Research Article

Cancer Prevention Research

Alpha-Blockers As Colorectal Cancer Chemopreventive: Findings from a Case–Control Study, Human Cell Cultures, and *In Vivo* Preclinical Testing

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Abstract

A retrospective case–controlled analysis was performed to identify drug candidates in the current use that may prevent colorectal cancer, outside of aspirin. A total of 37,510 patients aged ≥20 years were assessed to identify subjects who had been diagnosed with colorectal cancer by colonoscopy without a previous diagnosis of colorectal cancer, inflammatory bowel disease (IBD), or gastrointestinal symptoms; 1,560 patients were identified who were diagnosed with colorectal cancer by colonoscopy. The patients with colorectal cancer were matched with 1,560 age, gender, family history of colorectal cancer and comorbidity-matched control patients who were not diagnosed with colorectal cancer at colonoscopy. The medication histories were compared between the two groups. Next, candidate drugs that were more frequently used by the control patients were selected and their effects on human colorectal cancer cell lines *in vitro* and an inflammation-induced mouse model of colorectal cancer were tested. Putative colorectal cancer preventative agents were identified, including aspirin, vitamin D, vitamin B, vitamin C, vitamin E, xanthine oxidase inhibitor, alpha-blockers, angiotensin receptor blocker, nateglinide, probiotics, thienopyridine, folic acid, nitrovasodilators, bisphosphonates, calcium channel blockers, steroids, and statins ($P < 0.05$). Alpha-blockers and xanthine oxidase inhibitors were selected for further study because these agents have not been analyzed previously as factors that may affect colorectal cancer outcomes. *In vitro* doxazosin (alpha-blocker), but not febuxostat (xanthine oxidase inhibitor), suppressed the proliferation of human colorectal cancer cells. Doxazosin also decreased tumorigenesis in an AOM/DSS mouse colorectal cancer model. Alpha-blockers may prevent colorectal cancer.

Introduction

Drug repositioning involves using existing drugs for new indications. This strategy has attracted attention in recent years (1) as it is much more economical than developing new drugs. Colorectal cancer is the third most prevalent cancer worldwide; 1.36×10^6 people are affected with it and 0.69×10^6 people were expected to die from colorectal cancer in 2012 (2). As such, this disease places a huge social and economic burden on our communities, highlighting the significant benefit of new, low-cost chemopreventative agents for this disease. The colorectal cancer preventative properties of commonly used, existing drugs, aspirin, have been shown (3, 4).

Colorectal cancer is associated with chronic diseases such as obesity and diabetes (5–8). Patients with these conditions are continuously treated with various drugs, some of which are candidates for drug repositioning for colorectal cancer. As occurred with aspirin, a prospective intervention trial for any new agents to assess colorectal cancer outcomes will first require analysis of

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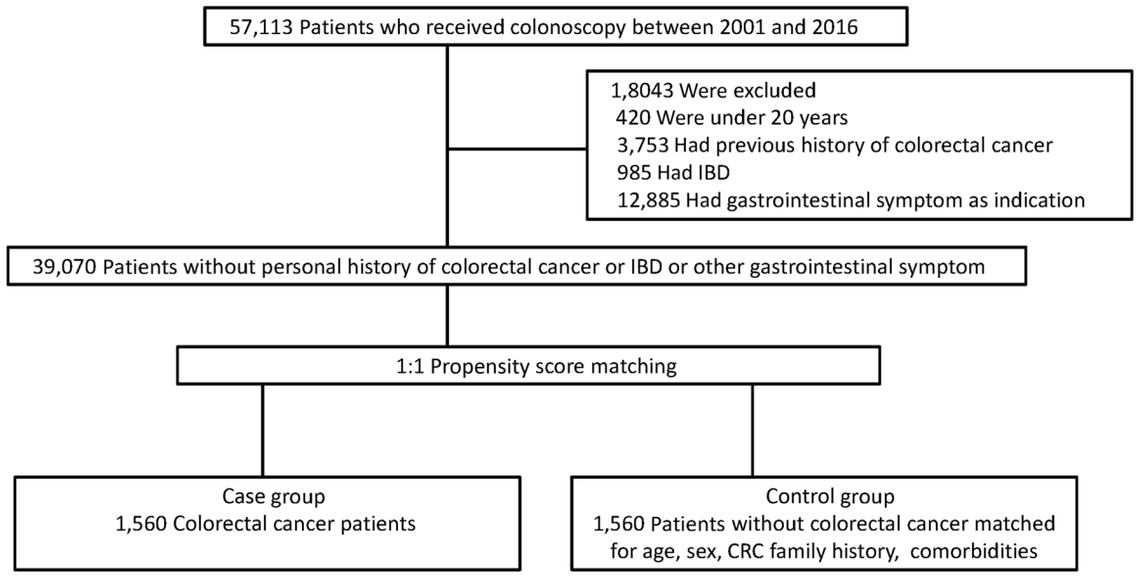

