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Comparison of pharmacokinetic and pharmacodynamic profiles of aspirin following oral gavage and diet dosing in rats

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ABSTRACT

Aspirin is one of the oldest drugs and has been purported to have multiple beneficial effects, including prevention of cardiovascular disease and cancer, in addition to its original indication for treatment of inflammation, fever and pain. In cancer chemoprevention studies using animal models, two methods of aspirin administration have been employed: oral gavage and diet. The untested assumption was that exposure and the resultant pharmacological effects are similar for these two administration methods when dosing is normalized on the basis of mg/kg body weight/day. This study examined and compared time-dependent plasma and colon mucosal concentrations of aspirin metabolite salicylate (aspirin concentrations were below level of quantification), plasma thromboxane B_2 concentrations, and colon mucosal prostaglandin E_2 concentration following these two different dosing paradigms in rats. Diet dosing yielded relatively constant plasma and colon salicylate concentration vs. time profiles. On the other hand, oral gavage dosing led to a rapid peak followed by a fast decline in salicylate concentration in both plasma and colon. Nevertheless, the exposure as measured by the area under plasma or colon concentration–time curve of salicylate was linearly related to dose irrespective of the dosing method. Linear relationships were also observed between colon and plasma salicylate areas under the curve and between colon prostaglandin E_2 and plasma thromboxane B_2 areas under the curve. Therefore, more easily accessible plasma salicylate and thromboxane B_2 concentrations were representative of the salicylate exposure and prostaglandin E_2 pharmacodynamic biomarker in the target colon, respectively.

1. Introduction

Aspirin is the first drug in the non-steroidal anti-inflammatory (NSAID) class and has been in use for its anti-inflammatory, antipyretic, and analgesic properties dating back to 1899. More recently, aspirin is being evaluated in prevention of two leading causes of death, cardiovascular disease (including stroke, myocardial infarction and thromboembolism) [1,2] and cancer [2]. Aspirin and other NSAIDS are being studied in colorectal cancer prevention [3–5], and to a lesser extent also prostate [6,7] and pancreatic cancer [8].

The purpose of this study was to describe and compare pharmacokinetic and pharmacodynamic profiles of aspirin following oral and dietary dosing. Test agents are typically administered to rodents via diet in cancer chemopreventive studies [9]. Diet is the most prevalent dosing method in chemopreventive studies for a number of reasons, including ability and ease of dosing large numbers of animals, availability of historical data, and because many candidate chemopreventive agents are naturally found in the diet. On the other hand, preclinical toxicology studies normally employ the oral gavage method of test agent administration in standard rodent protocols which would better approximate dosing in man, based on taking a pill, than via diet. The two approaches for administering the test agent can be expected to yield differences in pharmacokinetic and pharmacodynamic profiles as was recently shown for another NSAID, sulindac [10]. In animal models of colon cancer prevention, aspirin was administered via diet [11,12]. The present study examined effects of administration method of aspirin on its exposure and pharmacodynamic effects in rats. Pharmacokinetic markers of exposure were plasma and colon areas under the salicylate concentration–time curves. Areas under the concentration–time curve for plasma thromboxane B_2 (TXB_2) and colon mucosal prostaglandin E_2 (PGE_2) were utilized

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as pharmacodynamic markers of effect, as both are known to be responsive to aspirin. TXB_2 is a stable, measurable metabolite of thromboxane A_2, a prothrombic eicosanoid produced from arachidonate by cyclooxygenase (COX) and thromboxane synthase. COX inhibition by non-steroid anti-inflammatory drugs (NSAIDs) such as aspirin results in decreases in TXB_2. Similarly, PGE_2 is a pro-inflammatory prostaglandin produced by COX and prostaglandin E synthase that is decreased following COX inhibition by aspirin [13].

2. Experimental methods

2.1. Test article

Aspirin (Spectrum Chemical and Laboratory Products, New Brunswick, NJ) was stored at ambient temperature and ambient humidity, and protected from light. The purity determined by HPLC was >99.5% prior to initiation of dosing and after completion of the in-life phase of the study.

2.2. Animals

A total of 358 male Fisher 344 Viral Antibody Free (VAF) rats were obtained from Charles River Breeding Laboratories (Kingston, NY). The animals were approximately 8 weeks old and weighed 149–210 g at dosing initiation. Upon arrival at the University of Illinois AAALAC Intl.-accredited animal facility, animals were singly housed in polycarbonate cages with Anderson bed-o'cobs® bedding (Heinold, Kankakee, IL) in a temperature (18–26 °C) and humidity (30–70%) controlled room on a 14 h light/10 h dark cycle. The cage size, 840 cm^2 area and 20 cm height, was adequate to house rats in the upper weight range as described in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 1996. All animals were routinely transferred to clean cages with fresh bedding weekly.

