The dicarbonyl electrophile scavenger 2-hydroxybenzylamine (2-HOBA) prevents colorectal carcinogenesis and reduces tumor growth

Alain Gobert[1], Mohammad Asim[1], Thaddeus Smith[1], Kamery Williams[1], Daniel Barry[1], Margaret Allaman[1], Kara McNamara[1], Caroline Hawkins[1], Alberto Delgado[1], Shilin Zhao[1], M Piazuelo[1], M Washington[1], Lori Coburn[1], John Rathmacher[2], and Keith Wilson[1]

[1]Vanderbilt University Medical Center
[2]MTI Biotech

January 31, 2023

Abstract

Background and Purpose: Colorectal cancer (CRC) is a major health problem worldwide. Dicarbonyl electrophiles, such as isolevuglandins (isoLGs), are generated from lipid peroxidation and form covalent adducts with amine-containing macromolecules. We have shown high levels of adducts of isoLGs in colonic epithelial cells from patients with CRC. We thus investigated the role of these reactive aldehydes on colon cancer development. Experimental Approach: We investigated the effect of oral treatment with 2-hydroxybenzylamine (2-HOBA), a natural compound derived from buckwheat seeds that acts as a potent scavenger of electrophiles, on colon carcinogenesis using the azoxymethane-dextran sulfate sodium model of colitis-associated carcinogenesis and mice with epithelial-specific deletion of the adenomatous polyposis coli gene, as a model of sporadic cancer. We also tested 2-HOBA in a murine xenograft of human HCT116 CRC cells implanted into the flank of nude mice. Key Results: 2-HOBA is bioavailable in the colon of mice after supplementation in the drinking water and does affect the colonic microbiome. However, it reduced the level of isoLG adducts to lysine as well as tumorigenesis in both models of CRC. In parallel, we found that NRF2 activation and signaling was decreased in the colon of 2-HOBA-treated mice. Last, the growth of human tumors is significantly attenuated by 2-HOBA supplementation. Conclusion and Implications: 2-HOBA, which has been shown to be safe in humans, reduces colon tumorigenesis and growth of tumor cells in three distinct models of CRC. Thus, 2-HOBA represents a promising natural compound for the prevention and treatment of CRC.

The dicarbonyl electrophile scavenger 2-hydroxybenzylamine (2-HOBA) prevents colorectal carcinogenesis and reduces tumor growth

Running title: Colorectal cancer development is reduced by 2-HOBA

Alain P. Gobert [1,2], Mohammad Asim[1], Thaddeus M. Smith [1], Kamery J. Williams [1], Daniel P. Barry [1], Margaret M. Allaman [1], Kara M. McNamara[1,3], Caroline V. Hawkins [1], Alberto G. Delgado [1], Shilin Zhao [6], M. Blanca Piazuelo [1,2], M. Kay Washington[2,4], Lori A. Coburn [1,2,3,5], John A. Rathmacher [7,8], Keith T. Wilson[1,2,3,4,5,*]

[1] Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA.

[2] Center for Mucosal Inflammation and Cancer, Vanderbilt University Medical Center, Nashville, TN, USA.

[3] Program in Cancer Biology, Vanderbilt University Medical Center, Nashville, TN, USA.

[4] Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA.

[5] Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, USA.

[6] Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37232, USA.

[7] MTI BioTech Inc., Iowa State University Research Park, Ames, IA, USA.

[8] Department of Animal Science, Iowa State University, Ames, IA, USA.

Correspondence

Keith T. Wilson, Vanderbilt University Medical Center, 2215B Garland Ave., 1030C MRB IV, Nashville, TN 37232-0252. Phone 615-343-5675; Email: keith.wilson@vumc.org;

Word count: 2936.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING INFORMATION

This work was funded by NIH grants R41CA257262 (K.T.W. and J.A.R.), R01DK128200 (K.T.W.), P01CA116087 (K.T.W.); Department of Defense grant W81XWH-18-1-0301 (K.T.W.); Veterans Affairs Merit Review grants I01CX002171 (K.T.W.), and I01BX004366 (L.A.C.); Senior Research Award 703003 from the Crohn's and Colitis Foundation (K.T.W.); the Thomas F. Frist Sr. Endowment (K.T.W.); and the Vanderbilt Center for Mucosal Inflammation and Cancer (K.T.W.). Histopathology studies were supported in part by the Tissue Morphology Subcore of the Translational Analysis Core of NIH grant P30DK058404.

AUTHOR CONTRIBUTIONS

Conceptualization: APG, JAR, and KTW. Investigation: APG, MA, TMS, KJW, DPB, MMA, KMM, CVH, ADG, LAC. Formal Analysis: APG, SZ, MBP, MKW, JAR. Data Curation: SZ. Writing – Original Draft: APG. Writing – Review & Editing: APG, LAC, JAR, KTW. Visualization: APG. Funding Acquisition: APG, JAR, KTW

CONFLICTS OF INTEREST

APG and KTW are named inventors on a Vanderbilt University patent application for the use of electrophile scavengers. In addition, APG and KTW are named on a licensing agreement between Vanderbilt University and MTI Biotech for the future use of electrophile scavengers. All other authors have declared that no conflict of interest exists. JAR is an employee of MTI BioTech and is listed as an inventor on 2-HOBA patent applications. MTI BioTech intends to market/license 2-HOBA for commercial purposes.

ETHICS APPROVAL

All the mice were used under protocol M2000047, which was approved by the Institutional Animal Care and Use Committee at Vanderbilt University, the Vanderbilt University Institutional Biosafety Committee, and the Research and Development Committee of the Veterans Affairs Tennessee Valley Healthcare System. Procedures were performed in accordance with institutional policies, AAALAC guidelines, the AVMA Guidelines on Euthanasia, NIH regulations (Guide for the Care and Use of Laboratory Animals), and the United States Animal Welfare Act (1966).

Abstract

Background and Purpose: Colorectal cancer (CRC) is a major health problem worldwide. Dicarbonyl electrophiles, such as iso-levuglandins (isoLGs), are generated from lipid peroxidation and form covalent adducts with amine-containing macromolecules. We have shown high levels of adducts of isoLGs in colonic

epithelial cells of patients with CRC. We thus investigated the role of these reactive aldehydes in colorectal cancer development.

Experimental Approach: We investigated the effect of oral treatment with 2-hydroxybenzylamine (2-HOBA), a natural compound derived from buckwheat seeds that acts as a potent scavenger of electrophiles, on colon carcinogenesis using the azoxymethane-dextran sulfate sodium model of colitis-associated carcinogenesis and mice with epithelial-specific deletion of the adenomatous polyposis coli gene, as a model of sporadic cancer. We also tested 2-HOBA in a murine xenograft of human HCT116 CRC cells implanted into the flank of nude mice.

Key Results: 2-HOBA is bioavailable in the colon of mice after supplementation in the drinking water and does affect the colonic microbiome. However, it reduced the level of isoLG adducts to lysine as well as tumorigenesis in both models of CRC. In parallel, we found that NRF2 activation and signaling was decreased in the colon of 2-HOBA-treated mice. Last, the growth of human tumors is significantly attenuated by 2-HOBA supplementation.

Conclusion and Implications: 2-HOBA, which has been shown to be safe in humans, reduces colon tumorigenesis and growth of tumor cells in three distinct models of CRC. Thus, 2-HOBA represents a promising natural compound for the prevention and treatment of CRC.

KEYWORDS

Reactive aldehydes, lipid peroxidation, oxidative damage, inflammation, colon cancer, xenograft.

