Carcinogenesis vol.17 no.8 pp.1757–1760, 1996

SHORT COMMUNICATION

Sulindac suppresses tumorigenesis in the Min mouse

Yasmin Beazer-Barclay, Daniel B.Levy, Amy R.Moser[3], William F.Dove[4], Stanley R.Hamilton[1], Bert Vogelstein[2] and Kenneth W.Kinzler[5]

Oncology Center, [1]Department of Pathology and [2]The Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21231, [3]Department of Human Oncolgy, University of Wisconsin, Madison, WI 53792 and [4]McArdle Laboratory for Cancer Research, Madison, WI 53706, USA

[5]To whom correspondence should be addressed

The Min mouse provides a genetically defined model for inherited and sporadic forms of human colorectal tumorigenesis. To test the suitability of this model for the evaluation and optimization of chemopreventive agents, we examined the effects of sulindac on tumorigenesis in Min mice as this compound can inhibit colorectal tumorigenesis in human familial adenomatous polyposis patients. Treatment of Min mice with sulindac in their drinking water (84 mg/l) or diet (167 and 334 p.p.m.) resulted in a significantly decreased average tumor load. The conservation of sulindac activity in the Min mouse provides an opportunity to explore the mechanism of sulindac suppression as well as to test other potential chemopreventive agents.

Alterations of the adenomatous polyposis coli (*APC**) gene play a critical role in the development of both inherited and sporadic forms of colon cancer. Germline mutations of the *APC* gene result in familial adenomatous polyposis (FAP), an autosomal dominant inherited predisposition to colorectal cancer (1–4). Patients with FAP develop hundreds of colorectal adenomas, some of which inevitably progress to cancer. Although FAP patients with germline mutations of *APC* account for less than 1% of colorectal cancer in the USA, somatic mutations of the *APC* gene occur in the vast majority of sporadic colorectal cancers (5–7). Such alterations can be found in the smallest lesions examined, suggesting that they are an early event, if not the initiating event, in colorectal tumorigenesis (8,9). Like human FAP patients, mice with germline mutations of the murine homolog of the *APC* gene develop intestinal neoplasias (10–12). The Min mouse was the first such mouse described (10) and contains a nonsense mutation of the *Apc* gene at codon 850 (11). Min mice on a standardized sensitive genetic background (C57BL/6) develop an average of 30–60 macroscopically visible intestinal tumors by the age of 90 days (13). Thus, these mice provide a good model for colorectal tumorigenesis at both the genotypic and phenotypic levels.

A growing number of studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of colorectal cancer. Several epidemiological studies have found that aspirin use is associated with a reduced risk of death due to colorectal cancer (14–19). Additionally, colorectal tumorigenesis can be suppressed in FAP patients by treatment with the NSAID sulindac (20–24). To practically demonstrate the utility of the Min mouse model in chemoprevention, we evaluated whether sulindac could suppress tumorigenesis in Min mice.

In an initial study, we administered sulindac in the drinking water. Ninety nine Min mice were randomly divided among three treatment groups, maintaining sexual balance in each group. Mice were maintained from weaning (28 days of age) until necropsy (94 days of age) on buffered drinking water (4 mM sodium phosphate, pH 7.4) containing 0, 21 or 84 mg/l sulindac (Sigma). Mice had *ad libitum* access to both buffered water and chow (Purina 5010). Water consumption studies conducted prior to initiation of the study indicated that the sulindac did not have an adverse effect on the drinking behavior and that the expected drug levels would correspond to doses of ~0, 5 and 20 mg/kg/day sulindac. After sacrifice, the entire intestines were removed, opened, spread out on filter paper with the lumen side up and fixed in 3.7% formaldehyde. Macroscopically visible tumors along the entire length of the small and large intestines were counted by an observer blind to the treatment. While sulindac at 21 mg/l produced no effect, sulindac at 84 mg/l produced a statistically significant decrease in tumor number ($P < 0.001$, Student's *t*-test; Table I). In a fourth treatment group, 36 animals were exposed to sulindac starting *in utero*; their pregnant mothers were maintained on sulindac 7 days prepartum and throughout nursing. In this case, whole litters were randomly assigned to treatment groups and necropsied at 104 days of age. Interestingly, this treatment produced a significantly improved response over the same dose given from weaning to 90 days ($P < 0.05$, one-tailed Student's *t*-test; Table I). This finding suggests that the timing of drug administration can affect the efficacy of sulindac treatment. This is consistent with studies that suggest that the first 2 weeks of life are especially critical for tumor initiation in Min mice (25).