AIN-76A semi-purified rodent diet (PMI Feeds, Inc., St. Louis, MO) was provided *ad libitum* from one week prior to dosing until termination. For the animals in the low and high dose dietary administration groups, the diet during the dosing period was supplemented with aspirin to achieve target concentrations of 300 ppm and 3000 ppm, respectively. The supplemented diet was provided *ad libitum* until termination for these groups. Tap water from an automatic watering system in which the room distribution lines were flushed daily was provided *ad libitum*.

All animals were quarantined for at least eight days prior to dosing. During that time, the animals were observed daily for signs of illness. Animals were examined during quarantine and approved for use by the Clinical Veterinarian prior to being placed on test.

2.3. Experimental design

Three hundred twenty five male rats were randomly assigned to five treatment groups using a computer-generated randomization program, stratified on the basis of body weight as shown in Table 1. The study was stagger-started with the low dose gavage and low dose diet groups on one day, followed by the control, high dose gavage and high dose diet groups two days later.

Animals in Groups 2 and 3 received the test article vehicle by gavage for 3 days prior to initiation of dosing to acclimate them to the gavage procedure. The test article was administered once daily by gavage to animals in Groups 2 and 3 at a dosing volume of 5 mL/kg/day. The specific volume administered was adjusted on the basis of the animal's most recent body weight. The dose level in group 3 (300 mg/kg/day gavage) was decreased to 90 mg/kg/day on day 7 due to six deaths and clinical signs of severe toxicity in the remaining animals. Study animals were dosed at approximately the same time in the morning on days 1–14 (except on day 6 when surviving Group 3 animals were not dosed to allow them to recover). On day 15, all gavage animals, except the time zero group, received one last gavage dose at the same time as the previous day. Blood and tissue sampling took place at approximately 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 14 and 24 h after the last dose. Animals in Groups 4 and 5 had *ad libitum* access to diet supplemented with aspirin (300 ppm and 3000 ppm, respectively) starting with day 1 until termination. The aspirin dose level was calculated as:

$$\frac{[\text{dose concentration in ppm} \times \text{daily food consumption (g/day)}}{\text{average weekly body weight (kg)} \times 1000\,\text{g/kg}}$$

Animals in Groups 1 (control), 2 and 3 had *ad libitum* access to plain diet.

The gavage test article vehicle was 1% aqueous carboxymethylcellulose/0.2% Tween 80 in deionized distilled water. Carboxymethylcellulose was purchased from Sigma. Tween 80 was obtained from Fluka. The distilled deionized water used to prepare the test article formulations and the test article vehicle was collected from an in-house Barnstead Mega-Pure Water System.

Each test article dosing suspension was prepared individually by adding the appropriate amount of test article with the required volume of vehicle in a pre-calibrated beaker. The contents were mixed with an Omni-Mixer homogenizer for at least 2 min and stirred continuously during the dosing procedure. The test article gavage formulation for the 300 mg/kg/day dose level was prepared weekly; the formulations for the 30 and 90 mg/kg/day dose level were prepared approximately twice weekly based on the aspirin stability in dosage formulation determined prior to the study start. Aspirin concentration in representative formulations was analyzed prior to use, and all analyzed formulations were within 10% of mean target concentrations. The oral formulation was stored at 2–8 °C, and it was allowed to warm up to room temperature before use.

Table 1
Experimental design.

Group no.	No. of animals	Treatment	Dose level (mg/kg/day)	Dose volume	Dose concentration
1	65[a]	Control (diet)	0 mg/kg/day	*ad libitum*	0 mg/mL
2	65[a]	Aspirin (gavage)	30 mg/kg/day	5 mL/kg/day	6 mg/mL
3	65[a]	Aspirin (gavage)	300/90 mg/kg/day[b]	5 mL/kg/day	60/18 mg/mL[c]
4	65[a]	Aspirin (diet)	17.2 mg/kg/day[d]	*ad libitum*	300 ppm
5	65[a]	Aspirin (diet)	192.1 mg/kg/day[d]	*ad libitum*	3000 ppm

[a] 5 rats per time-point.
[b] The dose level in group 3 was decreased from 300 to 90 mg/kg/day from day 7 until termination. These animals were not dosed on day 6.
[c] The dose formulation concentration in group 3 was decreased from 60 to 18 mg/mL because of the change in dose level.
[d] The actual dose levels were calculated weekly based on the animal's body weight and food consumption during the study. The calculated dose levels of 17.2 and 192.1 mg/kg body weight/day were based on data for week 2 when the blood and tissue samples were collected.

