Cancer Chemother Pharmacol (2010) 66:755–764
DOI 10.1007/s00280-009-1220-5

ORIGINAL ARTICLE

Inhibition of experimental colorectal cancer and reduction in renal and gastrointestinal toxicities by copper–indomethacin in rats

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Received: 31 July 2009 / Accepted: 9 December 2009 / Published online: 25 December 2009

Abstract
Purpose To evaluate, for the first time, the efficacy of copper–indomethacin in the inhibition of aberrant crypt foci formation using the azoxymethane-induced adenocarcinoma model, to examine cell viability in the HCT-116 colorectal cancer cell line, gastrointestinal permeability, mitochondrial oxidative damage, and renal toxicity in rat models.
Methods Azoxymethane-induced adenocarcinoma rats were dosed with indomethacin and copper–indomethacin for 28 days and aberrant crypt foci were evaluated. HCT-116 colorectal cancer cells were exposed to indomethacin and copper–indomethacin at 0–250 μg/mL (0–698 μM for indomethacin, and 0–147 μM for copper–indomethacin), and cell viability was measured. Acute gastrointestinal toxicity was measured using gastrointestinal permeability markers, gastrointestinal ulceration and bleeding, and measurement of an acute-phase protein haptoglobin. Effects of acute and chronic administration of indomethacin and copper–indomethacin on urinary electrolyte concentrations were examined.
Results Both indomethacin and copper–indomethacin resulted in a significant reduction in aberrant crypt foci in azoxymethane-treated rats. In parallel, high concentrations of indomethacin and copper–indomethacin also reduced cell viability in HCT-116 colorectal cancer cells. However, copper–indomethacin was considerably safer in all measures of gastrointestinal toxicity compared to indomethacin. In addition, indomethacin reduced urinary electrolytes at an ulcerogenic dose of 10 mg/kg acutely and chronically at 3.0 mg/kg for 28 days, whereas copper–indomethacin at equimolar doses of indomethacin affected urine electrolytes after acute dosing but not after chronic dosing for 28 days.
Conclusions Copper–indomethacin has both gastrointestinal and renal sparing properties while maintaining efficacy in experimental adenocarcinoma.

Keywords NSAID · Colon · Cancer · Metal

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Introduction

The long-term use of NSAIDs, such as aspirin and indomethacin, has been shown to reduce significantly the incidence of colon cancer in humans and experimental animals [1, 2]. However, NSAID use is also associated with gastrointestinal and renal side effects, which limit their utility [3, 4]. In the stomach, NMR spectroscopic studies indicated that glycolytic and Krebs cycle enzymes were partially inhibited in the ulcerated stomach by the NSAID, as shown by changes in the lactate/glucose ratio [5]. Several approaches to the problems of NSAID-induced gastrointestinal toxicity have been developed which include: selective cyclooxygenase-2 inhibitors, nitric oxide releasing NSAIDs; [2] as well as NSAIDs with slow release of gastroprotective gaseous mediators, such as nitric oxide or hydrogen sulfide; [6] phosphatidylcholine releasing NSAIDs; [2] the design of NSAID prodrugs by temporarily blocking the free carboxylic group in the molecule until they are systemically

absorbed; [7] and NSAIDs with selective inhibition of terminal prostaglandin synthases. [6] Although these are promising approaches to reducing GI toxicity, selective COX-2 inhibitors are the only group to be currently marketed clinically, and concerns over their renal and cardiovascular toxicities still remain [8–11]. Hence, the search and development for safe and effective NSAIDs still continues.

NSAID coordination complexes are a class of potentially GI and renal sparing drugs that are in experimental and clinical development [12–15]. Copper–indomethacin (Fig. 1) is currently marketed in Australia and New Zealand for veterinary use and has successfully completed Phase 1 human clinical trials for proctitis [14]. There have been no studies on the efficacy of NSAID coordination complexes in models of experimental colorectal cancer, but the low gastrointestinal toxicities of such complexes [12–15] suggested that they would offer substantial advantages over the free NSAIDs for such applications. This study was undertaken to compare the abilities of indomethacin and copper–indomethacin to inhibit the formation of carcinogen-induced aberrant crypt foci (ACF) in the well-characterized azoxymethane-induced colonic cancer model in rats [2]. ACF are early pre-neoplastic microscopic lesions that have consistently been observed in a number of experimental models of colon carcinogenesis and are also present in the mucosa in human colon cancer, where they have been suggested to be precursor lesions from which adenomas and carcinomas develop [2]. In addition, there have been no previous studies designed to evaluate the renal side effects or gastrointestinal permeability profile of NSAID coordination complexes.

Methods and materials

Indomethacin, methylcellulose, phosphate-buffered saline, fetal bovine serum, trypsin–EDTA, halothane, HEPES, penicillin–streptomycin, sodium bicarbonate, sucrose, McCoy's 5A medium, methylene blue, and dimethylsulfoxide (DMSO) were obtained from Sigma Aldrich (St. Louis Mo. USA). Copper–indomethacin $[Cu_2(Indo)_4(DMF)_2]$ ($C_{82}H_{76}Cl_4Cu_2N_6O_{18}$) [16] was used as supplied by Biochemical and Veterinary Research Limited (Mittagong, NSW, Australia) and Vetafarm (Wagga Wagga, Australia). HCT-116 cells were obtained from American Type Culture Collection (Manassas, Virginia, USA) and Alamar Blue from Trek Diagnostic Systems (Cleveland, Ohio, USA).