Figure 1.
Study flowchart.

retrospectively collected data from existing cohorts (9, 10). Patients with colorectal cancer often suffer from multiple chronic diseases, and it is not unusual for them to take more than one drug. Therefore, it can be difficult to conduct retrospective analyses on such a diverse group of patients. Nevertheless, if other existing drugs are identified with colorectal cancer preventive effects similar to aspirin, it could be useful. Using drugs that are already approved for use is far more economical than developing new drugs.

To discover novel drug repositioning candidates for preventing colorectal cancer, we conducted a retrospective multi-center propensity-matched study. On the basis of our findings, we selected an alpha-adrenoceptor blocker and a xanthine oxidase inhibitor as drug repositioning candidates, with aspirin serving as a positive control and beta-blockers as a negative control. We then examined the effect of these agents on human colorectal cancer cells *in vitro* and an inflammation-associated mouse model of colorectal cancer.

Materials and Methods

Data source

We previously developed a Colorectal Cancer Endoscopy (CCE) Database at Tokyo University. This was a retrospectively recorded database of patients who underwent colonoscopy at one referral hospital and four territorial hospitals in Japan; data were compiled from 57,113 patients between 2001 and 2016 (11). The database includes the following information: patient characteristics, indications for colonoscopy, colonoscopy findings, and colorectal cancer data including the site of cancer, the Union for International Cancer Control cancer stage, cancer therapy, and drug history.

Study design, setting, and participants

We performed a retrospective case–control analysis of Japanese adults using the CCE database. Data were extracted from this database from 37,555 patients aged $\geq$20 years, including those without a previous diagnosis of colorectal cancer, inflammatory bowel disease (IBD), or other gastrointestinal symptoms. Next, 1,560 patients who were diagnosed with colorectal cancer from a colonoscopy and a control group of 1,560 patients not diagnosed with colorectal cancer were selected (Fig. 1). Colorectal cancer was diagnosed at colonoscopy and confirmed by pathology. The human investigations were performed after approval by Institutional Review Boards of all participating institutions (the University of Tokyo, Tokyo, Japan; the Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan; Japanese Red Cross Medical Center, Tokyo, Japan; JR Tokyo General Hospital, Tokyo, Japan; and Yaizu City Hospital, Shizuoka, Japan), according with the policy of the Japanese Ministry of Health, Labor and Welfare. Written informed consent from patients was obtained from each subject in accordance with Declaration of Helsinki.

Outcomes and variables

The primary outcome measure was the risk of colorectal cancer incidence associated with drug exposure, as determined by ORs. Colorectal cancer cases were categorized according to the location of the tumor (all colorectal, right-sided colon, and left-sided colon/rectal).

Use of the following 40 classes of drugs was assessed: antihypertensives (angiotensin-converting enzyme inhibitor, alpha-blocker, beta-blocker, angiotensin II receptor blocker, and calcium channel blocker), antidiabetics (biguanide, pioglitazone, insulin, sulfonylurea, alpha-glucosidase inhibitor, dipeptidyl peptidase-4 [DPP4] inhibitor, and nateglinide), diuretics (loop diuretic, benzothiazide diuretic, and thiazide diuretic), xanthine oxidase inhibitor (allopurinol and febuxostat) fibrates, statin, low-dose aspirin, selective COX-2 inhibitors (celecoxib), non-steroidal anti-inflammatory drugs (NSAID; loxoprofen, diclofenac sodium, and others), acetaminophen, thienopyridines (ticlopidine and clopidogrel), cilostazol, non-aspirin antiplatelet drugs (dipyridamole and eicosapentaenoic acid), anticoagulants [warfarin or non-vitamin K antagonist oral anticoagulants (NOAC) including dabigatran, rivaroxaban, and edoxaban], steroids, vitamins (vitamins B, C, D, E, and K), calcium, folic acid, probiotics, thiamazole, and potassium sodium hydrogen citrate. Use of a medication was defined as oral administration starting at least 4 weeks before colonoscopy. Use of NSAIDs included intermittent use within 4 weeks of colonoscopy.

Confounders including age, sex, family history of colorectal cancer, and other comorbidities were evaluated. Age was categorized into quintiles. Comorbidities were evaluated using the Charlson Comorbidity Index (12).