To confirm these promising initial results, a second study was initiated to explore the efficacy of higher doses of sulindac. While no toxicity was observed after administration of sulindac at the highest doses used in the first study, the solubility of sulindac limited the maximum dosage. Therefore, sulindac was administered in the diet, rather than in the drinking water, for the second study. Furthermore, to eliminate potential differences due to variation in standard chow preparations, the mice were maintained on the purified diet AIN-93G (26,27). Although no contemporaneous studies have been done, this study and others (28; unpublished data) suggest that Min mice fed purified diets develop fewer tumors than Min mice maintained on standard chow. One hundred and two mice were randomly divided among three treatment groups maintaining sexual balance in each group. Mice were maintained from weaning (27 days of age) until necropsy (86 days of age) on a diet containing 0, 167 or 334 p.p.m. sulindac. Diet consumption

*Abbreviations: *APC*, adenomatous polyposis coli; NSAIDs, non-steroidal anti-inflammatory drugs.

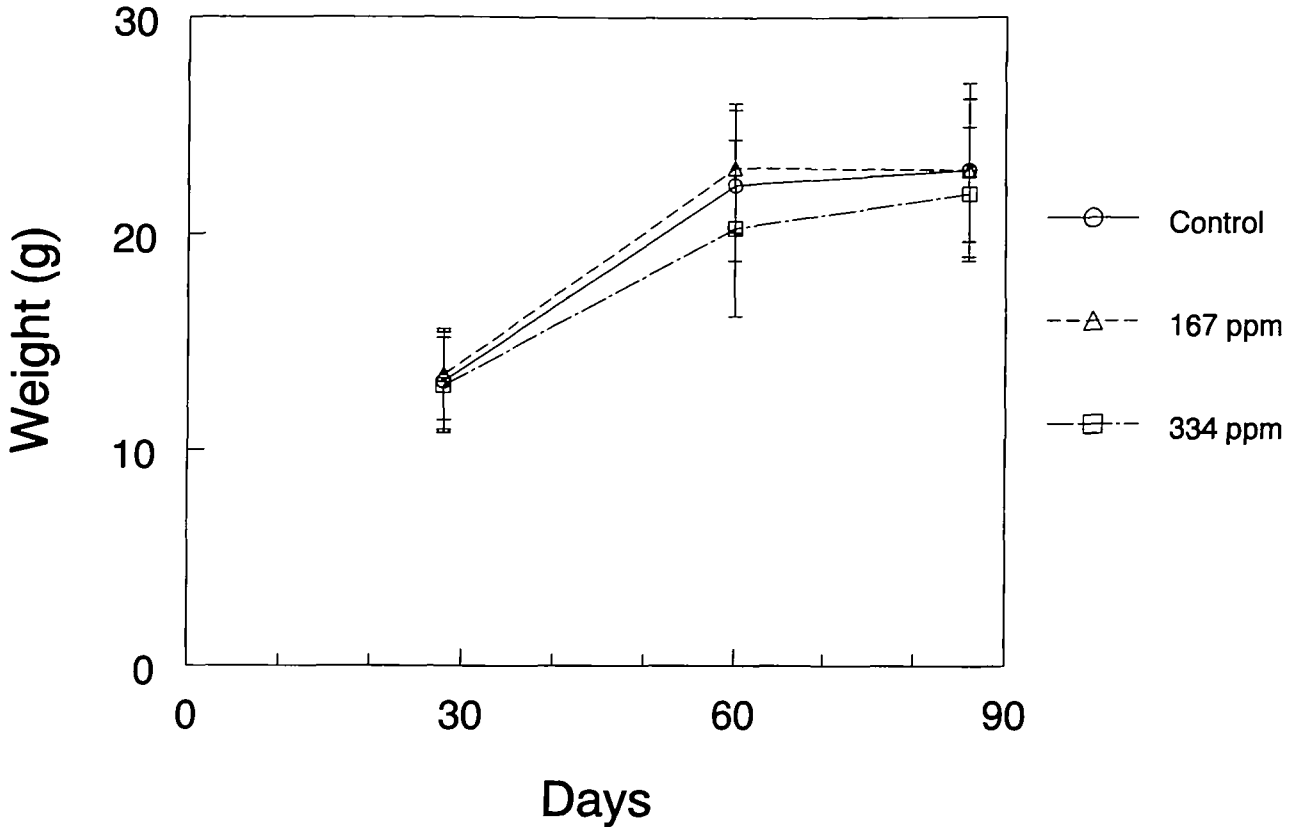

Fig 1. Effects of sulindac in the diet on Min mice. Min mice were fed a diet containing sulindac as described in the text and outlined in Table II. The mean body weights of the mice in each treatment group were plotted at 30, 60 and 90 days. The error bars are the standard deviations of the means.