Sprague Dawley male rats weighing 200–250 g were used throughout these studies. Animals were housed in polypropylene cages and allowed free access to standard laboratory rat chow (Purina Rat Chow, Ralston Purina, St. Louis MO, USA) and tap water. Animals were housed in an animal care facility at ambient temperature and humidity with a 12-h light–dark cycle. The experimental animal protocols were approved by animal ethics committees at The University of Sydney (L24/7-99/3/2972) and Washington State University (#3204).

Gastric mucosal damage

Rats were divided into three groups ($n = 4$ each) and were orally gavaged with either indomethacin at 10 mg/kg, copper–indomethacin at 13.3 mg/kg or vehicle (2% carboxymethylcellulose, CMC). Since copper–indomethacin consists of a copper moiety and an indomethacin moiety, a higher dose of this compound was given so that an equimolar

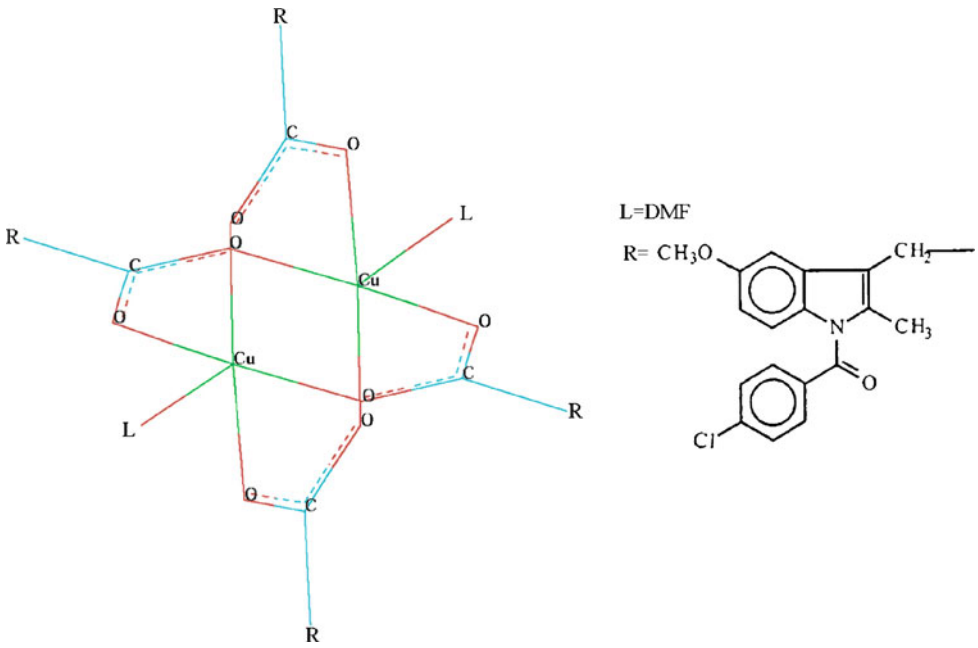

Fig. 1 Chemical structure of copper–indomethacin, $[Cu_2(Indo)_4(dmf)_2]$

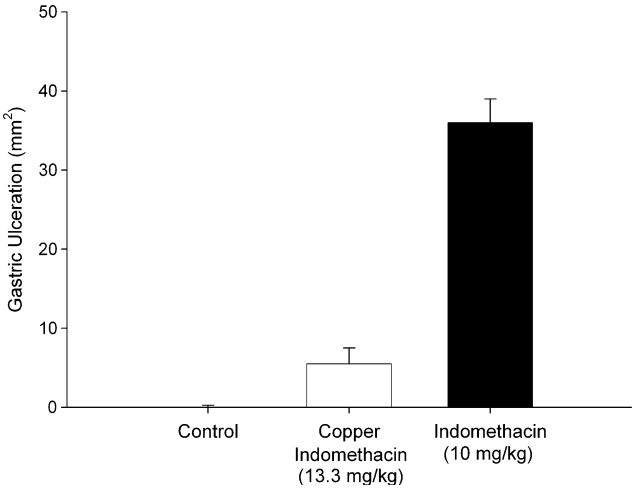

Fig. 2 Gastric ulceration of indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

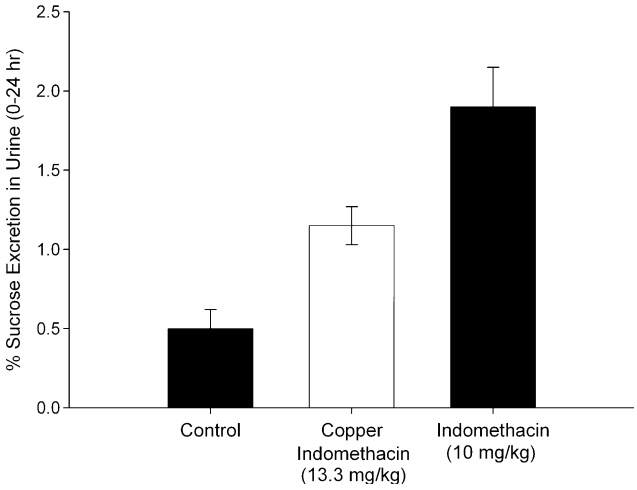

Fig. 3 Gastric permeability of indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

amount of the NSAID moiety was being delivered, as was the case with the indomethacin-treated rats. The compounds were suspended in 2% CMC. Rats were deprived of food, but not water, for 18 h prior to treatment and were orally administered by gavage one of indomethacin, copper–indomethacin, or vehicle (control). Animals were anesthetized with halothane 3 h after the dose, and the stomachs were excised and opened by an incision along the greater curvature for assessment of macroscopic damage by a trained observer blinded of the treatment groups using a method described previously for the macroscopic assessment [17]. This method involved the measurement of the length of lesions in millimeters (mm) using digital calipers, and then the lengths of all the lesions observed in each stomach were summed (Fig. 2).