Statistical analysis

Propensity scores were estimated using a logistic regression model for colorectal cancer cases as a function of patient demographics. Eighteen factors were included as potentially clinically significant variables: age, sex, ischemic heart disease, chronic heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic obstructive pulmonary disease, collagen disease, peptic ulcer, diabetes mellitus, chronic kidney disease, paresthesia, leukemia, malignant lymphoma, liver cirrhosis, acquired immunodeficiency syndrome (AIDS), and a family history of colorectal cancer. One-to-one matching analyses were also performed between the two groups (colorectal cancer and non-colorectal cancer) using the nearest neighbor method within a caliper width of 0.2 of the SD of the logit of the propensity score. After propensity matching, differences in the prevalence of drug exposure were compared between the two groups' ORs as an estimate of whether colorectal cancer incidence was associated with drug exposure. We did not perform double adjustment after propensity score matching because covariate balance is already achieved in matched population.

Statistical analyses were performed using SAS software version 9.4 (SAS Institute). $P < 0.05$ was considered statistically significant.

Mouse tumor assays

Both experimental and control groups consisted of 6 (3 males, 3 females) C57BL6/J (CLEA, Japan) littermate mice. Primary outcome was set to tumor incidence per mouse. Then, the sample power (6 vs. 6) size was calculated by Power and Sample Size Calculation software provided from University of Vanderbilt (Nashville, TN) and approved by animal ethics committee of Asahi Life. Mice were injected intraperitoneally with 12.5 mg/kg AOM (Sigma-Aldrich) on day 1. After 5 days, mice received water supplemented with 2.5% DSS (MP Biomedicals) for 5 days, after which the mice were maintained on regular water for 14 days and then subjected to two identical additional DSS treatment cycles (13). On days 1–64, the experimental mice were injected intraperitoneally with doxazosin (Tokyo-Kasei; 5 mg/kg) 5 days a week. The control mice were injected with PBS. On day 64, the mice were sacrificed and colon tumors were analyzed. Macroscopic colon tumors were counted and the longest diameter of each tumor was measured using a digital caliper in a blinded fashion. The animal room was quarantined by airflow and maintained at a constant temperature and humidity under conventional conditions. All animal experiments were approved by the ethics committee of the Institute for Adult Diseases, Asahi Life Foundation, (Tokyo, Japan) and were performed according to the guidelines for the care and use of laboratory animals of the Institute for Adult Diseases, Asahi Life Foundation (Tokyo, Japan).

Cell culture and growth assays

HCT116 (RIKEN BRC, RCB2979) and RKO (ATCC, CRL2577) human colorectal cancer cells were cultured in McCoy's 5A and DMEM, respectively, containing 10% FBS. Additions to the culture medium included 0.6% DMSO (vehicle), doxazosin (1, 5, 15, and 30 μmol/L), febuxostat (1 and 10 μmol/L), aspirin (0.31 μmol/L, 0.62 μmol/L, 1.25 mmol/L, 2.5 mmol/L, and 5 mmol/L), and bisoprolol (12.5, 25, 50, and 100 μmol/L). Cell growth was measured using Cell Counting Kit-8 (CCK-8) from Dojindo Laboratories. Cells (1.5×10^4/mL) were seeded into 48-well plates and exposed to vehicle, febuxostat, doxazosin, aspirin, or bisoprolol. CCK-8 solution was added to each well at 24, 48, and 72 hours and the absorbance was read at 450 nm using a plate reader (SpectraMax, Molecular Devices). *Mycoplasma* negative of both cells was confirmed by MycoAlert Kit (Lonza) within a month from experiments.

Results

Case–Controlled analysis of patients with colorectal cancer

Between 2001 and 2016, 57,113 patients were enrolled in our database at five centers after receiving colonoscopy without any abdominal symptoms. A total of 18,043 patients aged <20 years or with a history of colorectal cancer or IBD were excluded. Of the patients in the database, 1,560 were diagnosed with colorectal cancer and 37,510 were not at the initial colonoscopy (Fig. 1). The