1 INTRODUCTION

Colorectal cancer (CRC) is the third most diagnosed cancer worldwide, with around two million new cases per year and will certainly continue to rise in the next decades, notably in countries with the largest population (Xi & Xu, 2021) and in younger adults (Bailey et al., 2015). Therefore, CRC is the second leading cause of death by cancer globally, accounting for over one million deaths yearly (Sung et al., 2021; Xi & Xu, 2021). This leads to the need for colonoscopic screening and frequent adenoma surveillance, and progression to surgical resections with discovery of CRC. New therapies that could be safe, effective, inexpensive, and rationally-based are needed to reduce the risk for neoplastic transformation in the colon and for adjunctive therapy for existing CRC.

The genetic alterations leading to CRC, also known as the classical Vogelgram, have been well characterized (Fearon & Vogelstein, 1990). Principally, the generation of early adenomas results from germline or somatic mutations in the gene encoding for the adenomatous polyposis coli (APC) protein, whose loss of function allows for the uncontrolled activation of β-catenin. This transcriptional activator induces in turn the expression of genes encoding for the Myc proto-oncogene protein (MYC) (He et al., 1998), a transcription factor controlling proliferation/differentiation and the production of reactive oxygen species (ROS) (Vafa et al., 2002) or the enzyme prostaglandin G/H synthase 2 (Araki et al., 2003; Castellone et al., 2005) that generates the prostaglandin H2, the precursor of all prostaglandins and thromboxanes. Additionally, patients with inflammatory bowel disease (IBD) have a high cumulative risk of developing colitis-associated carcinoma (CAC) (Eaden et al., 2001), and also exhibit an increase of ROS and prostaglandin synthesis in the colonic mucosa (Crifo & MacNaughton, 2022), although mutations in *TP53* are the main driver for dysplasia initiation (Beaugerie & Itzkowitz, 2015).

The nonenzymatic rearrangement of prostaglandins and/or the oxidative degradation of lipids leads to the formation of highly reactive aldehydes, termed dicarbonyl electrophiles, including isolevuglandins (isoLGs), malondialdehyde, 4-hydroxy-nonenal, 4-oxo-nonenal, methylglyoxal, and acrolein (Davies et al., 2020); this last molecule is also generated by β-elimination from 3-aminopropanal. These strong oxidants react principally with amines present in nucleic acid bases (Carrier et al., 2009) and lysine residues (Carrier et al., 2014; Uchida et al., 1998) to form irreversible covalent adducts, which may lead to changes in cell signaling, somatic genomic abnormalities (Esterbauer, 1993), and epigenetic alterations (Carrier et al., 2014). Of importance, we have reported high levels of nuclear adducts of isoLGs to lysine (isoLG-lysyl) are detected in colonic

epithelial cells (CECs) from patients with active colitis, dysplasia, or CAC, as well as in adenomatous polyps with areas of high-grade dysplasia, and in CRC tumor tissues (Gobert et al., 2021). Moreover, we have shown that treatment with an experimental scavenger of electrophiles prevented tumorigenesis in a murine model of CAC (Gobert et al., 2021).

The natural product 2-hydroxybenzylamine (2-HOBA) is derived from buckwheat seeds (Koyama et al., 1971) and reacts with all electrophiles at a rate 3 orders of magnitude faster than with lysine, thus preventing adduct formation with macromolecules (Zagol-Ikapitte et al., 2010). It is not toxic (Fuller et al., 2018a; Fuller et al., 2018b; Pitchford et al., 2018) or mutagenic (Fuller et al., 2018c) in mice, rats, or rabbits, and protects mice from oxidative damage in models of hypertension (Wu et al., 2016), pulmonary arterial hypertension (Egnatchik et al., 2017), atrial fibrillation (Prinsen et al., 2020), atherosclerotic cardiovascular disease (Tao et al., 2020), and Alzheimer's disease (Davies et al., 2011). In addition, two Phase 1 clinical trials have demonstrated its safety in humans (Pitchford et al., 2020; Pitchford et al., 2019). Therefore, 2-HOBA is well positioned as chemopreventive agent for the development of neoplastic transformation in the colon, notably in high-risk populations, such as IBD patients, individuals with congenital *APC* mutation, and patients with a history of high-risk/advanced adenomas. In this context, our goal was to test the role of 2-HOBA on colon carcinogenesis in models of CAC and sporadic CRC. We also sought to determine the efficacy of 2-HOBA on altering the growth of tumors in a model of human CRC cell xenografts.

2 METHODS

2.1 Materials

2-HOBA (as the acetate salt, CAS 1206675–01-5) was obtained from TSI Co., Ltd. (Shanghai, China). A commercial production lot was used (Lot SAA20200727). The purity of the commercial lot was verified by HPLC to be $>$ 99% at MTI BioTech. Microbial and analytical tests were within all specification limits. Azoxymethane (AOM) and tamoxifen (TAM) were purchased from Sigma-Aldrich (St. Louis, MO). Dextran sulfate sodium (DSS) was obtained from TdB Labs (Uppsala, Sweden). Reagents for cell culture were purchased from Gibco (Waltham, MA).

2.2 Cell culture

The human colon cancer cell line HCT116 (RRID: CVCL_0291) was purchased from ATCC (Manassas, VA) and used between passage 3 and 13. Cells were maintained in DMEM with GlutaMAX supplemented with 10% fetal bovine serum, 10 mM HEPES, 1 mM sodium pyruvate, 100 U.ml^{-1}penicillin, and 100 mg.ml^{-1} streptomycin.

2.3 Mice, models of colon cancer, and treatment with 2-HOBA

Age-matched (8 wk) male C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). As a model of CAC, mice were treated with one intraperitoneal injection of 12.5 mg.kg^{-1}AOM followed by 3 cycles of 4% DSS for 4 complete days each, beginning at day 5, 26, and 47, as described (Gobert et al., 2021). The experiment was stopped 56 days after AOM injection.

As a model of sporadic CRC, we used transgenic C57BL/6 mice with a tamoxifen-inducible disruption of *Apc* using the intestinal epithelial cell-specific, caudal type homeobox 2 (CDX2) *Cre*(*CDX2P -CreER*^{T2} ;*Apc*$^{fl/fl}$) (Feng et al., 2013; Triner et al., 2019), which recapitulates genetically-driven CRC associated with *APC* loss (Powell et al., 1992). Animals were house-bred in our animal facility. Male and female mice were i.p. injected with 4.5 mg.kg^{-1} TAM dissolved in a corn oil:ethanol (9:1) solution; control mice were given the corn oil:ethanol solution without TAM. Animals were sacrificed after 35 days. Mice were treated with or without 1 mg.ml^{-1}2-HOBA in the drinking water beginning one day after AOM or TAM injection and maintained throughout the experiment, except during the DSS administration periods, as reported (Gobert et al., 2021).

For heterotopic generation of human colon cancer xenografts, we used 7-8 wk-old nude female mice (The Jackson Laboratory, Bar Harbor, Maine). HCT116 cells were harvested, washed in PBS, resuspended in

50% PBS 50% Matrigel (Corning, NY), and implanted in the right flank region of nude mice (10^6 cells per mouse). Tumors were measured weekly using a digital caliper and tumor volume was calculated using the following formula: Volume = 0.5 x Length x Width^2. Mice were given 1 mg.ml^{-1} 2-HOBA in the drinking water when the first mouse per experiment exhibited a tumor with a volume of 100 mm^3. Animals were sacrificed after 35 days, or earlier if ulceration was observed. The tumor growth inhibition (TGI) index was calculated using this formula: TGI = (1 – (mean volume of treated tumors)/(mean volume of control tumors)) × 100.