In order to achieve target concentrations in the diet (300 and 3000 ppm), aspirin was mixed with the AIN-76A diet. This diet was chosen to stabilize the aspirin while mixed in the diet. Animals in Groups 4 and 5 received 300 ppm and 3000 ppm of aspirin in the diet, respectively, during the dosing period. Diet formulations were prepared approximately twice weekly based on the aspirin stability in the diet determined prior to the study start. The concentration of aspirin in representative batches of diet was analyzed prior to use. The dietary formulations were stored in the refrigerator.

Body weights were recorded at randomization (day −5 for Groups 2 and 4, day −7 for Groups 1, 3 and 5), and on days 1, 8 and 15. All animals were observed once daily for clinical signs of toxicity, approximately 1–2 h after gavage dosing. All animals were also observed for moribundity/mortality twice daily, at least 6 h apart. All animals were subjected to a physical examination (clinical observations), which included examination of eyes and all orifices, in week −1, and on days 1, 8 and 15. Food consumption for all animals was measured weekly commencing in week −1. Food consumption was accomplished by weighing, on-line, a full feeder for each animal at the start of the interval to be measured and re-weighing the same feeder, on-line, at the end of the interval. Average daily food consumption for each animal was calculated by the Labcat® Body Weight Module.

All rats were euthanized by CO_2 asphyxiation and sacrificed starting on the 15th day. In Groups 2 and 4, five animals per group were sacrificed in parallel at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 14 and 24 h after the last gavage dose in Group 2. Animals in Groups 1, 3 and 5 were sacrificed in parallel at the same time points on their day 15. Animals in Groups 4 and 5 had continual access to diet supplemented with aspirin until sacrifice. At the time of sacrifice, blood and target organ (colon) were collected for each animal. A blood sample from the vena cava (∼3 mL) was collected in heparin tubes. The blood was promptly centrifuged at 3000 rpm for 15 min. The harvested plasma was transferred to labeled vials and placed on dry ice until stored at approximately −80 °C. The colon was opened longitudinally and immediately rinsed with cold saline to remove contents. After scraping an approximately 4–5 cm segment of colon mucosa using glass slides, colon tissue and mucosa samples were snap frozen in liquid nitrogen until stored at approximately −80 °C.

2.3.1. Analysis of salicylate in plasma and colon tissue

Salicylate was extracted from plasma samples by combining 50 μL plasma, 100 μL internal standard solution and 125 μL 0.5% formic acid, and vortexing with two, 500 μL aliquots of MTBE. The MTBE layers were combined, evaporated to dryness under nitrogen, and reconstituted in 500 μL of HPLC mobile phase for analysis. Salicylate was extracted from colon samples by homogenizing samples (0.1–0.2 g) in 1.0 mL ice-cold formic acid (0.5%) for 20–30 s. The homogenate was combined with two washings of the homogenizer probe (1 mL each), and internal standard was added. The homogenate was extracted with 5–10 mL MTBE. The MTBE layer was removed, evaporated to dryness under nitrogen, and reconstituted in 150 μL of HPLC mobile phase for analysis.

The concentration of salicylate in plasma and colon was determined using high performance liquid chromatography and tandem mass spectrometry (LC/MS/MS). Chromatographic separation was achieved using Zorbax Eclipse® XDB-C18 (150 mm × 2.1 mm, 5 μm) (Agilent Technologies, Inc., Santa Clara, CA) analytical and Thermo Hypersil Gold (10 mm × 2.1 mm, 3 μm) (Thermo Fisher Scientific, Inc., Walthman, MA) guard columns at ambient temperature with linear gradient elution (mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile). Detection was performed using multiple reaction monitoring mode with negative polarity on a Micromass Quatro Micro™ API (Waters, Milford, MA) unit. The following parent–daughter transitions were monitored 179–137 (aspirin), 137–93 (salicylate and 4-hydroxybenzoate; internal standard for plasma samples), and 151–107 (3-methylsalicylate; internal standard for colon samples). Quantification was based on peak areas. The standard curve for salicylate was linear over the range of 5.85–750 ng/mL, with an R^2 coefficient = 0.99. The lower limit of quantitation (LLOQ) was 5.85 ng/mL in plasma and 5.85 ng/g in colon mucosa.

2.3.1.1. Analysis of eicosanoids. Thromboxane B_2 in plasma was quantified using the commercially available Biotrak thromboxane B_2 enzyme immunoassay system from Amersham Biosciences (Piscataway, NJ), following method validation. Blood samples were collected into tubes containing EDTA and indomethacin for measurement of thromboxane B_2. Plasma was isolated following centrifugation. Solid phase extraction procedures were performed using Amprep™ minicolumns (Amprep C2 100 mg, Amersham Biosciences, Piscataway, NJ). Following plate preparation, the optical density of samples was measured at 450 nm. The linear range for thromboxane E_2 was 0.01–1.3 ng/mL, with an R^2 coefficient = 0.999.