Gastric permeability

Sucrose permeability changes were measured using a previously reported method [18]. Rats ($n = 4$ for each treatment) were deprived of food, but not water, for 18 h prior to treatment. The rats received molar equivalent oral NSAID doses of either indomethacin at 10 mg/kg or copper–indomethacin at 13.3 mg/kg via gavage. Two hours post-dosing, 0.5 mL of a solution containing 0.5 g/mL of sucrose was orally administered via a curved feeding needle to each rat. Urine was collected for a 24-h period following the administration of the sucrose solution. Relative permeability was determined by calculating the sucrose present in each urine sample, and the value was expressed as a percentage of the administered dose (Fig. 3).

Plasma haptoglobin

Rats ($n = 4$ for each treatment) were deprived of food, but not water, for 18 h prior to treatment. The rats received oral doses of either indomethacin at 10 mg/kg or copper–indomethacin at 13.3 mg/kg via gavage. Whole blood was obtained by cardiac puncture using a 23 gauge (G) needle attached to a 10-mL syringe, under halothane anesthesia. The blood was then placed into EDTA vacutainer tubes, the contents were thoroughly mixed, then centrifuged to obtain the plasma fraction. Haptoglobin concentration (milligrams per milliliter) was measured using a commercially available kit (Dade Behring; Mannheim, Germany), and the value was determined by radial immunodiffusion after pipetting 5-μL samples of plasma into each well. Normal range values correspond to a diffusion zone of approximately 6 mm of diameter (1.22 g/L). The diameter of the precipitin zone is directly proportional to the concentration of the haptoglobin protein in the sample [19] (Fig. 4).

Small intestinal ulceration

Rats ($n = 4$ for each treatment) received oral doses of either indomethacin at 10 mg/kg or copper–indomethacin at 13.3 mg/kg via gavage. In order to assess small intestinal damage, the animals were sacrificed 24 h after dosing and the intestines were removed. A vertical mid-line abdominal incision was made, and then the entire length of the small intestine was isolated, excised, and examined over a region that extended from 10 cm distal to the ligament of Treitz to the ileocecal junction. A 26-G needle attached to a 5-mL syringe was used to flush the intestine, in order to avoid distension. After expelling the intestinal contents, the entire intestine was submerged in 10% technical grade formaldehyde 37/7 for 1 h. The intestine was then opened along the anti-mesenteric side and pinned to a thin piece of cardboard, and intestinal ulceration was determined by measuring the length of lesions in mm using digital calipers, and the lengths of all lesions observed in each intestine were summed (Fig. 5) [20].

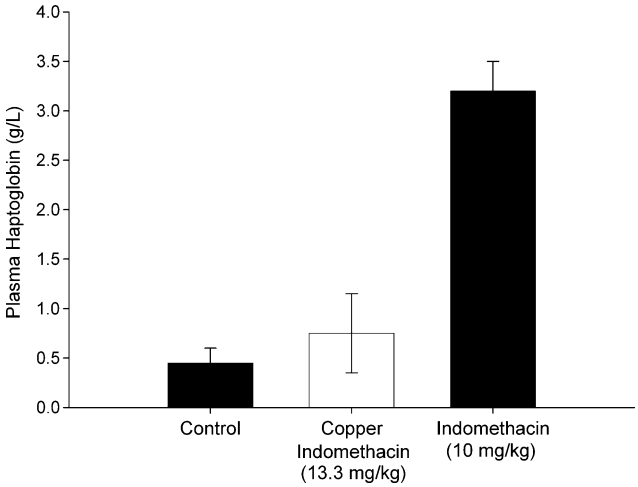

Fig. 4 Plasma haptoglobin after indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

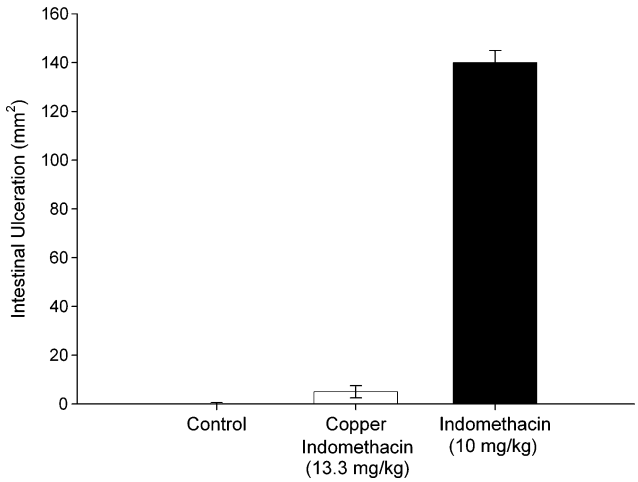

Fig. 5 Small intestinal ulceration after indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

Small intestinal permeability

Rats ($n = 4$ for each treatment) received either oral indomethacin at doses of 10 mg/kg or copper–indomethacin at 13.3 mg/kg via gavage. The intestinal permeability was tested with 0.5 mL of a solution containing 10 μCi/mL of ^{51}Cr-EDTA, which was administered orally 3 h following the dose of placebo or NSAIDs. Rats were housed in special metabolic cages, and urine and feces were collected separately. Urine (0–24 h) was collected following the administration of ^{51}Cr-EDTA. The urine was collected in cups and transferred to scintillation vials. Urine samples were counted by a gamma counter Beckman Gamma 8000 (Irvine, California) for 1 min in a counting window scanning within a range of 0–2 MeV. At least two standards of 100 μL of the administered ^{51}Cr-EDTA solution were counted with every set of urine samples. Relative permeability was determined by calculating the activity present in each urine sample and expressed as a percent of the administered dose after correcting for background radiation [21] (Fig. 6).