2.4 Assessment of tumorigenesis and histopathology

At sacrifice, tumors in the mid and distal colon were measured with an electronic caliper under a dissecting microscope to determine their area. The tumor burden corresponds to the sum of all tumor areas.

Swiss rolls of the colons were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin (H&E). Histologic assessment was then performed by our gastrointestinal pathologist (M.B.P.) in a blinded manner. Colon tissues were scored on a 0–40 scale based on the parameters of inflammation severity (0–3), inflammation extent (0–3), and crypt damage (0–4), each multiplied by the percent involvement (1 = 0–25%; 2 = 25–50%; 3 = 50–75%; and 4 = 75–100%), as reported (Gobert et al., 2021). The presence and severity of dysplasia was scored as described (Gobert et al., 2021).

2.5 Measurement of 2-HOBA

Concentrations of 2-HOBA were determined in the colon by liquid chromatography tandem mass spectrometry as described (Gobert et al., 2022a; Pitchford et al., 2019).

2.6 Analysis of mRNA levels

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Germantown, MD). mRNAs were reverse-transcribed using Superscript II Reverse Transcriptase and Oligo dT (Invitrogen, Waltham, MA), and cDNAs were amplified by real-time PCR using the PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA) and the primers listed in Table S1. We performed a semi-quantitative analysis using *Actb* as the housekeeping gene.

2.7 Immunostaining

The immunodetection of the isoLG-lysyl adducts was performed as we reported (Gobert et al., 2021). Briefly, tissue sections were blocked at room temperature with 3% hydrogen peroxide in PBS and then with a solution of 5% human/mouse serum and 5% BSA in PBS. Slides were then incubated overnight at 4degC with the D11 antibody (1:200) (Davies et al., 2004). Then, the DYKDDDDK Epitope Tag Antibody (1:300; Novus, Cat# NB600-345, RRID: AB_10001331) was used for 1 h at room temperature. Visualization was performed using 3,3'-diaminobenzidine, and tissues were counterstained by hematoxylin.

For immunofluorescence, deparaffinized sections of the mouse colon tissues were first treated with Protein Block (Dako, Santa Clara, CA) for 45 min at room temperature. Slides were then incubated overnight at 4degC with the anti-NRF2 polyclonal antibody (1:100; Proteintech, Cat# 16396-1-AP, RRID: AB_2782956) and for 45 min at room temperature with the donkey anti-rabbit IgG (H+L) Highly Cross-Adsorbed secondary Ab, Alexa Fluor Plus 488 (1:600; Invitrogen, Cat# A-21206, RRID: AB_2535792). The slides were then mounted with VECTASHIELD HardSet Antifade Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA) and confocal images were acquired using the Cytation C10 Confocal Imaging Reader (Agilent BioTek, Winooski, VT)

2.8 Analysis of the composition of the colonic microbiota

Littermate co-housed $CDX2P\text{-}CreER^{T2}$;$Apc^{fl/fl}$ mice (6-12 wks) were treated or not with 2-HOBA for 35 days. After euthanasia, the feces in the colon were harvested, weighed, and DNA was isolated using the QIAmp Fast DNA Stool Mini Kit (Qiagen, Germantown, MD). The V4 hypervariable region of 16S rRNA genes were amplified with MyTaq polymerase master mix (Bioline). In this step, amplicons of each sample

were barcoded with primers 515F/806R (Kozich et al., 2013). ZymoBIOMICS (Zymo, Irvine, CA) positive controls and extraction and PCR negative controls were run alongside the samples. PCR products were run on 1.2% TAE agarose gels to verify reaction success. Amplicons were cleaned and normalized with the SequalPrep Normalization Plate Kit (Invitrogen, Waltham, MA). Samples were pooled and cleaned with 1X Ampure XP Beads (Beckman Coulter, Indianapolis, IN). Sequencing was performed on an Illumina MiSeq with 2 X 250 bp reads. Sequences were processed with mothur and aligned to the SILVA database release 132 and taxonomically classified with the Ribosomal Database Project classifier 16. Non-bacterial sequences and chimeric sequences detected by UCHIME were removed. Operational Taxonomic Unit (OTU) clustering was performed with VSEARCH, using abundance-based greedy clustering.

2.9 Data and statistical analysis

The data and statistical analysis comply with the recommendations of the British Journal of Pharmacology on experimental design and analysis in pharmacology (Curtis et al., 2018). The study was designed to generate groups of equal size, using randomization and blinded analysis. Figures were generated and statistics analyzed using GraphPad Prism 9.5. Graphs represent the mean +- SEM. Outliers were identified using the ROUT test (Q = 5%) and removed from the analysis. Data that were not normally distributed according to the D'Agostino & Pearson normality test were log or square root transformed. Student's t test was used to determine significant differences between two groups, whereas analysis of multiple groups was performed using ANOVA followed by Tukey's or Dunnett's multiple comparisons tests. Contingency analyses were performed by Fisher's exact test or Chi-square test. All the statistical tests were two-sided.

The relative abundance of the genera of the colonic microbiota was analyzed by the Wilcoxon rank sum test after multiple comparison adjustment by the Benjamini & Hochberg method.

3 RESULTS

3.1 Tumorigenesis is reduced by 2-HOBA in a model of CAC

We first tested the effect of 2-HOBA on colon carcinogenesis in C57BL/6 mice treated with AOM-DSS, as a reliable model of inflammation-driven colon carcinogenesis (Gobert et al., 2021), as depicted in Fig. 1A. Mice that were given DSS lost weight significantly during each cycle compared to untreated mice, but there was no difference between animals treated or not with 2-HOBA (Fig. 1B). At the end of the experiment, we determined the concentration of 2-HOBA in the colonic tissues by LC-MS/MS. We did not detect this compound in the colon of naive mice or of those treated with AOM-DSS only (Fig. 1C). In contrast, 2-HOBA was found in the colon of animals that were given this scavenger, treated or not with AOM-DSS (Fig. 1C), demonstrating that a *per os* treatment with 2-HOBA efficiently increases its concentration in the colon. In AOM-DSS-treated mice, 2-HOBA had no effect on the number of tumors in the colon (Fig. 1D). However, mice that were given 2-HOBA had a significant reduction of tumor size (Fig. 1E-F) and total tumor burden per colon (Fig. 1G). The reduced tumor size in the 2-HOBA-treated group was confirmed microscopically by the analysis of the H&E staining (Fig. 1H). The level of histologic inflammation in the non-tumor areas was not affected by 2-HOBA supplementation (Fig. 1I).

3.2 2-HOBA dampens the immune response in AOM-DSS-induced tumors

An important part of inflammatory carcinogenesis in CAC is the immune response pattern. We therefore assessed the effect of 2-HOBA on colonic mucosal immune gene expression during AOM-DSS treatment. In the non-tumor area of AOM-DSS-treated mice, the genes *Nos2* ,*Cxcl1* , and *Ifng* were significantly upregulated compared to control animals, and there was no difference in the level of expression with 2-HOBA (Fig. 2). Strikingly, these 3 genes plus *Tnf* ,*Il1b* , and *Il17* were induced in the tumors of mice treated with AOM-DSS (Fig. 2). Unlike non-tumor areas, the expression of*Nos2* , *Tnf* , *Cxcl1* , and *Ifng* was significantly reduced in the tumors of mice that were given 2-HOBA.