Table 1. Baseline characteristics

	All patients			Propensity score–matched patients		
Variables	**Non–colorectal cancer (n = 37,510)**	**Colorectal cancer (n = 1,560)**	***P***	**Non–colorectal cancer (n = 1,560)**	**Colorectal cancer (n = 1,560)**	***P***
Age, years			<**0.001**			0.999
<50	7,743 (20.64)	278 (5.45)		83 (5.32)	85 (5.45)	
50–59	8,405 (22.41)	542 (16.54)		253 (16.22)	258 (16.54)	
60–69	10,427 (27.80)	942 (29.29)		462 (29.62)	457 (29.29)	
70–79	8,270 (22.05)	936 (31.86)		498 (31.92)	497 (31.86)	
≥80	2,665 (7.10)	490 (16.86)		264 (16.92)	263 (16.86)	
Male	24,383 (65.00)	1,003 (64.29)	0.565	1,008 (64.62)	1,003 (64.29)	0.852
Comorbidities						
Ischemic heart disease	4,838 (12.90)	347 (22.24)	<**0.001**	341 (21.86)	347 (22.24)	0.796
Congestive heart failure	3,540 (9.44)	280 (17.95)	<**0.001**	280 (17.95)	280 (17.95)	1.000
Peripheral vascular disease	1,870 (4.79)	112 (7.18)	<**0.001**	117 (3.75)	112 (3.59)	0.728
Cerebrovascular disease	2,288 (6.10)	189 (12.12)	<**0.001**	192 (12.31)	189 (12.12)	0.870
Dementia	402 (1.07)	48 (3.08)	<**0.001**	40 (2.56)	48 (3.08)	0.387
COPD	652 (1.74)	51 (3.27)	<**0.001**	50 (3.21)	51 (3.27)	0.919
Collagen disease	1,449 (3.86)	64 (4.10)	0.631	55 (3.53)	64 (4.10)	0.400
Peptic ulcer disease	11,419 (30.44)	710 (45.51)	<**0.001**	714 (45.77)	710 (45.51)	0.886
Diabetes	9,484 (25.28)	601 (38.53)	<**0.001**	612 (39.23)	601 (38.53)	0.686
Chronic kidney disease	654 (1.74)	38 (2.44)	**0.042**	44 (2.82)	38 (2.44)	0.502
Hemiplegia	745 (1.99)	80 (5.13)	<**0.001**	75 (4.81)	80 (5.13)	0.680
Leukemia	119 (0.32)	3 (0.19)	0.386	4 (0.26)	3 (0.19)	0.705
Malignant lymphoma	567 (1.51)	24 (1.54)	0.932	25 (1.60)	24 (1.54)	0.886
Liver cirrhosis	762 (2.03)	35 (2.24)	0.561	28 (1.79)	35 (2.24)	0.373
AIDS	155 (0.41)	27 (1.73)	<**0.001**	27 (1.73)	27 (1.73)	0.438
Family history of colorectal cancer	230 (0.61)	22 (1.41)	<**0.001**	23 (1.47)	22 (1.41)	0.881

NOTE: Bold indicates statistical significance ($P < 0.05$).
Abbreviation: COPD, chronic obstructive pulmonary disease.

baseline data for all patients and data after 1:1 power density matching are presented in Table 1.

The site of colorectal cancer was the right colon for 265 cases (17.0%), left colon for 521 cases (33.4%), and both left and right for one case (0.1%); 773 cases (49.6%) had no data for colorectal cancer site (Table 2). The use history for the 40 drug classes was compared between the colorectal cancer and non–colorectal cancer groups. Use of alpha-blockers (OR, 0.69), xanthine oxidase inhibitors (OR, 0.69), aspirin (OR, 0.63), vitamin D (OR, 0.36), vitamin B, vitamin C, vitamin E, ARB, nateglinide, probiotics, thienopyridine, folic acid, nitrovasodilators, bisphosphonates, Ca-blockers, calcium, steroids, and statins were significantly associated with a decreased risk of colorectal cancer (Table 3). In the CCE, doxazosin, febuxostat, and bisoprolol were the most frequently used alpha-blocker, xanthine oxidase inhibitor, and beta-blocker, respectively.

Cell proliferation assay

Doxazosin (alpha-blocker) suppressed the proliferation of RKO in a concentration-dependent manner (Fig. 2). There were significant differences at the following points (24 hours: vehicle > 15 μmol/L > 30 μmol/L, 48 and 72 hours: vehicle > 5 μmol/L > 15 μmol/L > 30 μmol/L; $P < 0.05$). At high concentrations, the proliferation of HCT116 cells was also significantly suppressed at 24, 48, and 72 hours; $P < 0.05$. In contrast, febuxostat (xanthine oxidase inhibitor) and bisoprolol did not inhibit the proliferation of either HCT116 or RKO cells. Aspirin suppressed the proliferation of RKO and HCT116 in a concentration-dependent manner. There were significant differences at the following points (RKO 24 hours: vehicle > 5 mmol/L, 42 hours: vehicle > 2.5 mmol/L > 5 mmol/L, 72 hours: vehicle > 1.25 mmol/L > 2.5 mmol/L > 5 mmol/L, HCT116 24 hours: vehicle > 5 mmol/L, and 42 and 72 hours: vehicle>2.5 mmol/L > 5 mmol/L; $P < 0.05$).

