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Nitric Oxide-releasing Medications and Colorectal Cancer Risk: The Framingham Study

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Abstract. *Background: The major sources of human exposure to nitric oxide (NO) are medicinal nitrovasodilators that release NO into the vasculature. Experimental NO-donating aspirin also releases NO in a similar manner, and is a potent in vitro inhibitor of colon cancer. Materials and Methods: The effects of nitrovasodilators on the risk of colorectal cancer was studied in the Framingham Heart and Offspring studies among 145 cases of colorectal cancer and 433 matched controls. Results: Eleven percent of controls reported currently using nitroglycerine or other long-lasting nitrates. In conditional logistic regression analysis, the odds ratio (OR) for colorectal cancer associated with nitrovasodilator use was 1.2 (95% confidence interval [CI] 0.6, 2.2). In subgroup analysis, the OR was 0.7 (95% CI 0.2, 2.2) in aspirin users and 1.6 (95% CI 0.8, 3.2) in subjects not taking aspirin. Conclusion: These data indicate that NO does not change the risk of colorectal cancer.*

Nitric oxide (NO) is a small biologically active molecule, whose production is determined by the expression of inducible nitric oxide synthase (iNOS or NOS2). The increase of NO biosynthesis in colonic tumor cells (1) is associated with cancer promotion and metastasis *via* increased oxidative DNA damage, cellular proliferation, angiogenesis and tumor growth (2). NOS2 activity is correlated with p53 mutations, and the generation of NO in "bursts" can reach local genotoxic concentrations. In addition, NOS2 inhibitors reduce the incidence of colon cancer incidence in laboratory rats, further indicating that NO promotes colon carcinogenesis (3). Paradoxically, NO can also inhibit colon carcinogenesis and induce apopotosis in tumor cells. Its effects appear to depend upon local conditions such as the level of expression of NOS2 and the differential sensitivity of tumor cells to NO-mediated actions (4).

The major endogeneous sources of human exposure to nitric oxide are nitrovasodilators, which include nitroglycerine (glycerol trinitrate), amyl nitrite, isosorbide mono- and di-nitrate, erythrityl tetranitrate and sodium nitroprusside. These medications are taken sublingually, orally or subcutaneously to treat angina pectoris and other manifestations of coronary artery disease. Nitroglycerine has been used effectively for over 100 years, and the other organic medicinal nitrates have been available since the 1930s. All routes of administration release the reactive free radical NO, which eventually relaxes the vascular smooth muscle (5-7). The multiple effects of NO on smooth muscles account to a large extent for their clinically useful effects as well as some side-effects. The so-called NO-donating nonsteroidal anti-inflammatory drugs (NO-NSAIDs) are a second class of medicinal NO-donors. NSAIDs inhibit the development of colorectal cancer (8,9) but their severe and sometimes fatal side-effects (10) and limited efficacy in interventional clinical trails (11) preclude their use as chemopreventive agents. NO-NSAIDs are novel experimental agents that have potent anticancer properties like NSAIDs but better gastric tolerability. They have a NO-releasing moiety covalently attached to a traditional NSAID such as aspirin (ASA). Although not yet approved by the Food and Drug Administration for consumer use, their potential clinical applications include the prevention of both cardiovascular disease and cancer (12). These compounds are considered safe based on short-term studies in humans (13) although their long-term safety has not been evaluated.

In theory, these medications can promote or inhibit the development of cancer. There is little data on the effects of NO-releasing vasodilators on cancer cell growth. The long-term feeding of glyceryl trinitrate in the diet of F344 rats induced hepatocellular carcinomas that were frequently characterized by K-ras point mutations (14). Consistent with

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this finding is data showing elevated blood levels of nitrates and nitrites in patients with hepatocellular carcinoma (15). In contrast, glyceryl trinitrate activated caspase activity to induce apoptosis in colon cancer cell lines (16), and inhibited tumorigenesis in murine skin (17). Isosorbite mononitrate and isosorbite dinitrate were observed to inhibit angiogenesis, tumor growth and metastasis in mice (18). We and other groups have shown that several NO-NSAIDs have strong chemoprevention properties against various cancers in preclinical studies (19, 20). Taken together, these findings perhaps underscore the diverse and potentially conflicting roles of NO in cancer.