3.3 2-HOBA limits tumorigenesis associated with *Apc* loss

We then assessed whether 2-HOBA is also protective in a model of sporadic CRC (Fig. 3A). Animals treated

with TAM or TAM + 2-HOBA had significant body weight loss compared to sham treatment throughout the time course of the experiment (Fig. 3B). As in the AOM-DSS model, we detected 2-HOBA in the colon of *CDX2P-CreER^{T2};Apc$^{fl/fl}$* mice supplemented with this scavenger, treated or not with TAM, but not in the tissues of mice not exposed to 2-HOBA (Fig. 3C). The number of tumors (Figs. 3D-E) and the tumor burden (Fig. 3F) observed in TAM-treated *CDX2P-CreER^{T2};Apc$^{fl/fl}$* mice were significantly reduced by 2-HOBA supplementation. Further, we found that tumor burden was inversely correlated with 2-HOBA levels (Fig. 3G). Thus, increased concentration of 2-HOBA in the colon is associated with decreased tumor development in this genetically-induced cancer model. Histology (Fig. 3H) highlighted fewer and smaller adenomas in the 2-HOBA-treated group, while the moderate inflammation of the non-tumor area was not affected (Fig. 3I).

3.4 The composition of the intestinal microbiota is not affected by 2-HOBA

We then considered the possibility that the protective role of 2-HOBA against colon carcinogenesis could be due to an effect on the intestinal microbiota. To test this hypothesis, we determined the gut microbiome of *CDX2P-CreER^{T2};Apc$^{fl/fl}$* mice treated or not with 2-HOBA for 35 days. The total number of bacteria in the gut (Fig. 4A) and the diversity of the microbial community, assessed by Simpson and Shannon indexes (Fig. 4B), were not affected by 2-HOBA supplementation.

The colon microbiota of *CDX2P-CreER^{T2};Apc$^{fl/fl}$* mice was dominated by the Bacteroidetes phylum (Fig. 4C), as we reported in wild-type C57BL/6 mice (Gobert et al., 2022b). Firmicutes and Proteobacteria were also detected in the microbiota in a proportion ranging from 5.8% to 14.1% (Fig. 4C). Overall, the microbiome at the phylum level was similar in mice that were given 2-HOBA (Fig. 4C; Table S2).

At the genus level, *Prevotella* , *Bacteroides* , and *Porphyromonadaceae* were dominant in mice treated or not with 2-HOBA (Fig. 4D). Similarly, the prevalence of the genus belonging to Firmicutes, such as *Lachnospiraceae* , and Proteobacteria, including *Helicobacter* and *Parasutterella* , was not altered by 2-HOBA supplementation (Fig. 4D; Table S2).

3.5 The formation of isoLG adducts in the colon is reduced by 2-HOBA

We have reported that isoLG-lysyl adducts are increased in the colon of mice treated with AOM-DSS and reduced by an electrophile scavenger (Gobert et al., 2021). Next, we questioned whether 2-HOBA has the same effect in mice with intestinal epithelial-specific *Apc* deletion. In the non-tumor areas, we observed that the frequency of cells exhibiting isoLG-lysyl adducts in the nuclei of CECs of TAM-treated mice was markedly enhanced compared to the control group (Fig. 5A). In TAM-treated mice, the nuclear staining was observed all along the crypts (Fig. 5A). There was less nuclear staining when animals with specific *Apc* deletion were given 2-HOBA (Fig. 5A). These observations were confirmed by the quantification on multiple mice per group, evidencing a significant increase of isoLG-lysyl adducts in *CDX2P-CreER^{T2};Apc$^{fl/fl}$* mice + TAM that was reduced with 2-HOBA supplementation (Fig. 5B).

3.6 2-HOBA reduces human tumor development in a xenograft model

Our current data demonstrate that 2-HOBA dampens carcinogenesis. To test the effect of 2-HOBA on growth of established tumors, we used xenografts of HCT116 cells in nude mice. First, we observed that 2-HOBA was bioavailable in the tumors of animals treated with this scavenger (Fig. 6A). The growth of tumors in 2-HOBA-treated nude mice was significantly slower compared to the sham group (Fig. 6B). At sacrifice, we confirmed that the xenografts from animals that were given 2-HOBA exhibited a smaller size (Fig. 6C) and therefore significantly less volume (Fig. 6D). The TGI rate was 42% at the end of the experiment. Lastly, we found a significant and inverse correlation between tumor volume and the concentration of 2-HOBA in the xenografts (Fig. 6E).

3.7 NRF2 activation is regulated by 2-HOBA

To further understand the molecular mechanism by which dicarbonyl electrophiles support colon carcinogenesis, we first analyzed the expression of various genes well-known for their role in neoplasia. In the *Apc*

deletion model, the genes encoding for the pro-inflammatory mediators IL-1β and CXCL1 were induced at the same level in tumors of TAM-treated mice ± 2-HOBA (Fig. 7A). The expression of the β-catenin target genes *Myc* , *Mmp7* , *Axin2* ,*Ccnd1* , and *Ptgs2* was similar in the tumors of mice receiving 2-HOBA or not (Fig. 7A). In contrast, the gene *Hmox1* , which is notably regulated by the nuclear factor erythroid 2-related factor 2 (NRF2), was induced in the non-tumor and tumor areas of TAM-treated mice, but was significantly less expressed in animals supplemented with 2-HOBA (Fig. 7A). Similarly, the NRF2-target gene*Slc7a11* was downregulated in the tumors of mice that were given 2-HOBA compared to sham treatment (Fig. 7A). In this context, we sought to determine the activation of NRF2 in the colon of$CDX2P\text{-}CreER^{T2}$;$Apc^{fl/fl}$ mice ± 2-HOBA. We observed a strong nuclear translocation of NRF2 in CECs from mice treated with TAM compared to controls (Fig. 7B). However, there was less nuclear translocation in 2-HOBA-treated mice (Fig. 7B). This result was confirmed by the quantification of NRF2-postivite nuclei in CECs (Fig. 7C).

4 DISCUSSION

CRC is a global public health problem worldwide. Systematic CRC screening has led to a reduction of the prevalence of CRC in older persons in developed countries, but in the last two decades the rate of CRC mortality in people less than 50 years-old has increased by more than 1% annually (Santucci et al., 2021). In this context, strategies that can prevent colorectal malignant transformation are also essential to manage the disease burden. In this report, we showed that the scavenger of reactive aldehydes, 2-HOBA, which is a natural compound found in buckwheat seeds, prevents nuclear accumulation of electrophile adducts in CECs, NRF2 activation and expression of NRF2-target oncogenes, and carcinogenesis in a murine model of genetically-mediated colon cancer. In addition, 2-HOBA has no major effect on the colonic microbiome. Further, 2-HOBA tumorigenesis in a model of CAC, and reduced the growth of xenografts of human CRC cells, suggesting a potential efficacy to limit the development of established colorectal tumors.

Removing polyps remains the most effective way to prevent the development of sporadic CRC, and in addition, regular surveillance colonoscopy with biopsies is utilized to detect dysplasia in the setting of colitis to prevent CAC. These approaches are expensive and incompletely available to all persons. Nutritional and/or chemopreventive strategies have the potential to diminish CRC incidence, recurrence, and mortality. They can be used in the general population, but also in high-risk individuals. CRC chemoprevention agents comprise aspirin (Drew et al., 2016) or other nonsteroidal anti-inflammatory drugs (Arber et al., 2006), which can also exhibit cardiovascular toxicity (Dogne et al., 2006). Although lifestyle and specific diets, such as Mediterranean or vegetarian, have been associated with low incidence of CRC (Divisi et al., 2006), to date only a few nutrients have been shown to exhibit anti-CRC activity. The omega-3 fatty acid eicosapentaenoic acid (West et al., 2010) or curcumin and quercetin (Cruz-Correa et al., 2006) have shown protection for adenoma development in familial adenomatous polyposis patients, and a combination of antioxidant vitamins and selenium limits the recurrence of polyps (Bonelli et al., 2013). We have previously reported that an experimental scavenger of reactive aldehydes derived from 2-HOBA reduces tumorigenesis in the AOM-DSS model (Gobert et al., 2021), but the present study highlights that the nutraceutical compound 2-HOBA that has been used in human Phase 1 trials, dampens not only CAC, but also sporadic CRC development and growth of established colon tumors.

