Schut HA, Conran P, Kramer PM, Lubet RA, Steele VE, et al. Prevention by aspirin and its combination with alpha-difluoromethylornithine of azoxymethane-induced tumors, aberrant crypt foci and prostaglandin E2 levels in rat colon. Carcinogenesis 1999;20:425–430.
60. Rao CV, Indranie C, Simi B, Manning PT, Connor JR, Reddy BS. Chemopreventive properties of a selective inducible nitric oxide synthase inhibitor in colon carcinogenesis, administered alone or in combination with celecoxib, a selective cyclooxygenase-2 inhibitor. Cancer Res 2002;62:165–170.
61. Suh N, Reddy BS, DeCastro A, Paul S, Lee HJ, Smolarek AK, et al. Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/β-catenin/cyclin D1 signaling pathway in rats. Cancer Prev Res (Phila) 2011;4:1895–1902.
62. Kitamura T, Itoh M, Noda T, Tani K, Kobayashi M, Maruyama T, et al. Combined effects of prostaglandin E receptor subtype EP1 and subtype EP4 antagonists on intestinal tumorigenesis in adenomatous polyposis coli gene knockout mice. Cancer Sci 2003;94:618–621.
63. Kitamura T, Itoh M, Noda T, Matsuura M, Wakabayashi K. Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on intestinal tumorigenesis in adenomatous polyposis coli gene knockout mice. Int J Cancer 2004;109:576–580.
64. Teraoka N, Mutoh M, Takasu S, Ueno T, Nakano K, Takahashi M, et al. High susceptibility to azoxymethane-induced colorectal carcinogenesis in obese KK-A^y mice. Int J Cancer 2011;129:528–535.

65. Mutoh M, Niho N, Komiya M, Takahashi M, Ohtsubo R, Nakatogawa K, et al. Plasminogen activator inhibitor-1 (Pai-1) blockers suppress intestinal polyp formation in Min mice. Carcinogenesis 2008;29:824–829.
66. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 1993;328:1313–1316.
67. Ishikawa H, Akedo I, Suzuki T, Narahara H, Otani T. Adverse effects of sulindac used for prevention of colorectal cancer. J Natl Cancer Inst 1997;89:1381.
68. Kim B, Giardiello FM. Chemoprevention in familial adenomatous polyposis. Best Pract Res Clin Gastroenterol 2011;25:607–622.
69. Higuchi T, Iwama T, Yoshinaga K, Toyooka M, Taketo MT, Sugihara K. A randomized double blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. Clin Cancer Res 2003;9:4756–4760.
70. Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, et al. [Adenoma Prevention with Celecoxib (APC) Study Investigators]. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 2005;352:1071–1080.
71. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. [Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial Investigators]. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352:1092–1102.
72. West NJ, Clark SK, Phillips RK, Hutchinson JM, Leicester RJ, Belluzzi A, et al. Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. Gut 2010;59:918–925.
73. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. (CAPP2 Investigators). Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet 2011;378:2081–2087.

その他

厚生労働科学研究委託費
（革新的がん医療実用化研究事業）
「がん化学予防薬の実用化をめざした大規模臨床研究」

（研究代表者：石川秀樹）

大腸腫瘍患者へのアスピリン(100mg/day)による発がん予防大規模臨床試験

Japan colorectal tumor prevention study: Mega-trial by low-dose aspirin

略称

Ｊ－ＣＡＰＰ Study II

＜Japan Colon Aspirin Polyp Prevention StudyII＞

プロトコール

2015年2月12日 版

試験責任者：石川秀樹（京都府立医科大学　分子標的癌予防医学）

目次

1. 概要

研究者自主研究として、アスピリン腸溶錠（1錠100mg/day）による大腸腫瘍予防効果をhistorical controlを用いた大規模単一投与臨床試験により評価し、アスピリンによる大腸腫瘍抑制効果を検証するとともに、大腸がん予防のためにアスピリンを用いるべき集団の特定を目的とする。