Mouse tumor assay

Next, the inflammation-associated colorectal cancer mouse model, AOM/DSS, was used to test whether doxazosin treatment affected colorectal cancer *in vivo*. As shown in the schematic in Fig. 3A, wild-type C57BL6 mice received AOM/DSS to establish distal colon tumors. Doxazosin treatment began on day 1 and continued until the animals were sacrificed on day 64. Macroscopic observation of dissected colons from the mice revealed that a mean of three colon tumors were present in the middle to distal colon of the doxazosin-treated group; the average tumor size was 3 mm. In the control group, approximately six colon tumors sized approximately 5 mm were present (Fig. 3B). The tumor number and size were significantly smaller in the doxazosin group than the control group ($P < 0.05$; Fig. 3C). Both groups had tumors with similar pathology on hematoxylin and eosin (H&E) staining: tubular adenomas or well-differentiated adenocarcinomas (Fig. 3D).

In the AOM/DSS model, weight loss correlates with the severity of colitis. There was no significant difference in body weight between treatment groups (Fig. 3E). Because

Table 2. Site of cancer at diagnosis ($N = 1{,}560$)

Site	Number of patients
Right-sided colon	265 (17.0)
Appendix	2 (0.06)
Cecum	56 (1.79)
Ascending	133 (8.53)
Transverse	74 (4.74)
Left-sided colon	521 (33.4)
Descending	33 (2.12)
Sigmoid	252 (16.15)
Rectum	236 (15.13)
Double	
Cecum + Rectum	1 (0.06)
Data not provided	773 (49.55)

doxazosin inhibited colorectal cancer cell proliferation *in vitro*, Ki67 IHC was performed to assess cell proliferation in the mouse tumors; there was no difference between the treatment groups (Fig. 3F and G).

Discussion

This large case–controlled study suggests that, like aspirin and vitamin D, alpha-blockers, xanthine oxidase inhibitors, vitamin B, vitamin C, vitamin E, ARB, nateglinide, probiotics, thienopyridine, folic acid, nitrovasodilators, bisphosphonates, Ca-channel blockers, steroids, and statins may have colorectal cancer preventive effects. Aspirin is a well-validated colorectal cancer chemopreventative (3, 4, 9, 10). Also, vitamin D and calcium are supported by some large observational studies (14–19). However, recent clinical trial results did not show the association between vitamin D supplementation and invasive colorectal cancer (20). In this study, only 7 patients received calcium by prescription and it was not significantly associated with colorectal cancer prevention. Many of the remaining hits in this study have been previously assessed for colorectal cancer preventative effects, including vitamin B, vitamin

Table 3. Association between drug exposure and colorectal cancer

Drugs	Non–colorectal cancer ($n = 1{,}560$)	Colorectal cancer ($n = 1{,}560$)	OR (95% CI)	*P*
ACE	55	58	1.06 (0.73–1.54)	0.774
Acetaminophen	91	72	0.78 (0.57–1.07)	0.126
Pioglitazone	53	37	0.69 (0.45–1.06)	0.089
aGI	68	74	1.09 (0.78–1.53)	0.607
Alpha-blocker	103	73	**0.69 (0.51–0.95)**	**0.021**
Xanthine oxidase inhibitor	100	64	**0.63 (0.45–0.86)**	**0.004**
ARB	266	205	**0.74 (0.60–0.90)**	**0.002**
Aspirin	153	100	**0.63 (0.48–0.82)**	**0.001**
Beta-blocker	96	77	0.79 (0.58–1.08)	0.137
Biguanide	72	65	0.89 (0.64–1.26)	0.541
Bisphosphonates	44	21	**0.47 (0.28–0.80)**	**0.005**
Ca-blocker	308	239	**0.74 (0.61–0.89)**	**0.001**
Calcium	2	5	2.50 (0.49–12.9)	0.273
Cox2-inhibitor	26	15	0.57 (0.30–1.09)	0.088
DPP4-inhobitor	41	26	0.63 (0.38–1.03)	0.066
EPA	21	10	0.47 (0.22–1.01)	0.052
Nateglinide	31	16	**0.51 (0.28–0.94)**	**0.030**
Fibrates	24	19	0.79 (0.43–1.45)	0.446
Insulin	53	59	1.12 (0.77–1.63)	0.564
Nitrovasodilator	70	48	**0.68 (0.47–0.98**)	**0.040**
NOAC	8	4	0.50 (0.15–1.66)	0.257
NSAIDs	212	189	0.95 (0.80–1.13)	0.219
Dipyridamole	5	5	1.00 (0.29–3.46)	1.000
Cilostazol	23	20	0.87 (0.48–1.59)	0.646
Diuretics	124	99	0.79 (0.60–1.03)	0.083
Selective estrogen receptor modulators	9	4	0.43 (0.13–1.42)	0.164
Statin	216	165	**0.74 (0.59–0.91)**	**0.005**
Steroid	67	45	**0.66 (0.45–0.97)**	**0.006**
Sulfonylurea	113	125	1.12 (0.86–1.45)	0.419
Thienopyridines	59	37	**0.62 (0.41–0.94)**	**0.024**
Thiamazole	23	15	0.65 (0.34–1.25)	0.192
Potassium sodium hydrogen citrate	12	7	0.58 (0.23–1.48)	0.256
Vitamin B	135	91	**0.65 (0.49–0.86)**	**0.003**
Vitamin C	30	12	**0.40 (0.20–0.78)**	**0.007**
Vitamin D	54	20	**0.36 (0.22–0.61**	**<0.001**
Vitamin E	8	7	**0.44 (0.25–0.78)**	**0.005**
Warfarin	45	36	0.80 (0.51–1.24)	0.314
Probiotics	100	74	**0.73 (0.53–0.99)**	**0.043**
Folic acid	18	6	**0.33 (0.13–0.84)**	**0.019**
Uricosuric	12	21	1.76 (0.86–3.59)	0.120