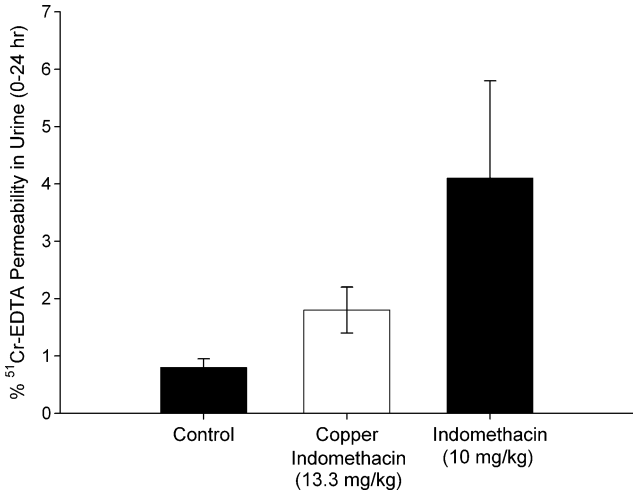

Fig. 6 % ^{51}Cr-EDTA excretion in urine 0–24 h after indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

Cecal hemoglobin

The cecum ($n = 4$) was excised from rats dosed with indomethacin (10 mg/kg), copper–indomethacin (13.3 mg/kg), or controls (no treatment). The contents were removed and diluted with distilled water up to 5 mL and vortex-mixed to form a homogeneous consistency. The contents of each test tube were divided into two tubes, each was centrifuged for 10 min at 3,000*g*, and the supernatants were stored at −20°C. Aliquots (0.5 mL) were assessed for the quantitative colorimetric determination of hemoglobin concentration, which was measured spectrophotometrically in accordance with Sigma Diagnostics Total Hemoglobin® (Sigma Chemical Co., St. Louis, MO, USA) [19] (Fig. 7).

Enteric bacterial numbers

Rats ($n = 4$ for each treatment) received either oral indomethacin at doses of 10 mg/kg or copper–indomethacin at 13.3 mg/kg via gavage. In order to assess enteric bacterial translocation, the animals were sacrificed 24 h after dosing and the intestines were removed. A vertical mid-line abdominal incision was made, and then the entire length of the small intestine was isolated, excised, and examined over the region that extended from 10 cm distal to the ligament of Treitz to the ileocecal junction. Intestinal tissue samples (~1 g) were obtained from the distal ileum, placed into Petri dishes containing sterile phosphate-buffered saline (pH 7.4), and then weighed. The tissues were homogenized, serial log dilutions were plated onto MacConkey agar No. 2

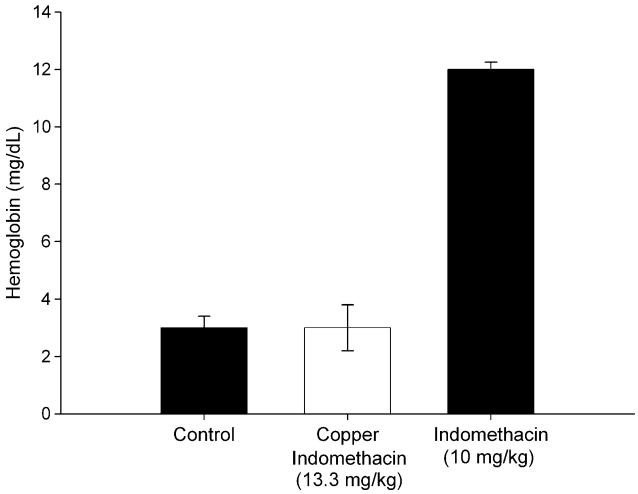

Fig. 7 Hemoglobin concentration in the cecum after indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

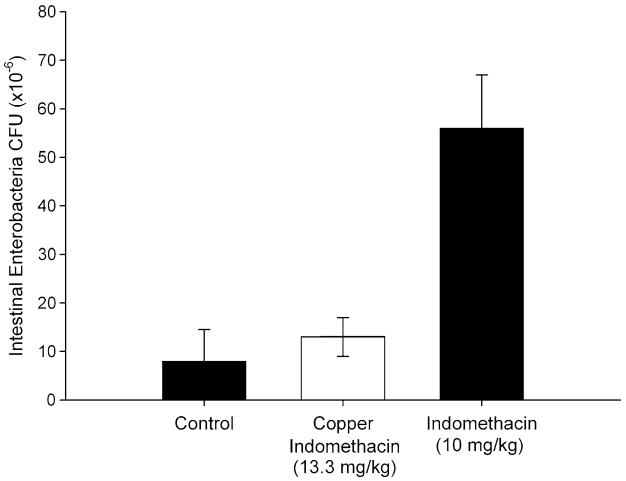

Fig. 8 Enteric bacteria numbers per gram of tissue following treatment with indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

(Oxoid, NSW, Australia), and the plates were incubated at 37°C for 24 h under aerobic conditions. Plates containing between 20 and 200 colony-forming units (CFU) were selected for counting to determine total enteric bacterial numbers per gram of tissue [22] (Fig. 8).