Lipid peroxidation-derived compounds are synthesized in the colon during DSS colitis (Lee et al., 2010) and in AOM-DSS-treated mice (Lei et al., 2021). We have reported that isoLG-lysyl adduct levels are increased in the colon of patients with IBD and CAC (Gobert et al., 2021). Similarly, the concentration of malondialdehyde is increased in primary colon tumors compared to normal colon (Skrzydlewska et al., 2005) and in the plasma of CRC patients versus normal individuals (Leung et al., 2008). Moreover, studies have shown that patients with CRC exhibit reduced levels of glutathione and lower expression of enzymes involved in reactive aldehyde detoxification, such as glutathione S-transferases (Leung et al., 2008). Interestingly, *Apc* mutation in mice leads to decreased maturation of aldehyde dehydrogenase-positive stem cells along the neuroendocrine cell lineage (Zhang et al., 2020) and has thus been associated with increased number of stem cells expressing aldehyde dehydrogenase (Huang et al., 2009). These data suggest that the fight against excessive dicarbonyl electrophiles is a physiological response to counteract oxidative injury. In this context,

the enhanced generation of dicarbonyl electrophiles and the decreased ability to degrade them supports the use of electrophile scavengers, such as 2-HOBA, to dampen this potent oxidative stress. Providing further evidence of the deleterious role of electrophiles, repeated intraperitoneal injection of the aldehyde epoxyketooctadecenoic acid exacerbates AOM-DSS-induced tumorigenesis in mice (Lei et al., 2021).

The transcription factor NRF2 is a master regulator of the inducible expression of genes encoding for enzymes involved in detoxification of reactive oxygen species and aldehydes and is therefore activated by oxidative species including dicarbonyl electrophiles (Ma, 2013). Herein, we observed that the nuclear translocation of NRF2 in CECs and the expression of NRF2-target genes are stimulated by *Apc* deletion and reduced by 2-HOBA treatment, suggesting that electrophiles might support colon carcinogenesis through a mechanism that involves this transcription factor. In fact, NRF2 is considered as an oncogene in different cancers due to the induction of genes involved in cell survival, proliferation, and repression of apoptosis. NRF2 level is increased in CRC tissues and is associated with poor prognosis (Torrente et al., 2020). In mice, the study of the role of NRF2 in carcinogenesis has led to contradictory results: In the AOM-DSS model, *Nfe2l2*deletion leads to increased tumorigenesis in BALB/C mice (Hammad et al., 2019) and to a reduction of tumor number in the C57BL/6 background (Song et al., 2021). Note that all these studies were performed with mice with total knockout of NRF2. In this context, it would be thus now interesting to decipher the specific role of NRF2 in CECs in colorectal carcinogenesis, notably mediated by lipid peroxidation products.

The use of classical antioxidants including N-acetylcysteine, vitamins, or selenium, have shown moderate or no effect on CRC development, recurrence, or treatment in the general population (Papaioannou et al., 2011). The thiol of N-acetylcysteine serves as an excellent scavenger of nitric oxide, hypochlorous acid, and hydroxyl radicals, whereas vitamin E acts in the defense against free radicals. However, these antioxidants are not specific to lipid aldehyde species. Moreover, their rate constant of reactions with free radicals is 10^5-10^6 faster than with aldehydes (Roberts et al., 2007). Lastly, unlike 2-HOBA, classical antioxidants do not react with all electrophiles (Davies et al., 2020). In this context, the use of 2-HOBA in prevention or treatment of CRC deserves further investigation.

In conclusion, 2-HOBA, which has been shown to be safe in humans, reduces colon tumorigenesis in murine models of CAC and sporadic CRC, as well as growth of human CRC tumor cells in xenografts in nude mice. Thus, 2-HOBA represents a promising natural compound for the prevention and treatment of CRC. Because 2-HOBA also prevents gastric carcinogenesis (Gobert et al., 2022a), this scavenger holds promise as a gastrointestinal cancer agent with a favorable safety profile. Thus, Phase 1 and ultimately Phase 2 trials are warranted in gastrointestinal cancers, including stomach and colorectal.

References

Araki, Y., Okamura, S., Hussain, S. P., Nagashima, M., He, P., Shiseki, M., Miura, K., & Harris, C. C. (2003). Regulation of cyclooxygenase-2 expression by the Wnt and Ras pathways. *Cancer Research***63,** 728-734.

Arber, N., Eagle, C. J., Spicak, J., Racz, I., Dite, P., Hajer, J., Zavoral, M., Lechuga, M. J., Gerletti, P., Tang, J., Rosenstein, R. B., Macdonald, K., Bhadra, P., Fowler, R., Wittes, J., Zauber, A. G., Solomon, S. D., Levin, B., & Pre, S. A. P. T. I. (2006). Celecoxib for the prevention of colorectal adenomatous polyps. *The New England Journal of Medicine* **355,** 885-895. 10.1056/NEJMoa061652

Bailey, C. E., Hu, C. Y., You, Y. N., Bednarski, B. K., Rodriguez-Bigas, M. A., Skibber, J. M., Cantor, S. B., & Chang, G. J. (2015). Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975-2010. *JAMA Surgery* **150,** 17-22. 10.1001/jamasurg.2014.1756

Beaugerie, L., & Itzkowitz, S. H. (2015). Cancers complicating inflammatory bowel disease. *The New England Journal of Medicine***372,** 1441-1452. 10.1056/NEJMra1403718

Bonelli, L., Puntoni, M., Gatteschi, B., Massa, P., Missale, G., Munizzi, F., Turbino, L., Villanacci, V., De Censi, A., & Bruzzi, P. (2013). Antioxidant supplement and long-term reduction of recurrent adenomas of the large bowel. A double-blind randomized trial.*Journal of Gastroenterology* **48,** 698-705. 10.1007/s00535-012-0691-z

Carrier, E. J., Amarnath, V., Oates, J. A., & Boutaud, O. (2009). Characterization of covalent adducts of nucleosides and DNA formed by reaction with levuglandin. *Biochemistry* **48,** 10775-10781. 10.1021/bi9015132

Carrier, E. J., Zagol-Ikapitte, I., Amarnath, V., Boutaud, O., & Oates, J. A. (2014). Levuglandin forms adducts with histone h4 in a cyclooxygenase-2-dependent manner, altering its interaction with DNA.*Biochemistry* **53,** 2436-2441. 10.1021/bi401673b

Castellone, M. D., Teramoto, H., Williams, B. O., Druey, K. M., & Gutkind, J. S. (2005). Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science***310,** 1504-1510. 10.1126/science.1116221

Crifo, B., & MacNaughton, W. K. (2022). Cells and mediators of inflammation as effectors of epithelial repair in the inflamed intestine. *The American Journal of Physiology-Gastrointestinal and Liver Physiology* **322,** G169-G182. 10.1152/ajpgi.00194.2021

Cruz-Correa, M., Shoskes, D. A., Sanchez, P., Zhao, R., Hylind, L. M., Wexner, S. D., & Giardiello, F. M. (2006). Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis.*Clinical Gastroenterology and Hepatology* **4,** 1035-1038. 10.1016/j.cgh.2006.03.020

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., Hoyer, D., Insel, P. A., Izzo, A. A., Ji, Y., MacEwan, D. J., Sobey, C. G., Stanford, S. C., Teixeira, M. M., Wonnacott, S., & Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. *The British Journal of Pharmacology* **175,**987-993. 10.1111/bph.14153