Prostaglandin E_2 levels were measured in colon mucosa using the commercially available Biotrak Prostaglandin E_2 enzyme immunoassay system from Amersham Biosciences (Piscataway, NJ), following method validation. Approximately 4–5 cm of the proximal colon was collected and the colon mucosa was removed using a glass slide to scrape the mucosa from the colon. Colon mucosa samples were homogenized in 1.5 mL Tris–HCl homogenate buffer for 10 s four times each on wet ice. Samples were then sonicated for 10 s in ice-cold water. An aliquot of 0.1 mL for each control or ASA treated homogenate samples was removed for determination of protein concentration using the Bio-Rad Protein Assay kit (Pierce Biosciences, Rockford, IL). Aliquots of 0.5 mL of each homogenized sample, were used for column extraction in duplicate Amprep™ minicolumns (Amprep C18 100 mg, Amersham Biosciences, Piscataway, NJ). Following plate preparation, the optical density of samples was measured at 450 nm. The linear range for prostaglandin E_2 was 50–6400 pg/mL, with an R^2 coefficient = 0.999.

2.4. Pharmacokinetic analyses

The area under the concentration–time curve (AUC) for salicylic acid was estimated using Microsoft Excel 2003 (Microsoft Corp., Seattle, Washington). The area under the concentration–time curve (AUC_{0-24}) from time zero (0) to the last measured time point (24 h) was determined by the linear trapezoidal method using the Bailer method [14]. Standard errors for the estimated AUC values were obtained using the Bailer method [14]. The half-life was estimated by first calculating the terminal elimination rate constant (λz) obtained by nonlinear least squares regression of the terminal log-linear portion of the concentration–time curve. The terminal elimination half-life ($t_{1/2}$) was calculated by dividing the natural logarithm of 2 by λz.

2.5. Pharmacodynamic analysis

The pharmacodynamic effect was assessed by analyzing plasma thromboxane B_2 and colon mucosa prostaglandin E_2 concentrations. The area under the concentration–time curves for TXB_2 plasma concentration and PGE_2 colon mucosa concentration was estimated using Microsoft Excel 2003 (Microsoft Corp., Seattle, Washington). The area under the concentration–time curve from time zero (0) to the last measured time point (24 h) was determined by the linear trapezoidal method [14] and standard errors for the estimated AUC values were obtained using the Bailer method [14].

The relationship between aspirin dose and TXB_2 and PGE_2 was assessed using an inhibitory sigmoid E_{max} model using the Hill equation:

$$E = E_{max}\left(1 - \frac{D^{\gamma}}{ED_{50}^{\gamma} + D^{\gamma}}\right)$$

where E_{max} represents the maximum pharmacodynamic effect, D is the aspirin dose, ED_{50} is the aspirin dose resulting in 50% of the maximal response, and γ is the Hill coefficient representing the sigmoidicity of the curve.

The relationship between salicylic acid AUC and TXB_2 and PGE_2 was assessed using a sigmoid E_{max} model using the Hill equation:

$$E = E_0 + \left(\frac{E_{max} \cdot C}{EC_{50} + C}\right)$$

where E_{max} represents the maximum% change in the pharmacodynamic effect, C is the salicylic acid AUC, EC_{50} is the salicylic acid AUC resulting in 50% of the maximal response, and γ is the Hill coefficient representing the sigmoidicity of the curve.

2.6. Statistical analyses

Analysis of variance tests were conducted on body weight, food consumption, plasma salicylic acid levels, colon tissue salicylic acid levels, plasma TXB_2 levels and colon mucosa PGE_2 levels. Data were compared between gavage and dietary administration groups at each dose level (Groups 2 and 4 and Groups 3 and 5, respectively) and to the control group (Group 1). If a significant F ratio was obtained ($p < 0.05$), Dunnett's t-test was used for pair-wise comparisons of two dosing paradigms at each level of aspirin. The Bailer method was used for comparison of AUCs for plasma salicylic acid levels, colon tissue salicylic acid levels, plasma TXB_2 levels and colon mucosa PGE_2 levels.