(1) 対象条件

大腸に大腸腫瘍（粘膜内がん・腺腫）を1個以上持ちそれらをすべて内視鏡的に摘除できた40歳以上、70歳未満の患者である。

(2) 試験薬

低用量アスピリン（100mg/day）を1日1錠4年間服用する。

4年間試験薬を投与する。

(3) 主な評価項目

(i)主評価は、1年目までを除く4年間の全大腸内視鏡検査における新たなIndex Lesion(IL10mm以上の腺腫、高度異型腺腫、がん）の発生の割合とする。

副評価は、大腸腫瘍（腺腫、がん）の発生の有無、個数、直径、異型度、部位とする。

(ii) 試験期間内の有害事象発生率、他臓器がんの発生の割合。

(iii) エントリー時点の性、年齢、飲酒、喫煙、身長、体重、運動習慣調査、服薬歴、生化学的血液検査、大腸腫瘍既往、乾燥血液濾紙法による遺伝子多型(CYP2A6(喫煙)、ALDH2、ADH1B(飲酒)など、大腸発癌やアスピリン代謝に関係する酵素の遺伝子多型を測定する。

(4) 予定参加者数

試験参加人数7,000人を目標数とする。

(5) 研究期間

登録期間：　2015年5月1日～2017年3月31日（23ヶ月）

試験薬投与期間：　4年間

追跡期間：　最終患者投与終了後3年間

試験実施期間：　2015年5月1日～2024年3月31日

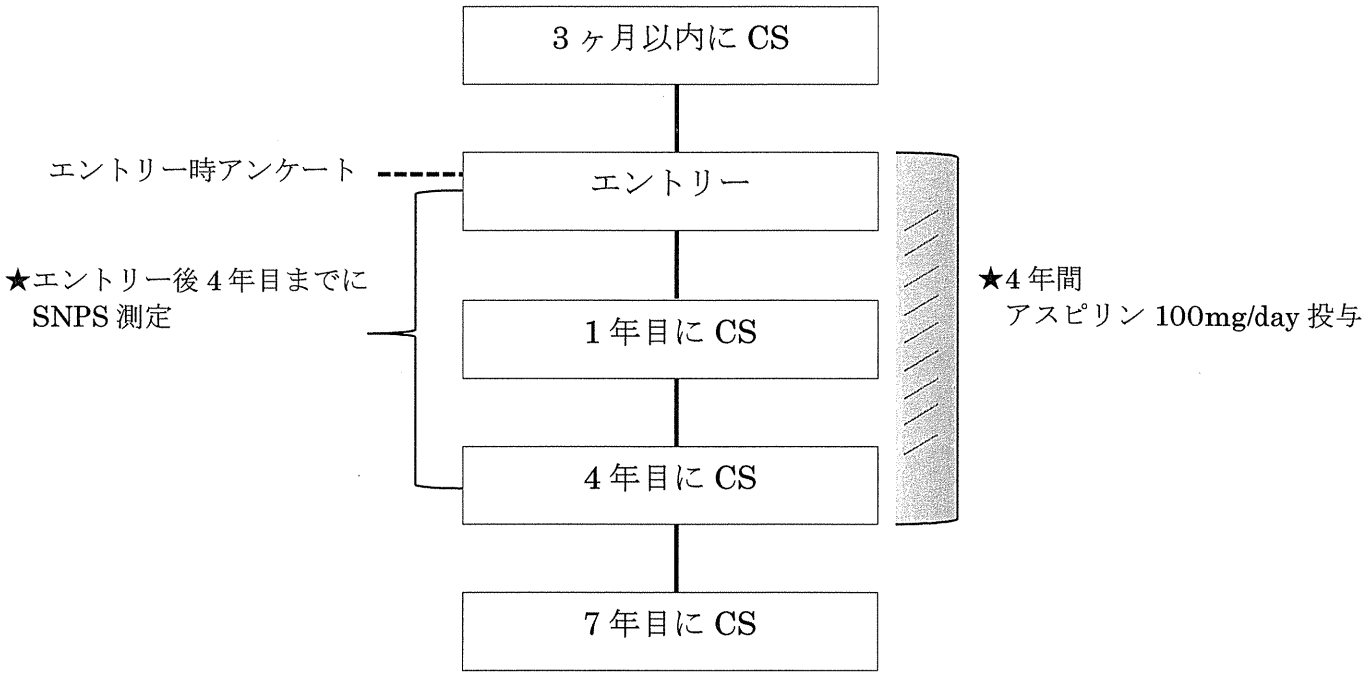
(6) 中間解析

中間解析は実施しない。

(7) 参加予定施設

全国の大腸専門21施設

本研究の試験計画



CS: Colonoscopy