Tumor-Suppressing Effects of Antioxidants from Tea[1,2]

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EXPANDED ABSTRACT

KEY WORDS: • *tea* • *sulindac* • *Apc* • *familial adenomatous polyposis*

Mutations in Apc or β-catenin occur early in colorectal cancer and activate the β-catenin/transcription factor (Tcf)[4] signaling pathway [reviewed by Morin et al. (1) and Polakis et al. 2)]. As a consequence, various downstream target genes (e.g., c-*myc*, c-*jun*, and *cyclin D1*) become strongly overexpressed in human colon cancers. A major goal of our laboratory is to find dietary factors that circumvent the abnormal β-catenin/Tcf signaling pathway and serve as cancer chemopreventive agents.

Inhibition of β-catenin signaling in vitro by tea polyphenols

We reported recently that isolated antioxidant polyphenols from tea, such as epigallocatechin-3-gallate (EGCG), as well as complete tea, inhibited the activity of β-catenin/Tcf signaling in vitro (3). Thus, when Tcf-4 and wild-type or mutant forms of β-catenin were overexpressed in HEK293 cells by transient transfection, a strong increase in reporter activity was detected using an optimal Tcf/Lef promoter–luciferase construct (TOPflash). However, physiologically relevant EGCG concentrations (2–25 μmol/L) strongly attenuated β-catenin/Tcf activity, and there was an apparent decrease in β-catenin protein levels in the cell lysates (3).

Inhibition of β-catenin signaling in vivo

To determine the possible in vivo relevance of our findings, we also studied the effects of tea on dysregulated β-catenin signaling in two mouse models, namely Apc^{min} and $A33^{\Delta N\beta\text{-cat}}$ mutant mice. The former species is well characterized with respect to spontaneous polyp formation in the small intestine and colon, resulting from a mutation in the murine *Apc* gene (4). Chemoprevention studies in Apc^{min} mice have excellent predictive value for clinical intervention studies of polyp recurrence in humans with familial adenomatous polyposis (5). $A33^{\Delta N\beta\text{-cat}}$ mice are new animal models engineered to conditionally express an *N*-terminally truncated form of β-catenin in the gastrointestinal (GI) tract (6). $A33^{\Delta N\beta\text{-cat}}$ mice have higher sensitivity than controls to colon polyp formation in response to carcinogen treatment. Carcinogen-treated $A33^{\Delta N\beta\text{-cat}}$ mice may prove to be an appropriate model for human sporadic colon cancer because tumors in this model result from a combination of genetic susceptibility and environmental exposure.

These 2 animal models of hereditary and sporadic colon cancer were used to determine whether teas [alone or in combination with the nonsteroidal antiinflammatory drug (NSAID) sulindac] are effective suppressing agents. Apc^{min} and $A33^{\Delta N\beta\text{-cat}}$ mice were treated with drinking water containing white or green tea (1.5%, 2 min brew), 80 μg/L sulindac, a combination of 80 μg/L sulindac in 1.5% white tea, or buffered water.

After 12 wk of treatment, Apc^{min} mice fed white tea, green tea, or sulindac had significantly fewer intestinal tumors than controls ($P < 0.05$) with protection provided by either tea alone comparable to that of 80 μg/L sulindac (7,8). Mice treated with a combination of white tea plus sulindac had fewer tumors than those fed either treatment alone ($P < 0.05$). β-Catenin and β-catenin/Tcf-4–regulated proteins cyclin D_1 and c-Jun were reduced in normal intestinal mucosa of tea-treated animals and almost eliminated in mice fed the combination treatment.

In contrast to the results obtained in Apc^{min} mice, 8 wk of treatment with white tea or sulindac alone did not provide notable protection against tumor multiplicity in the $A33^{\Delta N\beta\text{-cat}}$ mice (**Fig. 1**). However, $A33^{\Delta N\beta\text{-cat}}$ mice treated with the combination of 1.5% white tea plus 80 μg/L sulindac had significantly fewer polyps ($P < 0.05$) than control mice or

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[4] Abbreviations used: EGCG, epigallocatechin-3-gallate; GI, gastrointestinal; NSAID, nonsteroidal anti-inflammatory drug; Tcf, transcription factor.

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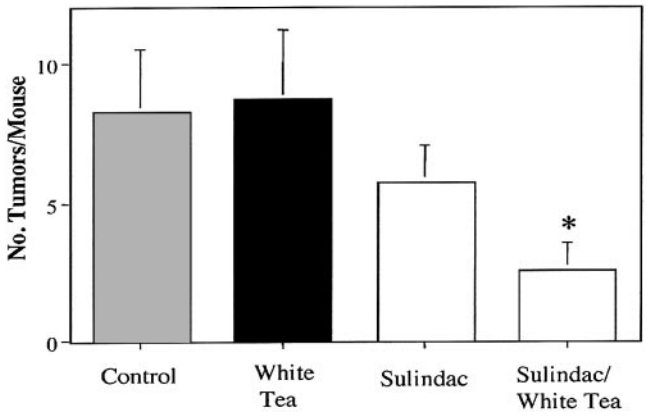

FIGURE 1 The combination of white tea plus sulindac suppresses colon polyp formation in *A33*$^{\Delta N\beta\text{-cat}}$ mice. Mice were initiated with azoxymethane (5 mg/kg body wt, 2 times/wk for 6 wk, i.p.) then beginning 1 wk postinitiation were treated with the test agents for an additional 8 wk.

mice fed either tea or sulindac alone. As in the *Apc*^{min} mice, sulindac treatment reduced levels of β-catenin and target proteins; however, in the *A33*$^{\Delta N\beta\text{-cat}}$ mice, the reduction occurred in the polyps rather than in the normal intestinal mucosa. Polyps from sulindac-treated mice had lower levels of β-catenin and target proteins than polyps from control or tea-treated mice (**Fig. 2**).