3. Results

The study was designed to compare diet and oral gavage administration with nominal doses of 30 and 300 mg/kg body weight/day. These doses overlap doses used in animal chemoprevention models. However, unexpected circumstances prevented carrying out the study with these doses. Treatment-related clinical signs of toxicity were observed in the high gavage dose of 300 mg/kg body weight/day and included six animal deaths. Consequently, the high gavage dose was lowered to 90 mg/kg body weight/day on day 7 of the study in surviving animals, after allowing one day for recovery from the 300 mg/kg body weight/day dose. Food consumption was significantly decreased, unexpectedly, in high and low dose diet group animals. As a result calculated mean dietary aspirin doses based on the last 7 days of food consumption were 17.2 and 192 mg/kg body weight/day, lower than the nominal doses of 30 and 300 mg/kg body weight/day, respectively.

Aspirin concentrations in plasma and colon samples were below the lower limit of quantitation (5.85 ng/mL or 5.85 ng/g), therefore the aspirin metabolite, salicylate (salicylic acid) was measured instead. The concentration–time profile of salicylic acid in plasma and colon mucosa is depicted in Fig. 1a and b, respectively, for low and high doses following gavage and dietary administration of aspirin on the last day of the study (Day 15). As may be expected, dietary treatment resulted in relatively constant salicylate concentrations in both plasma and colon over the 24-h period while gavage led to an early peak followed by a fast decline in salicylate concentrations. The elimination half-life for salicylate following low and high oral gavage dose, respectively, was calculated to be 8.5 and 9.4 h in plasma and 8.9 and 6.5 h in colon mucosa. Due to

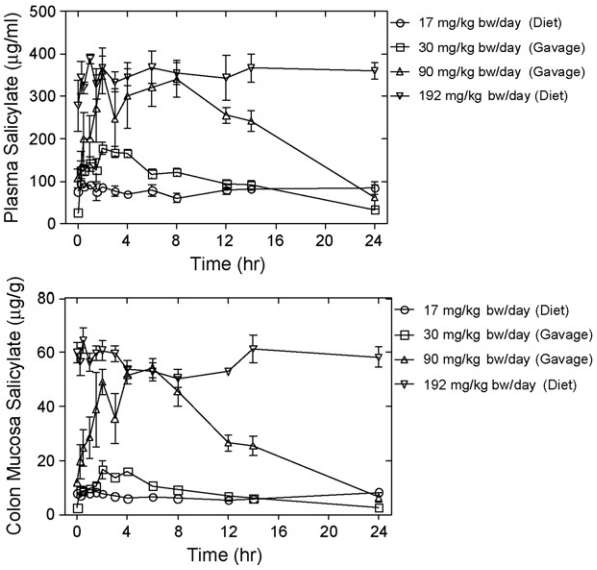

Fig. 1. Plot of (a) plasma (top) and (b) colon mucosal (bottom) salicylate concentration vs. time following administration of aspirin by oral gavage and diet. Data are means ±S.D. of salicylate concentrations from five animals.

constant availability of aspirin via the diet, it was not possible to calculate salicylate half-lives in animals administered aspirin in this manner. Even though the concentration–time profiles were markedly different visually between the two methods of aspirin administration, the resultant areas under the concentration curves were linearly related to the doses irrespective of the method of administration (Fig. 2). Furthermore, there was a linear relationship between plasma and colon AUCs for salicylate (Fig. 3a). The pharmacodynamic biomarkers, plasma TXB_2 and colon PGE_2, were decreased about 60% at the lowest dose of aspirin, 17.2 mg/kg body weight/day (Table 2). Further decreases in both biomarkers appeared to reach a plateau of about 90% at 90 mg/kg body weight/day. As was the case for plasma and colon salicylate AUCs, a linear relationship was observed between plasma TXB_2 and colon PGE_2 AUCs (Fig. 3b). The relationship between aspirin dose and both pharmacodynamic markers followed an inhibitory E_{max} model as presented in Fig. 4a and b. The E_{max} and ED_{50} for TXB_2 were −6.837 ng × h/mL and 11.44 mg/kg/day, respectively. The E_{max} and

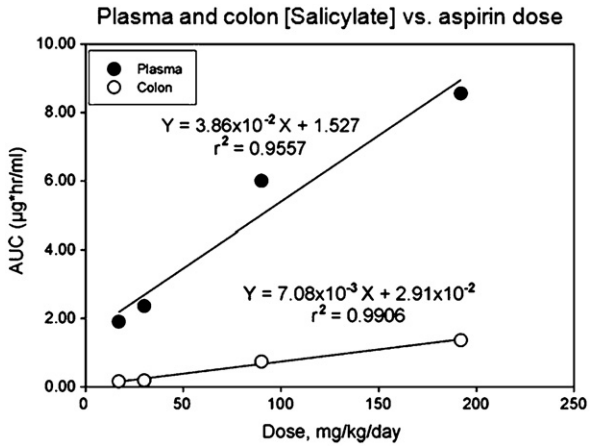

Fig. 2. Relationship between salicylate areas under plasma and colon concentration–time curve and dose of aspirin irrespective of its method of administration. Dietary doses were 17.2 and 192 mg/kg body weight/day; gavage doses were 30 and 90 mg/kg body weight/day.