Davies, S. S., Bodine, C., Matafonova, E., Pantazides, B. G., Bernoud-Hubac, N., Harrison, F. E., Olson, S. J., Montine, T. J., Amarnath, V., & Roberts, L. J., 2nd (2011). Treatment with a gamma-ketoaldehyde scavenger prevents working memory deficits in hApoE4 mice. *Journal of Alzheimers Disease* **27,** 49-59. 10.3233/JAD-2011-102118

Davies, S. S., May-Zhang, L. S., Boutaud, O., Amarnath, V., Kirabo, A., & Harrison, D. G. (2020). Isolevuglandins as mediators of disease and the development of dicarbonyl scavengers as pharmaceutical interventions. *Pharmacology & Therapeutics* **205,** 107418. 10.1016/j.pharmthera.2019.107418

Davies, S. S., Talati, M., Wang, X., Mernaugh, R. L., Amarnath, V., Fessel, J., Meyrick, B. O., Sheller, J., & Roberts, L. J., 2nd (2004). Localization of isoketal adducts in vivo using a single-chain antibody.*Free Radical Biology and Medicine* **36,** 1163-1174. 10.1016/j.freeradbiomed.2004.02.014

Divisi, D., Di Tommaso, S., Salvemini, S., Garramone, M., & Crisci, R. (2006). Diet and cancer. *Acta Biomedica* **77,** 118-123.

Dogne, J. M., Hanson, J., Supuran, C., & Pratico, D. (2006). Coxibs and cardiovascular side-effects: from light to shadow. *Current Pharmaceutical Design* **12,** 971-975. 10.2174/138161206776055949

Drew, D. A., Cao, Y., & Chan, A. T. (2016). Aspirin and colorectal cancer: the promise of precision chemoprevention. *Nature Reviews Cancer* **16,** 173-186. 10.1038/nrc.2016.4

Eaden, J. A., Abrams, K. R., & Mayberry, J. F. (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut***48,** 526-535. 10.1136/gut.48.4.526

Egnatchik, R. A., Brittain, E. L., Shah, A. T., Fares, W. H., Ford, H. J., Monahan, K., Kang, C. J., Kocurek, E. G., Zhu, S., Luong, T., Nguyen, T. T., Hysinger, E., Austin, E. D., Skala, M. C., Young, J. D., Roberts, L. J., 2nd, Hemnes, A. R., West, J., & Fessel, J. P. (2017). Dysfunctional BMPR2 signaling drives an abnormal endothelial requirement for glutamine in pulmonary arterial hypertension. *Pulmonary Circulation* **7,** 186-199. 10.1086/690236

Esterbauer, H. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *The American Journal of Clinical Nutrition* **57,**779S-785S.

Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* **61,** 759-767.

Feng, Y., Sentani, K., Wiese, A., Sands, E., Green, M., Bommer, G. T., Cho, K. R., & Fearon, E. R. (2013). SOX9 induction, ectopic Paneth cells, and mitotic spindle axis defects in mouse colon adenomatous epithelium arising from conditional biallelic *Apc* inactivation. *The American journal of Pathology* **183,** 493-503. 10.1016/j.ajpath.2013.04.013

Fuller, J. C., Jr., Pitchford, L. M., Abumrad, N. N., & Rathmacher, J. A. (2018a). Subchronic (90-day) repeated dose oral toxicity study of 2-hydroxybenzylamine acetate in rabbit. *Regulatory Toxicology and Pharmacology* **100,** 52-58. 10.1016/j.yrtph.2018.10.017

Fuller, J. C., Jr., Pitchford, L. M., Abumrad, N. N., & Rathmacher, J. A. (2018b). Subchronic (90-day) repeated dose toxicity study of 2-hydroxybenzylamine acetate in rats. *Regulatory Toxicology and Pharmacology* **99,** 225-232. 10.1016/j.yrtph.2018.09.025

Fuller, J. C., Jr., Pitchford, L. M., Morrison, R. D., Daniels, J. S., Flynn, C. R., Abumrad, N. N., Oates, J. A., Boutaud, O., & Rathmacher, J. A. (2018c). In vitro safety pharmacology evaluation of 2-hydroxybenzylamine acetate. *Food and Chemical Toxicology* **121,** 541-548. 10.1016/j.fct.2018.09.047

Gobert, A. P., Asim, M., Smith, T. M., Williams, K. J., Barry, D. P., Allaman, M. M., McNamara, K. M., Hawkins, C. V., Delgado, A. G., Blanca Piazuelo, M., Rathmacher, J. A., & Wilson, K. T. (2022a). The nutraceutical electrophile scavenger 2-hydroxybenzylamine (2-HOBA) attenuates gastric cancer development caused by *Helicobacter pylori* . *Biomedicine & Pharmacotherapy* **158,** 114092. 10.1016/j.biopha.2022.114092

Gobert, A. P., Boutaud, O., Asim, M., Zagol-Ikapitte, I. A., Delgado, A. G., Latour, Y. L., Finley, J. L., Singh, K., Verriere, T. G., Allaman, M. M., Barry, D. P., McNamara, K. M., Sierra, J. C., Amarnath, V., Tantawy, M. N., Bimczok, D., Piazuelo, M. B., Washington, M. K., Zhao, S., Coburn, L. A., & Wilson, K. T. (2021). Dicarbonyl electrophiles mediate inflammation-induced gastrointestinal carcinogenesis. *Gastroenterology* **160,** 1256-1268. 10.1053/j.gastro.2020.11.006

Gobert, A. P., Latour, Y. L., Asim, M., Barry, D. P., Allaman, M. M., Finley, J. L., Smith, T. M., McNamara, K. M., Singh, K., Sierra, J. C., Delgado, A. G., Luis, P. B., Schneider, C., Washington, M. K., Piazuelo, M. B., Zhao, S., Coburn, L. A., & Wilson, K. T. (2022b). Protective role of spermidine in colitis and colon carcinogenesis. *Gastroenterology* **162,** 813-827. 10.1053/j.gastro.2021.11.005

Hammad, A., Zheng, Z. H., Gao, Y., Namani, A., Shi, H. F., & Tang, X. (2019). Identification of novel NRF2 target genes as prognostic biomarkers in colitis-associated colorectal cancer in *Nrf2* -deficient mice. *Life Sciences* **238,** 116968. 10.1016/j.lfs.2019.116968

He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., & Kinzler, K. W. (1998). Identification of c-MYC as a target of the APC pathway. *Science* **281,** 1509-1512. 10.1126/science.281.5382.1509

Huang, E. H., Hynes, M. J., Zhang, T., Ginestier, C., Dontu, G., Appelman, H., Fields, J. Z., Wicha, M. S., & Boman, B. M. (2009). Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Research* **69,** 3382-3389. 10.1158/0008-5472.CAN-08-4418

Koyama, M., Obata, Y., & Sakamura, S. (1971). Identification of Hydroxybenzylamines in Buckwheat Seeds (*Fagopyrum esculentum*Moench). *Agricultural and Biological Chemistry* **35,**1870-1879.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology* **79,** 5112-5120. 10.1128/AEM.01043-13

Lee, I. A., Bae, E. A., Hyun, Y. J., & Kim, D. H. (2010). Dextran sulfate sodium and 2,4,6-trinitrobenzene sulfonic acid induce lipid peroxidation by the proliferation of intestinal gram-negative bacteria in mice. *Journal of Inflammation (Lond)* **7,** 7. 10.1186/1476-9255-7-7