NOTE: Forty Drugs. Bold indicates statistical significance ($P < 0.05$).

Abbreviations: ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; aGI, alpha-glucosidase inhibitor; Ca-blocker, calcium channel blocker; DPP4-inhibitor, dipeptidyl peptidase-4 inhibitor; EPA, eicosapentaenoic acid.

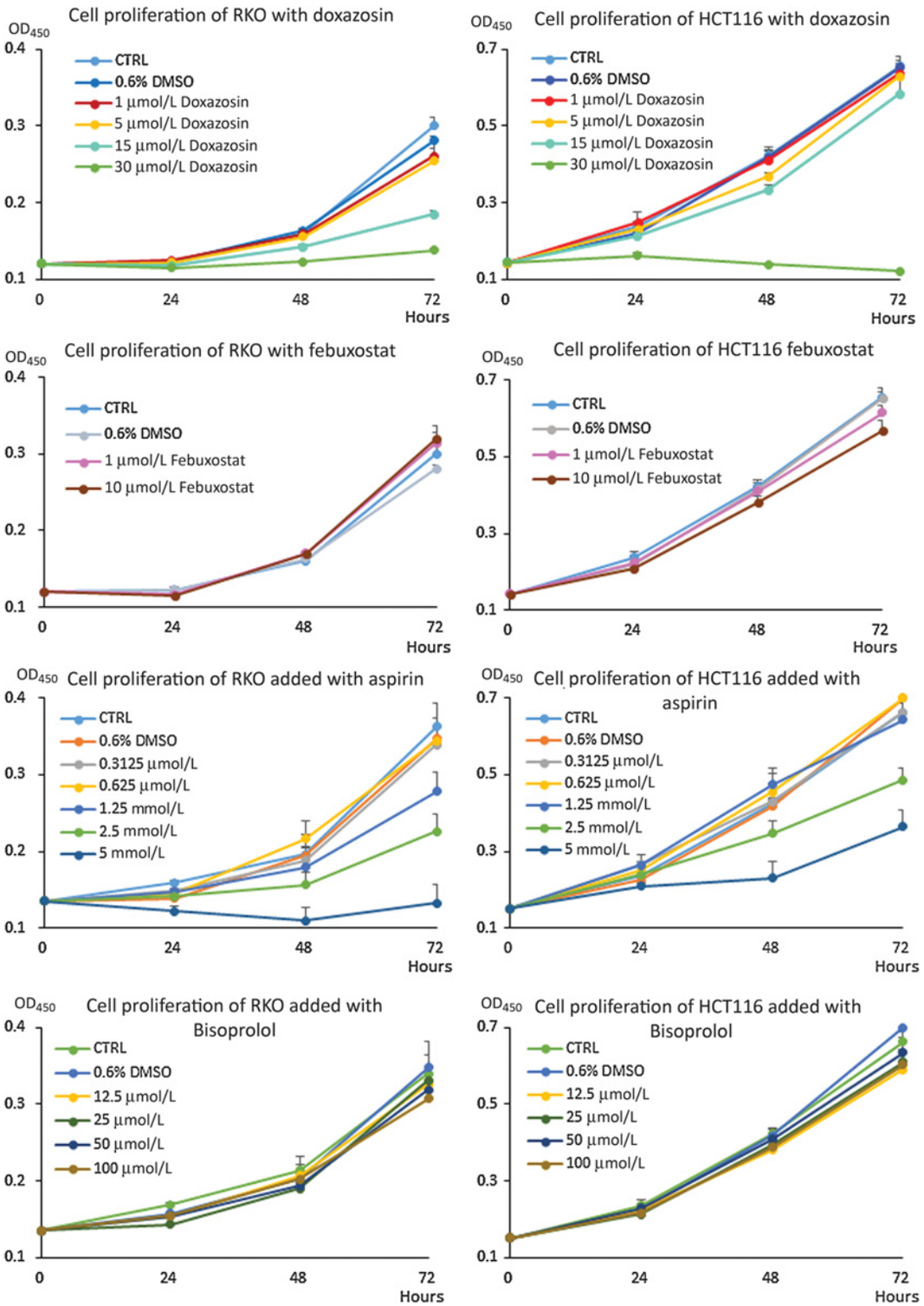

Figure 2.