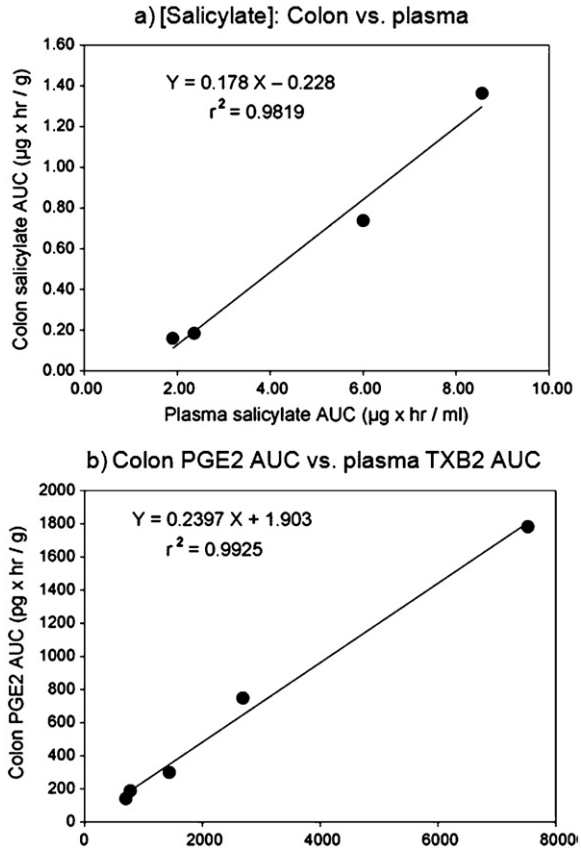

Fig. 3. Relationship between (a) colon and plasma salicylate (top) and (b) colon mucosal prostaglandin E_2 (PGE_2) plasma and thromboxane B_2 (TXB_2) (bottom) areas under concentration–time curves.

ED_{50} for PGE_2 were −1621 ng × h/g and 14.42 mg/kg/day, respectively. The absolute change in the AUC for TXB_2 and PGE_2 were similarly related to the plasma and colon mucosa salicylic acid AUC following an E_{max} model as presented in Fig. 4c and d. The E_{max} and EC_{50} for the absolute % change in TXB_2 were 90.53% and 1634 ng × h/mL, respectively. The E_{max} and EC_{50} for the absolute % change in PGE_2 were 90.50% and 152.8 ng × h/g, respectively.

4. Discussion

The elimination half-life of aspirin was reported to be 7–12 min in rats, with significant presystemic hydrolysis occurring in the gut to the extent that only approximately 25% of the oral dose is absorbed as intact parent compound [15]. In this study, concentrations of the parent compound were below the lower limit of quantification in plasma and the target organ colon mucosa during the entire 24-h measuring period. Consequently, the principle aspirin metabolite, salicylate, was measured. Salicylate is found in plants where it is believed to function to protect against pathogens, environmental stress and oxidative stress [16]. Salicylate itself was reported to possess a number of chemopreventive activities such as the induction of cellular growth arrest and apoptosis, and in fact may be responsible for some attributed to the parent drug, aspirin [16–25].

As shown in Fig. 1a and b, concentration–time profiles for salicylate in both plasma and colon were markedly different between gavage and dietary dosing. While dietary administration resulted in relatively constant concentrations over the 24-h testing period, gavage administration yielded a fast absorption peak followed by decline with the estimated elimination half-lives ranging from 6.5 to 9.4 h. Nevertheless, the AUCs in plasma and colon were linearly related to the dose irrespective of the method of administration. Therefore, all subsequent data analyses were based on the dose regardless of the method of administration. This differs from the case with another NSAID [10], sulindac, in which the route of administration was found to significantly impact pharmacokinetic and pharmacodynamic AUC's. Together these studies support the contention that equivalency between different methods of administration cannot be assumed or generalized *a priori*, but should be evaluated for each drug individually.

