The ability of NSAIDs to inhibit polyp formation and stimulate Type I immunity is dose dependent

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Introduction

Colorectal cancer is the second most prevalent cancer in the developed world and the third most prevalent in developing nations. Prevention strategies are of great interest.

• Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be colorectal cancer (CRC) chemopreventive agents.

• NSAIDs demonstrate immune-stimulatory effects: activating local antigen presenting cells and limiting development of myeloid derived suppressor cells.

We compared various doses of two agents for CRC prevention, celecoxib and naproxen, and questioned whether the dose of drug was related to either clinical efficacy or potential immune stimulation.

Methods

Animals: All animal work was performed in accordance with the University of Washington Institute for Animal Care and Use Committee guidelines in a Specific Pathogen-Free environment. F1 Min mice were induced by breeding female APCR/l to male C3HBL/10AcPu/J (Jackson Laboratories). Offspring from breeding pairs were genotyped by PCR for the presence of the Min mutation using primers as follows:

Wild-Type: S-GCACCCTCCATATGTTA-G3’
Mutant: S-TGCTTAGAGAAGACAGAT7A

Both male and female mice testing positive for the Min mutation were included in the study and randomized into treatment groups.

Special Diet Preparation: Celecoxib and naproxen data were prepared by weighing the appropriate amount of drug and adding it to the appropriate amount of rodent diet in meal form (Diet 5053). The mixture was then sealed in two plastic bags and shaken by hand for 10 minutes. The final concentration of drug was validated by weighing the appropriate amount of drug and adding it to the appropriate amount of food bags and shaken by hand for 10 minutes. The final concentration of drug was validated each time to confirm proper dosing (Dr. Clinton Grubbs).

Experimental Design: Newborn aged 1 to 4 weeks were provided 30, 75, 150, or 450 ppm Naproxen or 30, 75, 125, 250, or 750 ppm Celecoxib daily, mixed with regular food taken at 7 weeks of age, mice were sacrificed and small bowel tumors quantified. The entire small intestine from F1 Min mice were removed at sacrifice and cut longitudinally with dissection scissors. Tissue samples were collected from both ends of the tissues, and tissues were fixed in formalin for a minimum of 24 hours. Intestinal tissues were removed from formalin and tumors were counted under a Nikon Eclipse microscope by the same observer.

Immunohistochemical Staining (IHC): Tumor sections were stained for CD8 positive cells by immunohistochemistry as previously described. Immediately after sacrifice, tumor were completely removed and placed in a Tris-Tau-Tryptic (T2T™) solution. Tissue sections were incubated with 10% normal donkey serum in PBS (0.1M, pH 7.4) for 1 hour followed by 10% normal donkey serum in PBS (0.1M, pH 7.4) for 1 hour at room temperature. The slides were incubated with rabbit polyclonal antibody anti-CD8 (AbD Serotec/Replace, NC) at 1:100 dilution overnight at 4°C. After washing with PBS three times, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature. The slides were washed with PBS 3 times. After washing with PBS, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature. The slides were washed with PBS 3 times. After washing with PBS, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature. The slides were washed with PBS 3 times. After washing with PBS, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature. The slides were washed with PBS 3 times. After washing with PBS, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature. The slides were washed with PBS 3 times. After washing with PBS, the slides were incubated with Alexa Fluor 488 goat anti-rabbit (Life Technologies, Carlsbad, CA) for 1 hour at room temperature.

Statistical Analysis: All statistical analysis was completed using GraphPad Prism v5.04 software (GraphPad Software, San Diego, CA). Small bowel tumor counts between treatment groups and inhibiting CD8 cells were compared by One-Way ANOVA with Tukey’s postest. Significance was considered at p<0.05.

Results

• Both celecoxib and naproxen demonstrated significant inhibition of polyp formation, which was dose dependent, when the treatment was initiated at 7 weeks.

• Celecoxib appeared to be more potent than naproxen, maintaining dose dependent efficacy even when the treatment was initiated at 18 weeks.

• Treatment with celecoxib increased infiltrating CD8+ T-cells into the polyps where they were found diffusely throughout the lesion.

Conclusions

• The data presented here suggests an immunologic effect of celecoxib in mediating regression of polyp formation.

Future studies will explore:

• Whether the ability to inhibit polyp formation is dependent on CD8 T cells

• Whether co-administration of celecoxib and active immunization against expressing by polyps will synergize to prevent tumor formation.

• Whether celecoxib has a role in prostate and/or immunotherapy in established colon cancer models.

References


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