Mitochondrial DNA

The small intestine ($n = 5$) was excised from rats dosed with indomethacin (10 mg/kg), copper–indomethacin (13.3 mg/kg), or controls (vehicle treatment). Quantitative polymerase chain reaction (QPCR) was used as previously described [23]. DNA was isolated from ulcerated intestinal tissue using a Qiagen® genomic tip and a genomic DNA buffer set kit for mammalian DNA extractions (Valencia, CA, USA). DNA quantitation utilized the PicoGreen®

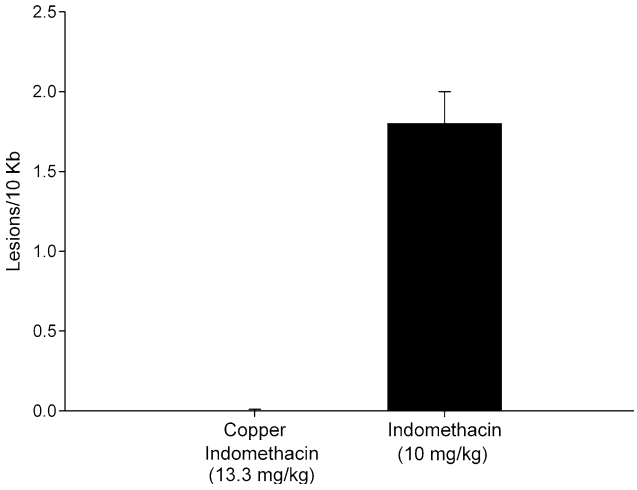

Fig. 9 Mitochondrial DNA damage in rat intestinal tissue after administration of single doses of indomethacin and copper–indomethacin ($n = 5$, mean ± SEM)

dsDNA Quantitation Kit (Molecular Probes, Eugene, OR, USA). PicoGreen® was used to quantify dsDNA fragment. QPCR involved the use of GeneAmp XL PCR kit (Applied Biosystems, Branchburg, NJ, USA) and dNTPs (Pharmacia, Peapack, NJ, USA). Primers were based on sequences already optimized by Van Houten [24, 25]. Fluorescent readings for all products were taken using a CytoFluor fluorescence multiwell plate reader Series 4000 (Applied Biosystems, Framingham, MMA, USA) and subtracted from the no-template controls and relative amplification was calculated (Fig. 9).

NAG (*N*-acetyl-*β*-D-glucosaminidase)

Rats ($n = 4$ for each treatment) received either oral indomethacin at doses of 10 mg/kg or copper–indomethacin at 13.3 mg/kg via oral gavage. Rats were housed in special metabolic cages where urine (0–24 h) and feces were collected separately. The colorimetric assay kit was obtained from Boehringer Mannheim. The instructions and procedures were followed as documented by the manufacturer. The test is based on the hydrolysis of 3-cresolsulfonphthalenyl-*N*-acetyl-*β*-D-glucosaminide, sodium salt by NAG with the release of 3-cresolsulfonphthalein, sodium salt (3-cresol purple), which was measured photometrically at 580 nm (Fig. 10).

Urine electrolytes

Rats ($n = 3$–5 for each treatment) received oral acute doses of either 10 mg/kg for indomethacin or 13.3 mg/kg for copper–indomethacin, and oral chronic doses of 3.0 mg/kg for indomethacin or 3.8 mg/kg for copper–indomethacin for 28 days. Rats were housed in special metabolic cages

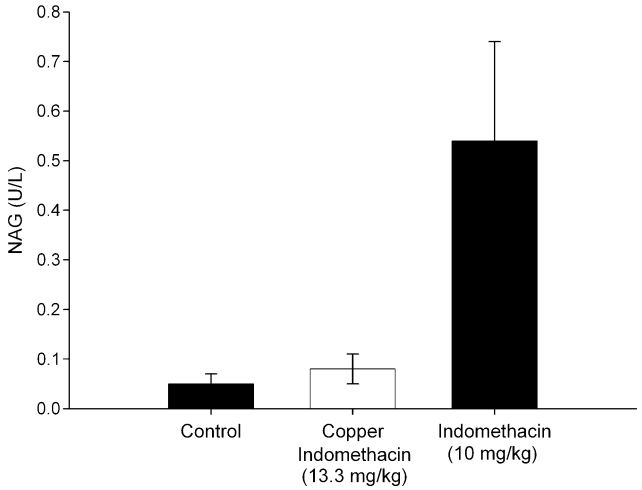

Fig. 10 NAG concentration in urine tissue following treatment with indomethacin and copper–indomethacin ($n = 4$; mean ± SEM)

where urine (0–24 h) and feces were collected separately. Sodium, potassium, chloride, and phosphate were assayed at the Veterinary Pathology Laboratory (Faculty of Veterinary Science, The University of Sydney), using commercially prepared reagents obtained from Beckman Coulter Inc. (Brea, CA USA), as well as a Beckman, Synchrome, and EI-ISE Electrolite system (Beckman Coulter Inc. Brea, CA USA) (Table 1).