The observed linear relationship in this study between plasma and colon salicylate AUCs as pharmacokinetic markers of exposure suggests that plasma exposure may be used as a surrogate for in the target colon mucosa. This provides obvious advantages in terms of the extent of procedural invasiveness and associated sampling issues when comparing blood versus tissue. Similarly, the linear relationship between AUCs for the two pharmacodynamic markers of exposure, plasma TXB_2 and colon mucosa PGE_2, demonstrates the same advantages for these measures. Good correlation between the two pharmacodynamic parameters was also confirmed by a marked similarity in the percent decrease of AUCs for plasma and colon biomarkers compared to control levels regardless of dose or mode of administration (Table 2). These observations were confirmed by very similar levels of aspirin ED_{50} in the sigmoid inhibitory model of aspirin dose vs. either PGE_2 or TXB_2 and a marked similarity of maximum percent inhibitory effect relative to control levels vs. salicylate concentrations (coincided at 90.5%) for both PGE_2 in colon and TXB_2 in blood. These correlations and modeling suggest that salicylic acid AUC in blood may be a good surrogate biomarker for both PGE_2 and TXB_2 pharmacodynamic parameters. It is however important to note that TXB_2 has a physiological role in coagulation homeostasis, which is different among species, i.e., an allometric scaling factor may be required for correlating rat and human data [26]. This study, as well as previous reports, indicate presystemic hydrolysis and rapid clearance of aspirin in the rat. Therefore, the pharmacodynamic measures reported here are presumed to arise from competitive inhibition of COX-1 by salicylate because TXB_2 is mediated by COX-1 in the platelets and because PGE_2, although it can be produced by either COX-1 or COX-2, appears to be primarily produced by COX-1 in normal mucosa. In contrast, COX-2

Table 2
Summary of pharmacokinetic and pharmacodynamic markers of exposure to aspirin following its administration by oral gavage or diet.

Dose (mg/kg/day)	Route	Salicylate AUC		TXB_2 AUC	PGE_2 AUC	TXB_2 AUC	PGE_2 AUC
		Plasma (µg h/mL)	Colon (µg h/g)	Plasma (ng h/mL)	Colon (pg h/g)	%Δ Control	%Δ Control
0	Gavage	N/A	N/A	7.53	1782	0	0
17.2	Diet	1.90	0.16	2.69	748	64	58
30	Gavage	2.36	0.18	1.44	299	81	83
90	Gavage	6.00	0.74	0.78	188	90	89
192.1	Diet	8.56	1.36	0.70	141	91	92

N/A—Not applicable. Salicylate was not measured in the control group.