Given the clinical importance and extensive use of NO-releasing vasodilators and also the emergence of NO-NSAIDS, we conducted a pilot study of the association between the commonly used NO-releasing nitrates and colorectal cancer risk using data from the Framingham Heart and Offspring studies.

Materials and Methods

The Framingham Heart study is an on-going population-based cohort of Caucasian middle class individuals that has made significant contributions to the etiology and prevention of cardiovascular disease (21). The initial study of 5,209 participants began in 1948, and a second cohort ("The Framingham Offspring Study") that included 5,124 children of the original participants and their spouses began in 1971 (22). The protocol of the Offspring study was modeled after the initial study and was based on an initial and biennial follow-up clinic visits. Each visit includes a medical examination and diagnostic measurements to detect heart disease. Participants also filled out a detailed structured questionnaire at each visit that includes medical history, the current use of specific medications and lifestyle information such as smoking habits. The Institutional Review Board for Human Research of the Boston Medical Center, USA, and the Human Investigation Research Committee of New England Medical Center approved the protocol. All participants provided written informed consent. A cancer diagnosis was determined by self-report at each examination, or by a telephone/mail questionnaire for subjects who missed their regularly scheduled examination. In addition, the study staff conducted surveillance of local hospital admissions and a review of all death records. The National Death Index was searched to identify subjects who had not been contacted or were lost to follow-up. If a death was identified, the cause of death was obtained and recorded. The medical and pathology records of each case were obtained from the treating hospitals and physician offices and the Framingham records were reviewed to confirm the location and earliest diagnosis of the cancer (23). The *International Classification of Disease for Oncology* (ICD-O codes 153.0-154.8) was used to code subjects with colorectal cancer. Ninety-four percent of cases were histologically verified (24). The study did not collect information on the diagnosis of adenomatous polyps in subjects without colorectal cancer.

The data used for this study included examination information that contained questions on current medicinal nitrovasodilator use, which started for the majority of participants in 1983/1984 and continued through November 1999 (Framingham exams 17-25 and Offspring exams 3-7). The year of the examination did not always match the year of cancer diagnosis, so we took data values from the examination date that was closest to the diagnosis date. We selected the biennial examination data prior to the diagnosis if it existed (*e.g.* within two years), and if not the closest examination data following the diagnosis. For the latter, if the closest examination date was the initial examination (17 or 3, respectively) we included only cases diagnosed two years before the examination. Three controls were matched to each case by study cohort, age at examination and sex. A total of 145 newly diagnosed incident cases of colorectal cancer and 433 matched controls were included in the final dataset.

Conditional logistic regression analysis was conducted to calculate odds ratios and 95 percent confidence intervals associated with current nitrovasodilator use including nitroglycerine and other long-lasting nitrates. Unconditional methods were used for subgroup analyses based on unmatched strata. All tests were 2-sided and performed using SAS statistical software (Cary, NC, USA).

Results

The ages, levels of education, smoking status and alcohol consumption of the subjects are shown in Table I. Sixty-seven percent of both cases and controls were original study participants and 33% were Offspring study participants. Among cases, 72.8% were diagnosed with colon cancer and 27.2% with rectal cancer.

About 12% of cases (13% of colon cancer cases and 8% of rectal cancer cases) and 10.6% of controls currently took nitrovasodilator medications. For subjects who participated in multiple examinations during this study period, none of them who were classified as nonusers at the latest examination had a past history of nitrovasodilator use in the previous examinations. The odds ratio for colorectal cancer associated with any current nitrovasodilator medication use was 1.18 (95% CI 0.64-2.17) (Table II). Similar risks were found for nitroglycerine use only, and for other long-lasting nitrates only (Table II). Thirty-five percent of controls and 28% of cases regularly used aspirin (≥1/week; OR=0.78, 95% CI 0.5, 1.20). The risk among subjects taking more than three times per week was the same (OR=0.78 (95% CI 0.49, 1.25; Table II). An interaction term for nitrate use and regular aspirin use was not statistically significant ($p=0.16$). In unconditional analysis, the odds ratio associated with nitrate medication was 0.69 (95% CI 0.21, 2.23) in regular aspirin users (≥1/week) and 1.55 (95% CI 0.75, 3.19) in subjects who were not regular aspirin users.