Doxazosin (alpha-blocker) but not febuxostat inhibits human colorectal cancer cell proliferation. **A,** Growth curves of RKO and HCT116 cells treated with 0.6% DMSO, doxazosin (1, 5, 15, or 30 μmol/L), febuxostat (1 and 10 μmol/L), aspirin (0.31 μmol/L, 0.62 μmol/L, 1.25 mmol/L, 2.5 mmol/L, and 5 mmol/L), bisoprolol (12.5, 25, 50, and 100 μmol/L) as determined using cell proliferation assays. Untreated cells were used as a control. Results depict a single biological replicate (n = 8). Data shown are means and SDs.

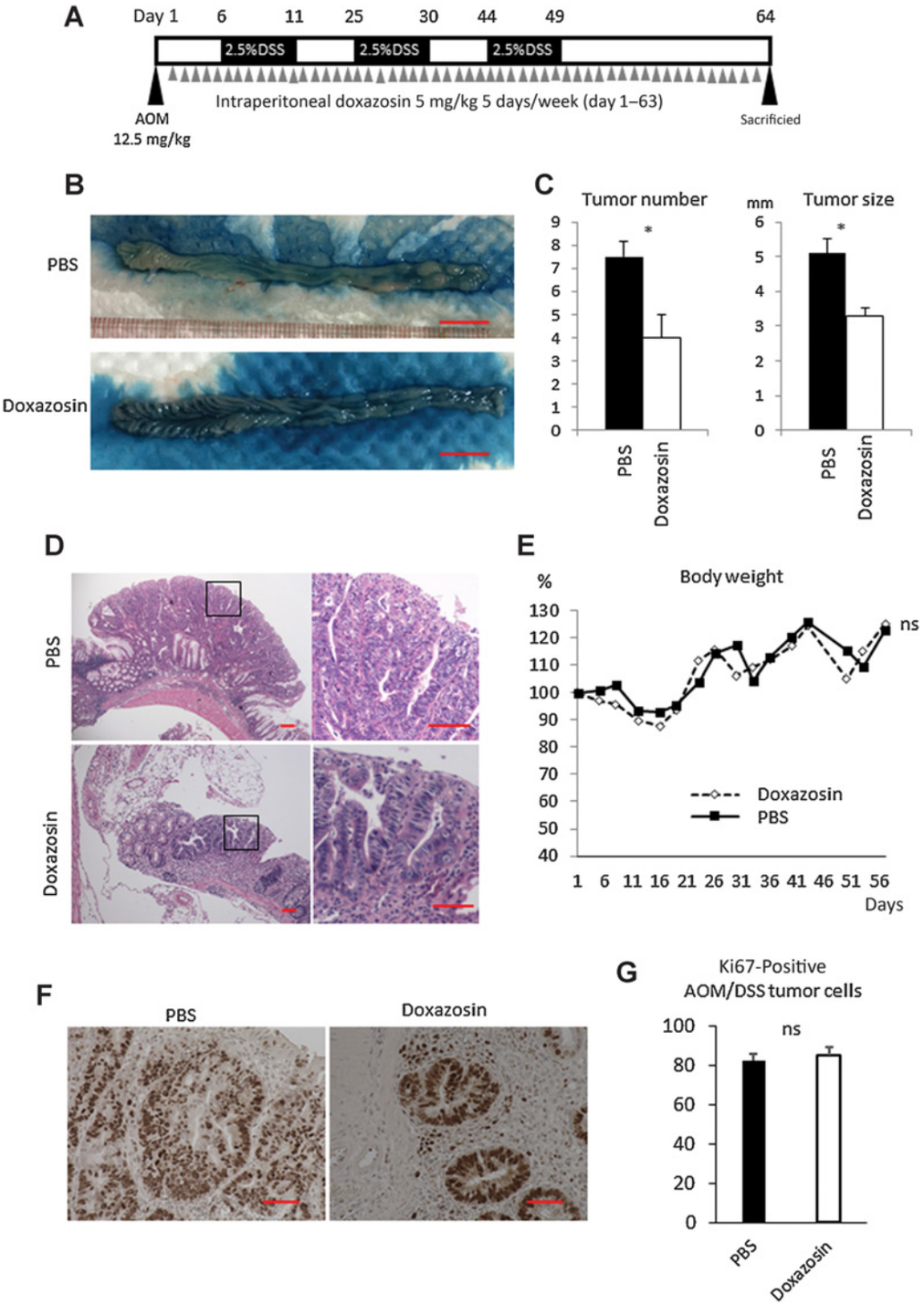

Figure 3.