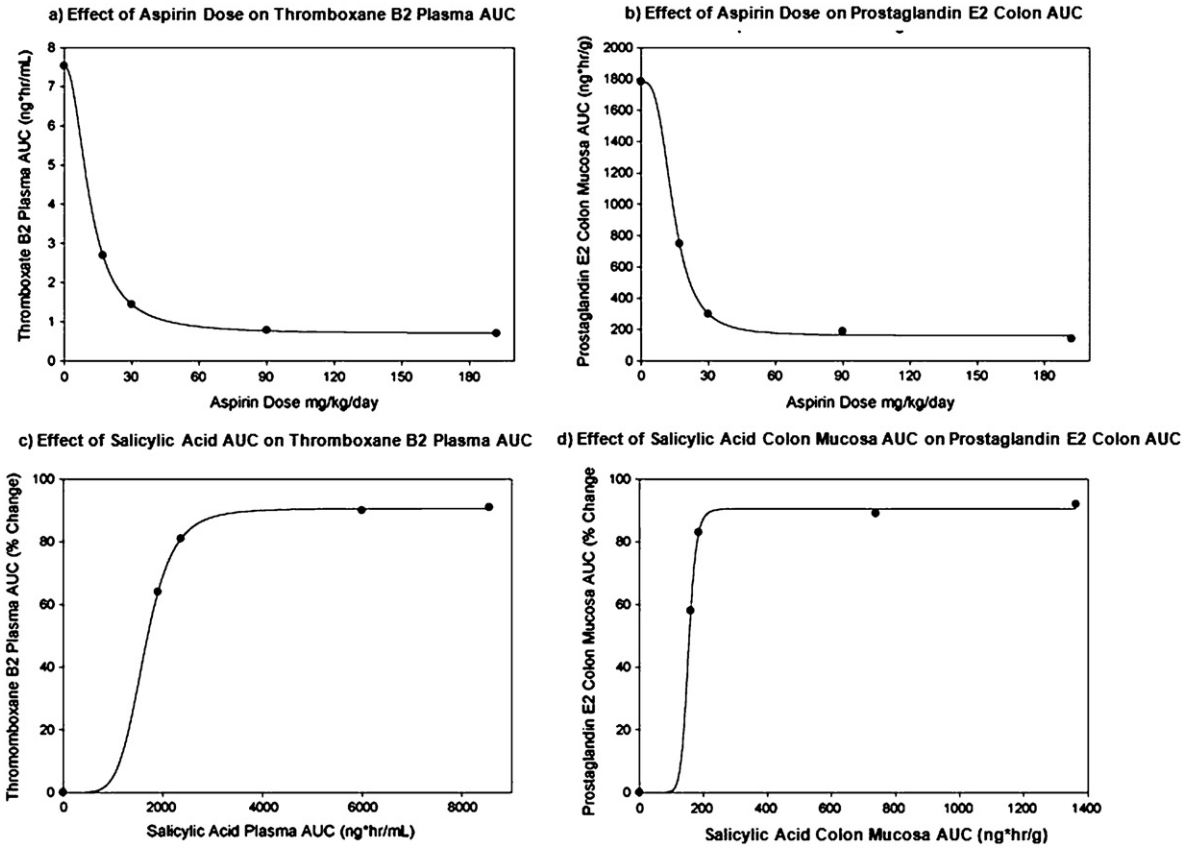

Fig. 4. Relationship between (a) plasma TXB_2 and aspirin dose (top left), (b) colon mucosal PGE_2 and aspirin dose (top right), (c) plasma TXB_2 and plasma salicylate (bottom left), and (d) colon mucosal PGE_2 and colon mucosa salicylate (bottom right) areas under concentration–time curves.

appears to be expressed in transformed epithelia and endothelial cells.

A large initial decrease of about 60% in AUCs for both PGE_2 and TXB_2 was observed with the lowest dose, 17.2 mg/kg body weight/day, followed by slow further decreases of about 80% for both pharmacodynamic parameters at low gavage dose (30 mg/kg body weight) that further reached a plateau at about 90% with the higher doses of 90 and 192 mg/kg body weight/day, either gavage or diet. A dose of roughly 30 mg/kg body weight reduced COX-1 mediated production of PGE_2 by roughly 80%. This bears some similarity to a clinical dose-finding study of aspirin using rectal mucosal PGE_2 levels as a biomarker [27]. In that study, the lowest dose of aspirin, 81 mg, caused about a 70% decrease in PGE_2 without additional effect with two higher doses up to 650 mg. This result would seem to be in agreement with the hypothesis discussed above that PGE_2 in normal mucosa is primarily COX-1. Thus aspirin is much more efficient in inhibiting COX-1 than COX-2 [28]. However, it should be kept in mind that PGE_2, particularly in normal mucosa, is not a validated biomarker for chemopreventive intervention and may not have a direct relationship to it. Although not directly addressed in this study, these doses of aspirin in diet have been employed in chemoprevention studies of both colon and bladder in rats. These two organ sites have proven to be sensitive to the preventive activities of various NSAIDS and COX-2 inhibitors. In the colon [11], we observed minimal chemopreventive activity at 200 ppm aspirin (approximately 16 mg/kg body weight, human equivalent 2.8 mg/kg body weight) which inhibited TXB_2 levels roughly 60% [11]. In contrast a dose of 1800 ppm (approximately 160 mg/kg body weight) was needed to achieve roughly a 60% decrease in tumor formation. We have recently observed in a rat bladder model that while a dietary dose of 300 ppm (26 mg/kg body weight; human equivalent 4 mg/kg) was totally ineffective, a dose of 3000 ppm inhibited the formation of palpable bladder cancers by roughly 60% [29]. These results show that doses of aspirin that strongly inhibit COX-1 (TXB_2 and probably PGE_2 in normal mucosa) are ineffective while higher doses which are likely to affect COX-2 activity do show significant efficacy. These interpretations would appear compatible with epidemiologic findings that aspirin activity in preventing colon cancer or even colon cancer-related death are associated with high doses of aspirin considerably above a baby aspirin.

Even though the 24-h exposure to salicylate was not dependent on the method of administration, gavage dosing tended to yield higher peak levels. Differences in peak levels were not reflected in the two pharmacodynamic biomarkers over the same 24-h period. However in the current study, it was not possible to determine what, if any, pharmacological and/or toxicological effects could result from these transient salicylate spikes following gavage dosing.

In summary, this study has demonstrated that pharmacokinetic and pharmacodynamic parameters for salicylates are independent of the method of aspirin administration (oral gavage or dietary) in rats. In addition, linear relationships between: (a) dose of aspirin and plasma and colon mucosal concentrations of salicylate, (b) plasma and colon mucosal AUCs of salicylate, and (c) AUCs of plasma TXB_2 and colon mucosal PGE_2 were shown. These results should provide basis for comparisons of published and future studies involving the two methods of aspirin dosing. They also may facilitate future studies suggesting that plasma may be used as a surrogate for colon mucosa.

Conflict of interest

The authors declare that there are no conflicts of interest.

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