Colonic aberrant crypt foci

The rats were randomized into treatment groups of five animals at the beginning of the experiment. For the first 4 weeks, rats received weekly intraperitoneal injections of azoxymethane (AOM) at a dose of 15 mg/kg for the induction of aberrant crypt foci ACF), as well as concurrent daily oral doses of 2% carboxymethylcellulose (CMC) vehicle, indomethacin (3.0 mg/kg), or an equimolar dose of copper–indomethacin (3.8 mg/kg). The dose of indomethacin was selected based on previous studies of the intestinal toxicity of indomethacin and significant reduction in colonic carcinogenesis in an animal model closely related to the one used in the present study [26, 27]. Six weeks after the final AOM injection, rats were sacrificed by an overdose of halothane, a laparotomy was performed and the entire colon was excised. After gentle flushing with 0.9% saline, the colon was tied at both ends with silk sutures and insufflated with 10% phosphate-buffered formalin (pH 7.4). After 2 h, the colons were opened along the mesenteric border, pinned flat, mucosal side up, and then submerged in formalin for a further 24 h. The tissues were stained with 0.2% methylene blue in 0.9% saline and then coded before scoring by a blinded observer. The colon from each animal was cut into convenient 2- to 3-cm lengths, and each length was placed

Table 1 Effect of acute and chronic indomethacin and copper–indomethacin on urine electrolytes composition

Drug	Na^+ Mmol/L	K^+ mmol/L	Cl^- mmol/L	Phosphate mmol/L
Acute Indomethacin 10 mg/kg	↓ Decreased from controls 0.31 ± 0.22 vs. 1.22 ± 0.25	↓ Decreased from controls 1.0 ± 0.45 vs. 2.3 ± 0.25	↓ Decreased from controls 0.25 ± 0.21 vs. 1.25 ± 0.35	↑ Increased from controls 0.07 ± 0.7 vs. 0.013 ± 0.003
Chronic indomethacin 3.0 mg/kg for 28 days	↓ Decreased from controls 0.85 ± 0.13 vs. 1.20 ± 0.3	↓ Decreased from controls 1.75 ± 0.23 vs. 2.5 ± 0.5	↓ Decreased from controls 0.6 ± 0.28 vs. 0.91 ± 0.25	↔No change from controls 0.012 ± 0.02 vs. 0.012 ± 0.003
Acute copper–indomethacin 13.3 mg/kg	↓Decreased from controls 0.33 ± 0.36 vs. 1.22 ± 0.25	↓Decreased from controls 1.2 ± 0.65 vs. 2.3 ± 0.25	↓ Decreased from controls 0.7 ± 0.41 vs. 1.25 ± 0.35	↔No change from controls 0.009 ± 0.002 vs. 0.013 ± 0.003
Chronic copper–indomethacin 3.8 mg/kg for 28 days	↔ No change from controls 1.19 ± 0.14 vs. 1.20 ± 0.3	↔No change controls 2.2 ± 0.23 vs. 2.5 ± 0.5	↔No change from controls 1.1 ± 0.28 vs. 0.91 ± 0.25	↔No change from controls 0.013 ± 0.01 vs. 0.012 ± 0.003

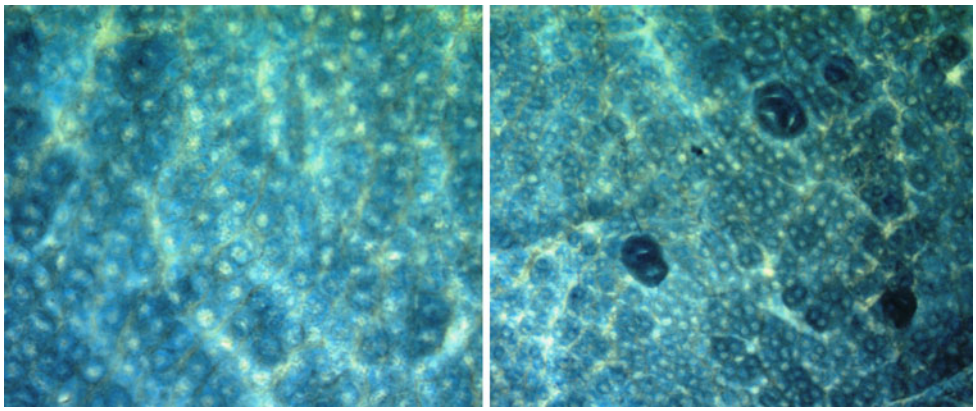

Fig. 11 Control rat colon (*left panel*) vs. azoxymethane-induced ACF (*right panel*)

"mucosal side up" between two slides as a wet mount and examined microscopically at 100× magnification. Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, bright blue staining, significantly increased distance from lamina to basal surface of cells, and the easily discernible pericryptal zone (Fig. 11). The parameters used to assess the aberrant crypts were occurrence and multiplicity. Crypt multiplicity was determined as the number of crypts in each focus and categorized as those containing up to three, or four or more aberrant crypts/focus (Fig. 12).

Cell culture

HCT-116 cells were cultured in McCoy's 5A medium containing sodium bicarbonate, penicillin–streptomycin, 25 mM HEPES and 10% heat-inactivated FBS in an atmosphere containing 5% CO_2 at 37°C. The cells were passed twice a week on reaching confluence. The cells were trypsinized, washed with PBS, and plated with fresh medium in 96-well plates at a density of 3×10^4 cells/well and cultured overnight. The culture medium was discarded, and the treatment was started by adding fresh medium with or without indomethacin or copper–indomethacin previously dissolved in dimethylsulfoxide (DMSO) at different concentrations (0, 10, 50, 100, and 250 μg/mL), and the cells were further incubated for 72 h. After incubation, the medium was discarded and fresh medium containing 10% Alamar Blue was added to each well, as well as blank wells, and the cells were further incubated for 3 h. The plate was placed at room temperature in the dark for 15 min, and the fluorescence was read with CytoFluor (PerSeptive Biosystems, MA) at Ex. 532/20, Em 590/50 gain 40.