Lei, L., Yang, J., Zhang, J., & Zhang, G. (2021). The lipid peroxidation product EKODE exacerbates colonic inflammation and colon tumorigenesis. *Redox Biology* **42,** 101880. 10.1016/j.redox.2021.101880

Leung, E. Y., Crozier, J. E., Talwar, D., O'Reilly, D. S., McKee, R. F., Horgan, P. G., & McMillan, D. C. (2008). Vitamin antioxidants, lipid peroxidation, tumour stage, the systemic inflammatory response and survival in patients with colorectal cancer. *International Journal of Cancer* **123,** 2460-2464. 10.1002/ijc.23811

Ma, Q. (2013). Role of NRF2 in oxidative stress and toxicity.*Annual Review of Pharmacology and Toxicology* **53,**401-426. 10.1146/annurev-pharmtox-011112-140320

Papaioannou, D., Cooper, K. L., Carroll, C., Hind, D., Squires, H., Tappenden, P., & Logan, R. F. (2011). Antioxidants in the chemoprevention of colorectal cancer and colorectal adenomas in the general population: a systematic review and meta-analysis.*Colorectal Diseases* **13,** 1085-1099. 10.1111/j.1463-1318.2010.02289.x

Pitchford, L. M., Driver, P. M., Fuller, J. C., Jr., Akers, W. S., Abumrad, N. N., Amarnath, V., Milne, G. L., Chen, S. C., Ye, F., Roberts, L. J., 2nd, Shoemaker, M. B., Oates, J. A., Rathmacher, J. A., & Boutaud, O. (2020). Safety, tolerability, and pharmacokinetics of repeated oral doses of 2-hydroxybenzylamine acetate in healthy volunteers: a double-blind, randomized, placebo-controlled clinical trial. *BMC Pharmacology and Toxicology* **21,** 3. 10.1186/s40360-020-0382-y

Pitchford, L. M., Rathmacher, J. A., Fuller, J. C., Jr., Daniels, J. S., Morrison, R. D., Akers, W. S., Abumrad, N. N., Amarnath, V., Currey, P. M., Roberts, L. J., Oates, J. A., & Boutaud, O. (2019). First-in-human study assessing safety, tolerability, and pharmacokinetics of 2-hydroxybenzylamine acetate, a selective dicarbonyl electrophile scavenger, in healthy volunteers. *BMC Pharmacology and Toxicology***20,** 1. 10.1186/s40360-018-0281-7

Pitchford, L. M., Smith, J. D., Abumrad, N. N., Rathmacher, J. A., & Fuller, J. C., Jr. (2018). Acute and 28-day repeated dose toxicity evaluations of 2-hydroxybenzylamine acetate in mice and rats.*Regulatory Toxicology and Pharmacology* **98,** 190-198. 10.1016/j.yrtph.2018.07.026

Powell, S. M., Zilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B., & Kinzler, K. W. (1992). APC mutations occur early during colorectal tumorigenesis. *Nature***359,** 235-237. 10.1038/359235a0

Prinsen, J. K., Kannankeril, P. J., Sidorova, T. N., Yermalitskaya, L. V., Boutaud, O., Zagol-Ikapitte, I., Barnett, J. V., Murphy, M. B., Subati, T., Stark, J. M., Christopher, I. L., Jafarian-Kerman, S. R., Saleh, M. A., Norlander, A. E., Loperena, R., Atkinson, J. B., Fogo, A. B., Luther, J. M., Amarnath, V., Davies, S. S., Kirabo, A., Madhur, M. S., Harrison, D. G., & Murray, K. T. (2020). Highly reactive isolevuglandins promote atrial fibrillation caused by hypertension.*JACC: Basic to Translational Science* **5,** 602-615. 10.1016/j.jacbts.2020.04.004

Roberts, L. J., 2nd, Oates, J. A., Linton, M. F., Fazio, S., Meador, B. P., Gross, M. D., Shyr, Y., & Morrow, J. D. (2007). The relationship between dose of vitamin E and suppression of oxidative stress in humans.*Free Radical Biology and Medicine* **43,** 1388-1393. 10.1016/j.freeradbiomed.2007.06.019

Santucci, C., Boffetta, P., Levi, F., La Vecchia, C., Negri, E., & Malvezzi, M. (2021). Colorectal cancer mortality in young adults is rising in the United States, Canada, United Kingdom, and Australia but not in Europe and Asia. *Gastroenterology* **160,** 1860-1862. 10.1053/j.gastro.2020.12.070

Skrzydlewska, E., Sulkowski, S., Koda, M., Zalewski, B., Kanczuga-Koda, L., & Sulkowska, M. (2005). Lipid peroxidation and antioxidant status in colorectal cancer. *World Journal of Gastroenterology***11,** 403-406. 10.3748/wjg.v11.i3.403

Song, C. H., Kim, N., Nam, R. H., Choi, S. I., Kang, C., Jang, J. Y., Nho, H., Shin, E., & Lee, H. N. (2021). Nuclear factor erythroid 2-related factor 2 knockout suppresses the development of aggressive colorectal cancer formation induced by azoxymethane/dextran sulfate sodium-treatment in female mice. *Journal of Cancer Prevention***26,** 41-53. 10.15430/JCP.2021.26.1.41

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians* **71,**209-249. 10.3322/caac.21660

Tao, H., Huang, J., Yancey, P. G., Yermalitsky, V., Blakemore, J. L., Zhang, Y., Ding, L., Zagol-Ikapitte, I., Ye, F., Amarnath, V., Boutaud, O., Oates, J. A., Roberts, L. J., 2nd, Davies, S. S., & Linton, M. F. (2020). Scavenging of reactive dicarbonyls with 2-hydroxybenzylamine reduces atherosclerosis in hypercholesterolemic *Ldlr* (-/-) mice.*Nature Communications* **11,** 4084. 10.1038/s41467-020-17915-w

Torrente, L., Maan, G., Oumkaltoum Rezig, A., Quinn, J., Jackson, A., Grilli, A., Casares, L., Zhang, Y., Kulesskiy, E., Saarela, J., Bicciato, S., Edwards, J., Dinkova-Kostova, A. T., & de la Vega, L. (2020). High NRF2 levels correlate with poor prognosis in colorectal cancer patients and with sensitivity to the kinase inhibitor AT9283*in vitro* . *Biomolecules* **10,** 1365. 10.3390/biom10101365

Triner, D., Devenport, S. N., Ramakrishnan, S. K., Ma, X., Frieler, R. A., Greenson, J. K., Inohara, N., Nunez, G., Colacino, J. A., Mortensen, R. M., & Shah, Y. M. (2019). Neutrophils restrict tumor-associated microbiota to reduce growth and invasion of colon tumors in mice.*Gastroenterology* **156,** 1467-1482. 10.1053/j.gastro.2018.12.003

Uchida, K., Kanematsu, M., Sakai, K., Matsuda, T., Hattori, N., Mizuno, Y., Suzuki, D., Miyata, T., Noguchi, N., Niki, E., & Osawa, T. (1998). Protein-bound acrolein: potential markers for oxidative stress.*Proceedings of the National Academy of Sciences of the United States of America* **95,** 4882-4887.

Vafa, O., Wade, M., Kern, S., Beeche, M., Pandita, T. K., Hampton, G. M., & Wahl, G. M. (2002). c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Molecular Cell* **9,**1031-1044.