Table I. Effects of sulindac in the drinking water on intestinal tumorigenesis in Min mice

Treatment[a]	No. of mice	Intestinal tumors[b]	Significance[c]
Control (buffered water)	34	48.6 ± 27.0	
Low dose (21 mg/l)[d]	33	52.3 ± 28.5	$P = 0.6$
High dose (84 mg/l)[d]	32	29.7 ± 17.9*	$P = 0.0008$
High dose (84 mg/l) 0–104 days[e]	36	22.3 ± 17.2*	$P = 0.000007$

[a]See text for details of treatment.
[b]Mean number of tumors ± SD.
[c]Two-tailed Student's *t*-test versus control.
[d]Mice were maintained on sulindac from weaning (28 days) until necropsy (94 days).
[e]Pregnant female mice were started on sulindac ~7 days prepartum. Mother and pups were maintained on sulindac through weaning and pups remained on sulindac until necropsy at 104 days.
*Significant at the $P < 0.001$ level versus control.

Table II. Effects of sulindac in the diet on intestinal tumorigenesis in Min mice

Treatment[a]	No. of mice	Intestinal tumors[b]	Significance[c]
Control (0 p.p.m.)	36	32.7 ± 17.5	
Low dose (167 p.p.m.)	32	12.4 ± 5.5*	$P = 0.00000008$
High dose (334 p.p.m.)	34	9.8 ± 5.4*	$P = 0.000000005$

[a]See text for details of treatment.
[b]Mean number of intestinal tumors ± SD.
[c]Two-tailed Student's *t*-test versus control diet.
*Significant at the $P < 0.001$ level versus control diet.

studies performed before and twice during the study indicated that the sulindac did not have an adverse effect on the feeding behavior and that the expected drug levels corresponded to doses of ~0, 40 and 80 mg/kg/day sulindac. Although there was a slight decrease (8%) in the mean weights of the high treatment group at 60 days, there was no significant difference in the weights at 90 days, suggesting minimal or no toxicity (Figure 1). However, both concentrations of sulindac produced a striking drop in tumor number (Table II), in agreement with the drinking water study. Histopathological examination of Swiss rolls (29) of the gastrointestinal tract from representative Min mice confirmed the macroscopic observations. Sulindac reduced both the number of adenomas and microscopic dysplastic foci (Table III).

The studies described above demonstrate that sulindac can inhibit the spontaneous intestinal tumorigenesis that occurs in the Min mouse. These results parallel studies in humans which demonstrate that sulindac can reduce both the number and size of adenomatous polyps in FAP patients (20–24). They are also consistent with studies in rodents which demonstrated the ability of sulindac and other NSAIDs to inhibit chemically induced tumorigenesis (30–36). Studies in tissue culture and patients suggest that sulindac and other NSAIDs promote apoptosis in epithelial cells, providing a potential explanation for their anti-neoplastic effects (37–40). Because the Min mouse model shares both genetic and phenotypic features with human FAP (11), it should provide a good system for studying NSAID and for the evaluation of other compounds for the treatment of FAP. This study and recent studies demonstrating the ability of Bowman–Birk inhibitor (28) and piroxicam

Table III. Effects of sulindac in the diet on adenomas and microscopic dysplastic foci in Swiss rolls of the intestines of Min mice[a]

Treatment[b]	No. of mice	Adenomas[c]	Significance[d]	Dysplastic foci[c]	Significance[d]
Control (0 p.p.m.)	10	7.1 ± 5.4		5.9 ± 4.3	
Low dose (167 p.p.m.)	11	1.8 ± 1.0*	$P = 0.01$	3.7 ± 2.3	$P = 0.18$
High dose (334 p.p.m.)	10	1.0 ± 1.4*	$P = 0.006$	2.3 ± 2.5*	$P = 0.04$

[a]For each mouse, a single eosin and hematoxylin stained section of a Swiss roll of the entire length of the intestines was examined microscopically. The number of observed tumors is less than those determined grossly because only a portion of the intestines was present in the examined section.
[b]See text for details of treatment.
[c]Mean number ± SD.
[d]Two-tailed Student's *t*-test versus control diet.
*Significant at the $P < 0.05$ level versus control diet.

(41) to inhibit tumorigenesis in the Min mouse support this contention. Moreover, because sporadic colorectal tumors apparently share similar initiating *APC* mutations with FAP patients and Min mice, insights into the prevention of tumorigenesis in FAP may apply to the general population.