Statistical analysis

All results are expressed as the mean ± standard error of the mean (SEM). Student's *t*-test, two-tailed ANOVA, and Bonferroni's multiple comparison test were used to determine significance levels. $P < 0.05$ was considered significant.

Results

Administration of indomethacin induced statistically significant gastric and intestinal damage in the rat; however, copper–indomethacin administration significantly attenuated the ulcerogenic properties of the parent compound (Figs. 1, 2, 3, 4, 5, 6, 7, 8 and 9). Overall, gastrointestinal toxicity data demonstrate attenuation of gastrointestinal permeability with the copper–indomethacin coordination complex that appears to ameliorate the toxicological sequalae of ulceration, bleeding, bacterial invasion, and mitochondrial damage to enterocytes.

Acute administration of ulcerogenic doses of indomethacin resulted in decreased urinary excretion of Na^+, K^+, and Cl^- and an increase in urinary excretion of NAG and phosphate (Table 1, Fig. 10). Acute administration of copper–indomethacin also decreased Na^+, K^+, and Cl^- but did not result in an increase in urinary NAG or phosphate excretion. The chronic administration of indomethacin at a non-ulcerogenic dose resulted in a decrease in Na^+, K^+, Cl^-, but not phosphate, whereas chronic administration of copper–indomethacin at 3.8 mg/kg for 28 days did not significantly change the concentrations of these urinary electrolytes. Taken together, these results demonstrate comparatively less tubular and glomerular toxicity to the kidneys with the exposure to copper–indomethacin versus indomethacin in rats.

Administration of AOM resulted in the development of widespread ACF, predominantly in the distal colon (Fig. 11). Figure 12 shows that both indomethacin and copper–indomethacin significantly decreased the number of induced ACFs compared with controls. Indomethacin was significantly more effective than copper–indomethacin; however, two animals in the indomethacin group died in the first week of treatment with this agent. Interestingly,

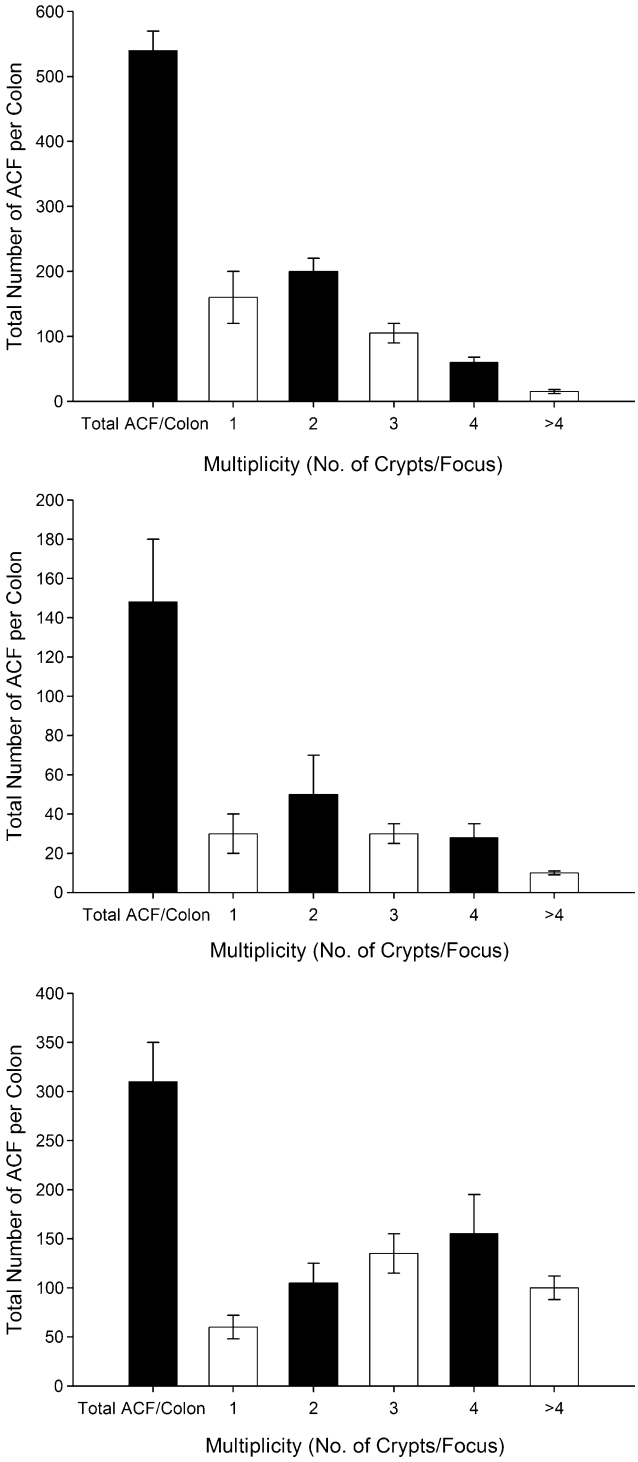

Fig. 12 Effect of indomethacin and copper–indomethacin on azoxymethane-induced ACF in rats ($n = 3$–5; mean ± SEM). *Upper panel*, control; *middle panel*, indomethacin 3.0 mg/kg; and *lower panel*, copper–indomethacin 3.7 mg/kg

there were proportionally more ACF with two crypts in both the control and indomethacin groups, whereas with the copper–indomethacin group, ACF with one and two crypts were approximately equal (Fig. 12). Both indomethacin and copper–indomethacin reduced the percentage survival of the colorectal cancer cell line HCT-116 in a concentration-dependent manner, but neither compound reached an IC_{50} value at concentrations as high as 250 μg/mL, suggesting this anti-cancer effect may not be due to strong cytotoxicity. At the highest concentrations tested, copper–indomethacin demonstrated significantly greater activity than indomethacin and even more so with respect to the amount of indomethacin in the complex.