Summary, conclusions, and future directions

Both mouse models responded well to the combined chemopreventive effects of the NSAID, sulindac, and white tea or green tea, with evidence for strong tumor suppression (i.e., decreased multiplicity of polyps). Interestingly, these treatments apparently decreased β-catenin expression, as well as β-catenin/Tcf targets (c-Jun, cyclin D1), in either the normal-looking intestinal mucosa or the adjacent polyps (7,8).

These studies support a role for combined NSAID-tea treatment in the inhibition of intestinal and colon polyps and suggest the need for further studies on combined drug–diet interactions as an improved strategy for tumor suppression in the GI tract.

Studies currently are in progress to determine the mechanisms of tumor suppression and the reasons for the differences in response in the 2 animal models. Additional questions for investigation include whether the β-catenin–suppressing properties of the tea polyphenols are related to their antioxidant properties and whether the combination of tea plus sulindac is effective in humans.

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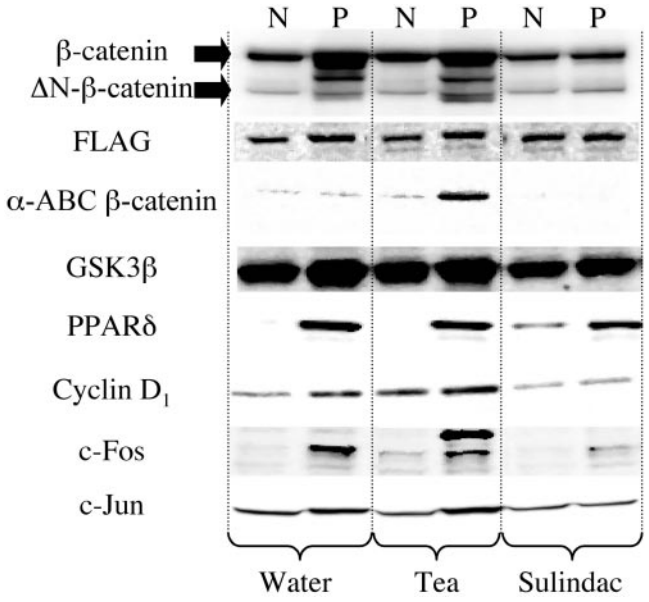

FIGURE 2 Sulindac lowers the expression of β-catenin and target proteins in the polyps of *A33*$^{\Delta N\beta\text{-cat}}$ mice treated with azoxymethane. Representative Western blots of normal-appearing colonic mucosa (*N*) and polyps (*P*) of *A33*$^{\Delta N\beta\text{-cat}}$ mice after 8 wk of treatment with drinking water (controls), white tea, or sulindac.

LITERATURE CITED

1. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B. & Kinzler, K. W. (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275: 1787–1790.

2. Polakis, P., Hart, M. & Rubinfeld, B. (1999) Defects in the regulation of beta-catenin in colorectal cancer. Adv. Exp. Med. Biol. 470: 23–32.

3. Dashwood, W. M., Orner, G. A. & Dashwood, R. H. (2002) Inhibition of beta-catenin/Tcf activity by white tea, green tea, and epigallocatechin-3-gallate (EGCG): minor contribution of H_2O_2 at physiologically relevant EGCG concentrations. Biochem. Biophys. Res. Commun. 296: 584–588.

4. Moser, A. R., Pitot, H. C. & Dove, W. F. (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247: 322–324.

5. Corpet, D. E. & Pierre, F. (2003) Point: from animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. Cancer Epidemiol. Biomark. Prev. 12: 391–400.

6. Tebbutt, N. C., Johnstone, C. N., Demmier, K., Grail, D., Heath, J. K. & Ernst, M. (2000) Generation of mice expressing conditional oncogenes in intestinal epithelial cells. Proc. Amer. Assoc. Cancer Res. 41: 848.

7. Orner, G. A., Dashwood, W. M., Blum, C. A., Diaz, G. D., Li, Q., Al-Fageeh, M., Tebbutt, N., Heath, J. K., Ernst, M. & Dashwood, R. H. (2002) Response of Apc(min) and A33 (delta N beta-cat) mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Mutat. Res. 506–507: 121–127.

8. Orner, G. A., Dashwood, W.-M., Blum, C. A., Diaz, G. D., Li, Q. & Dashwood, R. H. (2003) Suppression of tumorigenesis in the Apc^{min} mouse: down-regulation of β-catenin signaling by a combination of tea plus sulindac. Carcinogenesis 24: 263–267.