Acknowledgements

This work was supported by NIH grants CA50585, CA62924 and CA57345. BV is an investigator of the Howard Hughes Medical Institute. WFD acknowledges the early interest in studies of sulindac with Min mice from William Waddell, MD.

References

1. Groden,J., Thliveris,A., Samovitz,W. *et al.* (1991) Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, **66**, 589–600.
2. Joslyn,G., Carlson,M., Thliveris,A. *et al.* (1991) Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell*, **66**, 601–613.
3. Kinzler,K.W., Nilbert,M.C., Su,L.K. *et al.* (1991) Identification of FAP locus genes from chromosome 5q21. *Science*, **253**, 661–665.
4. Nishisho,I., Nakamura,Y., Miyoshi,Y. *et al.* (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science*, **253**, 665–669.
5. Miyoshi,Y., Nagase,H., Ando,H. *et al.* (1992) Somatic mutations of the *APC* gene in colorectal tumors: mutation cluster region in the *APC* gene. *Hum. Mol. Genet.*, **1**, 229–233.
6. Powell,S.M., Zilz,N., Beazer-Barclay,Y., Bryan,T.M., Hamilton,S.R., Thibodeau,S.N., Vogelstein,B. and Kinzler,K.W. (1992) *APC* mutations occur early during colorectal tumorigenesis. *Nature*, **359**, 235–237.
7. Smith,K.J., Johnson,K.A., Bryan,T.M. *et al.* (1993) The *APC* gene product in normal and tumor cells. *Proc. Natl Acad. Sci. USA*, **90**, 2846–2850.
8. Jen,J., Powell,S.M., Papadopoulos,N., Smith,K.J., Hamilton,S.R., Vogelstein,B. and Kinzler K.W. (1994) Molecular determinants of dysplasia in colorectal lesions. *Cancer Res.*, **54**, 5523–5526.
9. Smith,A.J., Stern,H.S., Penner,M., Hay,K., Mitri,A., Bapat,B.V. and Gallinger,S. (1994) Somatic *APC* and K-*ras* codon 12 mutations in aberrant crypt foci from human colons. *Cancer Res.*, **54**, 5527–5530.
10. Moser,A.R., Pitot,H.C. and Dove,W.F. (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science*, **247**, 322–324.
11. Su,L.K., Kinzler,K.W., Vogelstein.B., Preisinger,A.C., Moser,A.R., Luongo,C., Gould,K.A. and Dove,W.F. (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the *APC* gene. *Science*, **256**, 668–670.
12. Fodde,R., Edelmann,W., Yang,K. *et al.* (1994) A targeted chain-termination mutation in the mouse *Apc* gene results in multiple intestinal tumors. *Proc. Natl Acad. Sci. USA*, **91**, 8969–8973.
13. Dietrich,W.F., Lander,E.S., Smith,J.S., Moser,A.R., Gould,K.A., Luongo,C., Borenstein,N. and Dove,W. (1993) Genetic identification of *Mom*-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. *Cell*, **75**, 631–639.
14. Kune,G.A., Kune,S. and Watson,L.F. (1988) Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. *Cancer Res.*, **48**, 4399–4404.
15. Rosenberg,L., Palmer,J.R., Zauber,A.G., Warshauer,M.E., Stolley,P.D. and Shapiro,S. (1991) A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. *J. Natl Cancer Inst.*, **83**, 355–358.
16. Thun,M.J., Namboodiri,M.M. and Heath,C.W.,Jr (1991) Aspirin use and reduced risk of fatal colon cancer. *New Engl. J. Med.*, **325**, 1593–1596.
17. Logan,R.F., Little,J., Hawtin,P.G. and Hardcastle,J.D. (1993) Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case–control study of subjects participating in the Nottingham faecal occult blood screening programme. *Br. Med. J.*, **307**, 285–289.
18. Giovannucci,E., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Ascherio,A. and Willett,W.C. (1994) Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Annls Internal Med.*, **121**, 241–246.
19. Peleg,I.I., Maibach,H.T., Brown,S.H. and Wilcox,C.M. (1994) Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. *Arch. Internal Med.*, **154**, 394–399.
20. Waddell,W.R., Ganser,G.F., Cerise,E.J. and Loughry,R.W. (1989) Sulindac for polyposis of the colon. *Am. J. Surg.*, **157**, 175–179.
21. Labayle,D., Fischer,D., Vielh,P., Drouhin,F., Pariente,A., Bories,C., Duhamel,O., Trousset,M. and Attali,P. (1991) Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology*, **101**, 635–639.
22. Rigau,J., Pique,J.M., Rubio,E., Planas,R., Tarrech,J.M. and Bordas,J.M. (1991) Effects of long-term sulindac therapy on colonic polyposis. *Annls Internal Med.*, **115**, 952–954.
23. Giardiello,F.M., Hamilton,S.R., Krush,A.J., Piantadosi,S., Hylind,L.M., Celano,P., Booker,S.V., Robinson,C.R. and Offerhaus,G.J. (1993) Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *New Engl. J. Med.*, **328**, 1313–1316.
24. Winde,G., Gumbinger,H.G, Osswald,H., Kemper,F. and Bunte,H. (1993) The NSAID sulindac reverses rectal adenomas in colectomized patients with familial adenomatous polyposis: clinical results of a dose-finding study on rectal sulindac administration. *Int. J. Colorectal Dis*, **8**, 13–17.
25. Shoemaker,A.R., Moser,A.R. and Dove,W.F. (1995) *N*-Ethyl-*N*-nitrosourea treatment of multiple intestinal neoplasia (Min) mice: age-related effects on the formation of intestinal adenomas, cystic crypts, and epidermoid cysts. *Cancer Res.*, **55**, 4479–4485.
26. Reeves,P.G., Nielsen,F.H. and Fahey,G.C.,Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.*, **123**, 1939–1951.
27. Reeves,P.G, Rossow,K.L. and Lindlauf,J. (1993) Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. *J. Nutr.*, **123**, 1923–1931.
28. Kennedy,A.R., Beazer-Barclay,Y., Kinzler,K.W. and Newberne,P.M. (1996) Suppression of carcinogenesis in the intestines of Min mice by the soybean-derived Bowman–Birk Inhibitor. *Cancer Res.*, **56**, 679–682.
29. Park,C.M., Reid,P.E., Walker,D.C. and MacPherson,B.R. (1987) A simple, practical 'swiss roll' method of preparing tissues for paraffin or methacrylate embedding. *J. Microsc.*, **145**, 115–120.
30. Metzger,U., Meier,J., Uhlschmid,G. and Weihe,H. (1984) Influence of various prostaglandin synthesis inhibitors on DMH-induced rat colon cancer. *Dis. Colon Rectum*, **27**, 366–369.
31. Narisawa,T., Hermanek,P., Habs,M. and Schmahl,D. (1984) Reduction of carcinogenicity of *N*-nitrosomethylurea by indomethacin and failure of resuming effect of prostaglandin E2 (PGE2) against indomethacin. *J. Cancer Res. Clin. Oncol.*, **108**, 239–242.
32. Reddy,B.S., Maruyama,H. and Kelloff,G. (1987) Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. *Cancer Res.*, **47**, 5340–5346.
33. Moorghen,M., Ince,P., Finney,K.J., Sunter,J.P., Appleton,D.R. and Watson,A.J. (1988) A protective effect of sulindac against chemically-induced primary colonic tumours in mice. *J. Pathol.*, **156**, 341–347.
34. Pollard,M. and Luckert,P.H. (1989) Prevention and treatment of primary intestinal tumors in rats by piroxicam. *Cancer Res.*, **49**, 6471–6473.
35. Skinner,S.A., Penney,A.G. and Pe,O.B. (1991) Sulindac inhibits the rate of growth and appearance of colon tumors in the rat. *Arch. Surg.*, **126**, 1094–1096.