West, N. J., Clark, S. K., Phillips, R. K., Hutchinson, J. M., Leicester, R. J., Belluzzi, A., & Hull, M. A. (2010). Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. *Gut* **59,** 918-925. 10.1136/gut.2009.200642

Wu, J., Saleh, M. A., Kirabo, A., Itani, H. A., Montaniel, K. R., Xiao, L., Chen, W., Mernaugh, R. L., Cai, H., Bernstein, K. E., Goronzy, J. J., Weyand, C. M., Curci, J. A., Barbaro, N. R., Moreno, H., Davies, S. S., Roberts, L. J., 2nd, Madhur, M. S., & Harrison, D. G. (2016). Immune activation caused by vascular oxidation promotes fibrosis and hypertension. *J Clin Invest* **126,** 50-67. 10.1172/JCI80761

Xi, Y., & Xu, P. (2021). Global colorectal cancer burden in 2020 and projections to 2040. *Translational Oncology* **14,** 101174. 10.1016/j.tranon.2021.101174

Zagol-Ikapitte, I., Amarnath, V., Bala, M., Roberts, L. J., 2nd, Oates, J. A., & Boutaud, O. (2010). Characterization of scavengers of gamma-ketoaldehydes that do not inhibit prostaglandin biosynthesis.*Chemical Research in Toxicology* **23,** 240-250. 10.1021/tx900407a

Zhang, T., Ahn, K., Emerick, B., Modarai, S. R., Opdenaker, L. M., Palazzo, J., Schleiniger, G., Fields, J. Z., & Boman, B. M. (2020).*APC* mutations in human colon lead to decreased neuroendocrine maturation of ALDH+ stem cells that alters GLP-2 and SST feedback signaling: Clue to a link between WNT and retinoic acid signalling in colon cancer development. *PLoS One* **15,** e0239601. 10.1371/journal.pone.0239601

Hosted file

`image1.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 1. Effect of 2-HOBA on inflammation-mediated colon carcinogenesis. (**A**) C57BL6 mice were treated with AOM-DSS and were given 2-HOBA throughout the experiments, except during DSS treatment. (**B**) Body weights were measured weekly and are depicted as percentage of initial body weight; $^{***}P < 0.001$ and $^{****}P < 0.0001$ compared to AOM-DSS-treated mice in the control and 2-HOBA groups. (**C**) After

56 days, colons were removed and the concentration of 2-HOBA was measured by LC/ESI/MS/MS. (**D**) The number of tumors was counted. (**E-F**) tumor size was assessed by the average tumor size per mouse (**E**) and as a percent of total tumors ($n = 106$ tumors for the AOM-DSS group and $n = 96$ for AOM-DSS + 2-HOBA;**F**). (**G**) Tumor burden was calculated. (**H**)Representative images of H&E staining and tumors are surrounded by dotted lines. The scale bars correspond to 200 μm. (**I**) The histological injury score was calculated from the H&E staining. In figures with dot plots, P was determined by two-way ANOVA and Tukey test in **B** , one-way ANOVA and Tukey test in **C** , or Student's t test (**D** , **E** , **G** ,**I**); we also used Chi-Square for the contingency analysis (**F**).

Hosted file

`image2.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 2. Colonic and tumoral immune response in the AOM-DSS model. The expression of the genes *Nos2* , *Tnf* , *Il1b* ,*Cxcl1* , *Ifng* , and *Il17* was analyzed by RT-real time PCR using RNA isolated from the non-tumor (NT) or tumor (T) areas isolated from the colon of mice ± AOM-DSS ± 2-HOBA. P was determined by ANOVA and Tukey test.

Hosted file

`image3.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 3. Effect of 2-HOBA on a model of sporadic colorectal cancer. (**A**)$CDX2P$ -$CreER^{T2}$;$Apc^{fl/fl}$mice were treated or not with TAM ± 2-HOBA. (**B**) Body weights were monitored weekly and are shown as percentage of initial body weight; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.0001$ compared TAM-treated mice, and §$P< 0.05$, §§$P < 0.01$, and §§§§$P< 0.0001$ compared to TAM-treated mice in the 2-HOBA group. (**C**) 35 days after TAM injection, colonic levels of 2-HOBA were measured by LC/ESI/MS/MS. (**D** -**F**) Tumor number (**D-E**) and burden (**F**) were determined in the mid and distal colon. Note that there were no tumors in animals that did not receive TAM, treated or not with 2-HOBA. (**G**) Correlation plots comparing tumor burden (from Fig. 3F) and 2-HOBA concentrations (from Fig. 3C). (**H**) The H&E staining shows a large tumor with a complex growth pattern indicating high-grade dysplasia in the TAM group and two smaller tumors formed by densely packed neoplastic crypts with low-grade dysplasia; scale bar, 100 μm. (**I**) The inflammation score was determined by histologic assessment. P values were calculated by two-way ANOVA and Tukey test (**B**), one-way ANOVA and Tukey test (**C**), and Student's t test (**E** ,**F** , **I)** . In (**G**), statistical analysis was performed using the Pearson correlation test.

Hosted file

`image4.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 4. Effect of 2-HOBA on the colonic microbiome. DNA was extracted from colonic feces of 4$CDX2P$ -$CreER^{T2}$;$Apc^{fl/fl}$mice and 5$CDX2P$ -$CreER^{T2}$;$Apc^{fl/fl}$mice treated with 2-HOBA. The copy number of 16S rRNA genes was determined by real-time quantitative PCR (**A**). The 16S rRNA genes were then sequenced and analyzed. Alpha diversity was evaluated by the Simpson and Shannon indexes (**B**). Colonic bacterial community composition at phylum (**C**) and genus (**D**) levels was expressed as a ratio to the total community. The Pvalues corresponding to Fig. **C** and **D** are provided in Table S1.

Hosted file

`image5.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-

and-reduces-tumor-growth

Figure 5. Electrophile adduct levels. The colon of *CDX2P -CreER^{T2} ;Apc$^{fl/fl}$* mice ± TAM ± 2-HOBA was immunostained with the D11 antibody (**A**) and the nuclear staining was quantified (**B**). Each dot represents a mouse, and 20 crypts per animal were assessed. Scale bars, 50 μm.

Hosted file

`image6.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 6. Regulation of NRF2 activation. (**A**) RNA was extracted from non-tumor areas (NT) or tumors (T) from *CDX2P -CreER^{T2} ;Apc$^{fl/fl}$* mice ± TAM ± 2-HOBA. Gene expression was then measured by RT-real-time PCR. (**B**) The expression of NRF2 was assessed by immunofluorescence and the images are representatives of 3 animals per group. Each dot represents a mouse, and 5-10 crypts per animal were assessed. Scale bars, 50 μm. *P* values were calculated by two-way ANOVA and Tukey test (**A**) or Dunnett's test (**B**).

Hosted file

`image7.emf` available at https://authorea.com/users/581352/articles/621972-the-dicarbonyl-electrophile-scavenger-2-hydroxybenzylamine-2-hoba-prevents-colorectal-carcinogenesis-and-reduces-tumor-growth

Figure 7. Treatment with 2-HOBA in mice with human xenografts. Human CRC HCT116 cells were injected in the flank of nude mice. Animals were then treated or not with 2-HOBA. (**A**) The levels of 2-HOBA were measured in the tumors after 35 days. (**B**) Tumor volume was measured weekly in sham-treated (left panel) or 2-HOBA-treated (middle panel) mice. The right panel depicts mean ± SEM; the fixed effects (type III) *P* value (time and treatment) was calculated by two-way ANOVA and Tukey test. (**C-D**) On day 35, tumors were harvested (**C**), measured, and the volume was calculated (**D**).*P* was determined by the Student's *t* test. Scale bar, 1 cm. (**E**) Correlation comparing tumor volume (from Fig. 5D) and 2-HOBA concentrations (from Fig. 5A, middle panel). Statistical analysis was performed using the Pearson correlation test.