Discussion

The Framingham study data indicates that nitrovasodilators, which have been used for over 100 years for the treatment of cardiovascular disease, do not increase the risk of colorectal cancer. The data suggests a possible synergistic chemoprotective protective effect of NO and aspirin use

Table I. *Characteristics of cases and controls in the Framingham studies.*

	Cases N=145	Controls N=433
Sex (men)	54%	54%
Mean age	71.4±10.7	72.0±10.5
Mean years of education	12.5±3.4	12.3±3.3
Smoking status at last exam*		
Non smoking	63.5%	79.2%
Current smoking	36.6%	20.8%
Alcohol drinking status*		
Non-drinker	41.3%	43.6%
<1 drink/day	12.6%	22.4%
1-2 drinks/day	12.6%	10.3%
≥3 drinks/day	33.6%	23.7%
Cancer site		
Colon cancer	72.8%	
Rectal cancer	27.2%	

*Within the past year. Subjects reported alcohol consumption in terms of bottles of beer, glasses of wine and cocktails. These drinks have approximately the same amount of ethanol, and the total number of drinks was calculated for each subject. Information on alcohol consumption was unavailable for 77 (13%) subjects.

Table II. *Nitrovasodilator use, aspirin and colorectal cancer risk in the Framingham studies.*

Medication	Cases N=145	Controls N=433	OR	95% CI
Nitrovasodilator				
No use	88.3%	89.4%	1.0	
Nitroglycerine/nitrates	11.7%	10.6%	1.18	0.64, 2.17
Nitroglycerine	6.9%	6.7%	1.08	0.50, 2.35
Longer-acting nitrates*	8.3%	7.4%	1.23	0.60, 2.54
Aspirin				
Never/<1/week	71.7%	65.1%	1.0	
1-3/week	5.7%	6.2%	0.74	0.32, 1.73
>3/week	22.8%	28.6%	0.78	0.49, 1.25

*Mono-,di, and tetranitrates. Odds ratios for nitrovasodilator use are adjusted for smoking and aspirin use. Odds ratios for aspirin use are adjusted for nitrovasodilator use and smoking.

which, although speculative because of the limited statistical power to detect interactions, seems consistent with an increased chemopreventive effect of NO-releasing aspirin compared to aspirin alone (25).

The validity of self-reported nitrovasodilator use has not been reported, but prescription drug use in general is usually reported accurately (26-28). In a study of medications that induce gastric reflux and esophageal carcinoma risk, an association was observed with several anticholinergics but not with nitroglycerine (29). The percent of controls that used nitroglycerine in this study was similar to that in our data (5.7% *vs.* 6.7%). The lack of an association with nitroglycerine use would also appear to be consistent with the conclusions from the current study.

As a secondary data analysis, the analysis had several limitations. Because some cases were enrolled only through the initial examination of the study period, misclassification might have affected the findings. For example, it is possible that a subject diagnosed with cancer in 1983 and reported taking nitrovasodilators in 1984 might have first started taking the medication after the cancer diagnosis. There was no baseline information on the age at first nitrovasodilator use, and it was not possible to examine this association by duration, dosage, or method of administration. Information was not gathered on the indications for nitrovasodilator use such as acute symptomatic relief or long-term prophylactic management of angina pectoris. We did not analyze information on dietary habits, which was obtained in earlier examinations but might not have reflected current eating habits (30). The current findings of an increased colorectal cancer risk associated with cigarette smoking, heavy alcohol consumption and low aspirin intake are consistent with previous reports.

The current findings seem consistent with data showing that NO-aspirin was non-toxic in a short-term clinical trial, and with studies showing no promoting effects for intestinal cancer in animals (31). Although the pharmacokinetics of organic nitrates and NO-NSAIDs differ in some respects, they share sufficient similarities that suggest that NO-NSAID use does not increase the risk of colorectal cancer. Since traditional NSAIDs are currently considered unacceptable choices for chemoprevention, and the newer COX-2 inhibitors have recently been shown to have adverse cardiovascular health effects, the potential use of NO-donating NSAIDs for the treatment of pain and chemoprevention will require further exploration of their health effects.

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