A, Schematic representation of the mouse assay of doxazosin administration on AOM/DSS-induced colon tumors. **B,** Typical macroscopic AOM/DSS-induced colorectal tumors in mice treated with doxazosin or PBS. Scale bars = 1 cm. **C,** Tumor number and tumor size were determined in PBS- ($n = 6$) and doxazosin-treated mice ($n = 6$). Data shown are means and SEM. *, $P < 0.05$ by Mann–Whitney U test. **D,** Representative histopathologic images of staining for H&E in doxazosin- or PBS-treated mice. Low magnification (100 ×; left) and high magnification (400 ×; right) images are shown. Scale bar = 200 μm (left), 50 μm (right). **E,** Body weight curves for each data point shown in the right. All data analyses were not significant (ns) according to Mann–Whitney U tests. **F,** Representative IHC images for Ki67 staining in AOM/DSS-derived colon tumors in mice treated with PBS or doxazosin. Scale bars = 50 μm. **G,** The proportion of Ki67-positive cells in AOM/DSS-derived tumors from PBS- and doxazosin-treated mice. Data shown are means ± SEM ($n = 3$; ns, no significant difference; Mann–Whitney *U* test).

C, vitamin E, folic acid, bisphosphonates, Ca-channel blockers, ARB, thienopyridine, nitrovasodilators, probiotics, statins, and steroids (21–32). Vitamin B, vitamin C, vitamin E, folic acid, bisphosphonates, and probiotics may have some colorectal cancer preventive effects, although the evidence is not yet strong.

Of the list of drugs that were potentially associated with colorectal cancer prevention in this study, only alpha-blockers and xanthine oxidase inhibitors have not been studied in detail in a colorectal cancer setting to date. Thus, we assessed their potential as colorectal cancer chemopreventative agents *in vitro* and *in vivo*. The alpha-blocker doxazosin both inhibited the growth of human colorectal cancer cell lines in culture and decreased tumor size and number in a colorectal cancer mouse model. Doxazosin targets alpha-adrenoceptor signaling and its use has not been studied in any great detail in colorectal cancer. Nevertheless, doxazosin suppressed bladder and prostate carcinogenesis in cohort studies (33, 34) and induced cancer cell apoptosis *in vitro* and *in vivo* (35, 36). According to a recent report, doxazosin reduced VEGF levels and angiogenesis in clinical prostate tumors (37). Importantly, tumor angiogenesis is maintained through nerve activation via adrenergic signaling (38). In this cell proliferation assays, doxazosin suppressed cancer cell proliferation; however, the proportion of Ki67-positive tumor cells in mice was not changed. Therefore, the cancer-suppressing effect of doxazosin might be due to apoptosis, necrosis, or changes to the microenvironment rather than cancer proliferation. This case–controlled study suggested that not only aspirin but also other drugs may be effective chemopreventative agents for colorectal cancer. Some drugs, such as Ca-channel blockers and steroids, had no such effect in previous studies (32, 39, 40). As these previous studies were retrospective analyses, they could have been affected by bias. To reduce the potential bias, we performed propensity matching using a comorbidity index.

Limitation

However, other factors also associated with colorectal cancer incidence were not accounted for, such as lifestyle factors including tobacco and alcohol use and body mass index (BMI) and socioeconomic status. Information regarding lifestyle factors and BMI were not collected for the CCE database, and the cohort does contain patients from diverse backgrounds. Patient economic status may be a strong bias as it affects the number of hospital visits and the ability of the patient to receive medications. As such, we used experimental approaches to assess the potential of doxazosin and febuxostat as chemopreventative agents for colorectal cancer. However, the concentration of these drugs in the cell proliferation assay was much higher than human plasma levels after administration. Doxazosin (30 μmol/L) corresponds to 13 ug/mL, which is higher than the interview form C_{max} 40 ng/mL. Although we validated doxazosin as an agent of interest for further testing, we have not verified its mechanism of action. As such, we plan to confirm this retrospective case–controlled study in a subsequent prospective trial and undertake further basic research in this area.

In conclusion, alpha-blockers are potential chemopreventatives for colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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Authors' Contributions

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