36. Reddy,B.S., Rao,C.V., Rivenson,A. and Kelloff,G. (1993) Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis*, **14**, 1493–1497.
37. Tsujii,M. and Dubois,R.N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*, **83**, 493–501.
38. Piazza,G.A., Rahm,A.L.K., Krutzsch,M. *et al.* (1995) Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. *Cancer Res.*, **55**, 3110–3116
39. Shiff,S.J., Qiao,L., Tsai,L.L. and Rigas,B. (1995) Sulindac sulfide, an aspirin-like compound, inhibits proliferation, causes cell cycle quiescence, and induces apoptosis in HT-29 colon adenocarcinoma cells. *J Clin. Invest.*, **96**, 491–503.
40. Pasricha,P.J., Bedi,A., O'Connor,K., Rashid,A., Akhtar,A.J., Zahurak,M.L., Piantadosi,S., Hamilton,S.R. and Giardiello,F.M. (1995) The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. *Gastroenterology*, **109**, 994–998.
41. Jacoby,R.F., Marshall,D.J., Newton,M.A. *et al.* (1996) Chemoprevention of spontaneous intestinal adenomas in the APC-Min mouse by the nonsteroidal anti-inflammatory drug piroxicam. *Cancer Res.*, **56**, 710–714.

Received on March 6, 1996; revised on May 8, 1996; accepted on May 23, 1996