Discussion

While it has been long known that NSAIDs can help prevent colorectal cancers, the major clinical issue has been the side effects of chronic doses with both non-selective and selective COX-2 inhibitors. If these side effects can be overcome, NSAIDs could become an important tool in reducing the incidence of such cancers. Here, we have demonstrated that copper–indomethacin is less ulcerogenic than indomethacin to the gastrointestinal tract of rats, as has been previously demonstrated [12, 13, 15, 28], at concentrations that are pharmacologically active at reducing the incidence of lesions in rat models of colorectal cancers. Importantly, here evidence is provided that formulating indomethacin in a coordination complex with copper also modulates the gastrointestinal permeability and, therefore, is expected to limit bacterial translocation across the intestinal mucosa through the tight junctions between enterocytes. Consequently, there is less ulceration and the sequalae of tissue damage in terms of up-regulated acute-phase proteins, such as haptoglobin and oxidative damage to the enterocytes and gastrointestinal bleeding as measured by cecal hemoglobin. Copper–indomethacin has been shown to have inhibitory effects against bacteria [29]. The potential for these complexes to be developed further for the treatment of colorectal cancers is supported by the lack of GI or other forms of toxicity in Phase 1 human clinical trials for the treatment of proctitis [14]. Thus, the lack of any apparent side effects for Cu–Indo in the human clinical trials augurs well for its application for the prophylaxis and treatment of colorectal and other cancers. Moreover, the chronic administration of copper–indomethacin appears to be much safer in terms of the renal toxicity of these compounds, which is a completely new and important finding for potential clinical applications. A plausible explanation is that copper may modulate prostaglandin production in the kidney, as has been previously demonstrated [30]. Copper can also preferentially inhibit COX-2 [31]. However, acute administration of high doses of copper–indomethacin does cause changes in urinary electrolyte composition, and this compound like all NSAIDs is not devoid of the potential for renal toxicity. Despite this, the

evidence provided here suggests that chronic dosing of patients at low therapeutic doses required for the prophylaxis or treatment of colorectal cancers may be safe in terms of renal and gastrointestinal toxicity. This study demonstrates for the first time that copper–indomethacin coordination compounds are effective in an experimental model of colorectal cancer. Interestingly, the majority of studies on anti-inflammatory efficacy have demonstrated equivalent or superior efficacy of copper-NSAIDs as compared to the parent NSAID [12, 13, 15, 28]. The solubility of copper–indomethacin results in slow dissolution in vitro which could in part account for a reduced topical gastric toxicity; however, the absorption of copper–indomethacin and circulating plasma concentrations of indomethacin are similar to that of indomethacin in the rat [32]. It is not clear whether the pattern of efficacy and safety demonstrated with copper–indomethacin compared to the parent compound in colorectal cancer is extendable to other NSAID coordination complexes including a range of complexes with Cu and Zn [12, 13, 33–36], which have also been shown to have reduced gastrointestinal toxicities compared with the parent NSAIDs [37–41]. Further tissue distribution and drug targeting studies with various formulations of copper-NSAIDs would be required to try and elucidate the relationship between drug concentrations at the effect-site of the colonic epithelium to enhance the inhibition of colorectal cancer. It should be emphasized that there has been no attempt to optimize the formulations that have produced these very promising results in order to optimize delivery to the colorectal region of the GI tract. The differences in efficacy of Indo and Cu–Indo may be due to the differences between the two forms of the drug with respect to the regions of maximum absorption within the GI tract due to differences in physicochemical properties such as solubility and dissolution [32]. Moreover, it has already been established that appropriate formulations can reduce the toxicity and increase the efficacy of NSAID coordination complexes even further [37]. Therefore, such an approach shows considerable promise for further investigation.

Conclusion

Copper–indomethacin is effective in significantly reducing the number of ACF in the rat colorectal cancer model and exhibits a dose-dependent effect on the viability of HCT-116 colorectal cancer cells at high concentrations. This compound appears to be safer for the gastrointestinal tract and is also sparing to kidneys, as indicated by some markers of renal toxicity. These data, if extendable to humans, may suggest one potential pharmacological approach to reducing the incidence of colon cancer with the added advantages of a significantly reduced incidence of concomitant gastrointestinal lesions and potentially less adverse effects in kidney function normally associated with this class of drug. Further studies are ongoing with other metallic NSAID coordination complexes.

Acknowledgments We gratefully acknowledge support from an Australian Research Council SPIRT grant (PAL and TWH) and a University of Sydney SESQUI grant (NMD). We are also grateful for the financial support from BVR and Nature Vet for this research and helpful discussions with Hub Regtop and Ray Biffin from BVR.

Financial interest statements Nature Vet has a commercial interest in Cu–Indo and the University of Sydney held shares in Medical Therapies Ltd that has been involved in human clinical trials of copper-indomethacin.

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