HHS Public Access

Author manuscript

Life Sci Space Res (Amst). Author manuscript; available in PMC 2023 January 03.

Published in final edited form as:

Life Sci Space Res (Amst). 2021 November ; 31: 85–91. doi:10.1016/j.lssr.2021.09.001.

Effects of dietary aspirin on high-LET radiation-induced prostaglandin E2 levels and gastrointestinal tumorigenesis in *Apc*$^{1638N/+}$ mice

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Abstract

Inevitable exposure to high-LET ionizing radiation (IR) present in galactic cosmic radiation (GCR) could enhance gastrointestinal (GI) cancer incidence among astronauts undertaking deep space exploration and GI-cancer mortality has been predicted to far exceed NASA's limit of < 3% REID (Radiation exposure-induced death) from cancer. Therefore, the development of countermeasure agents against high-LET radiation-induced GI cancer is needed to safeguard astronauts during and after an outer space mission. The cyclooxygenase-2/prostaglandin E2 (COX2/PGE2) mediated activation of pro-inflammatory and oncogenic signaling has been reported to play an important role in persistent inflammation and GI-tumorigenesis after high-LET radiation exposure. Therefore, aspirin, a well-known inhibitor of the COX/PGE2 pathway, was evaluated as a potential countermeasure against ^{28}Si-induced PGE2 and tumorigenesis in *Apc*$^{1638N/+}$, a murine model of human GI-cancer. Animals were fed either standard or aspirin supplemented diet (75, 150, or 300 mg/day of human equivalent dose) starting at the age of 4 weeks and continued till the end of the study, while mice were exposed to ^{28}Si-ions (300 MeV/n; 69 keV/μm) at the age of 8 weeks. Serum PGE2 level, GI tumor size (>2mm^2), number, and cluster (>5 adjoining tumors) were analyzed at 150 days post-exposure. Aspirin led to a significant reduction in PGE2 in a dose-dependent manner but did not reduce ^{28}Si-induced GI tumorigenesis even at the highest (300 mg/day) dose. In summary, this study suggests that aspirin could reduce high-LET IR-induced pro-inflammatory PGE2 levels, however, lacks the ability to reduce high-LET IR-induced GI tumorigenesis in *Apc*$^{1638N/+}$ mice.

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Keywords

High-LET radiation; Tumorigenesis; Aspirin; Chemoprevention; Cyclooxygenase; Prostaglandin E2

1. Introduction

Exposure to ionizing radiation (IR) is associated with a significantly greater risk of gastrointestinal (GI) tumorigenesis, therefore inevitable cosmic radiation exposure in the range of 0.1 to 0.2 Gy/y during outer-space exploration is a primary health concern for astronauts (Cragle *et al.*, 1988; Preston *et al.*, 2007; Boice *et al.*, 2006; Dupree-Ellis *et al.*, 2000; Cucinotta *et al.*, 2017; Sakata *et al.*, 2019). In contrast to low energy transfer (LET) radiation (γ or x-rays), exposure to densely ionizing high-LET heavy-ions present in the galactic cosmic radiation (GCR) poses greater cancer risk with relative biological effectiveness (RBE) in the range of 3.7 to 8 for GI-tumorigenesis (Shuryak *et al.*, 2017). Furthermore, human cancer risk prediction have also indicated a higher GI-cancer risk that far exceeds the current NASA limit of < 3% risk of exposure-induced death (REID) from cancers (Cucinotta *et al.*, 2017; Shuryak *et al.*, 2017). Although using appropriate radiation shielding is the best protective approach, current space craft shielding provides only modest protection from high-LET radiation (Naito *et al.*, 2020; Giraudo *et al.*, 2018). Therefore, there is an unmet need to develop safe and effective countermeasure agents against high-LET radiation-induced GI-tumorigenesis.

Molecular mechanisms implicated in high-LET ionizing radiation (IR)-induced GI-tumorigenesis includes chronic oxidative stress, persistent DNA damage, accelerated aging phenotype, and increased senescence-inflammatory response (SIR) (Datta *et al.*, 2012; Cheema *et al.*, 2014; Datta *et al.*, 2016; Suman *et al.*, 2018; Kumar *et al.*, 2018b; Kumar *et al.*, 2019; Suman *et al.*, 2020). SIR signaling is known to increase inflammation and tumorigenesis in many tissues, including the GI tract (Ruhland *et al.*, 2016; Frey *et al.*, 2018; Kumar *et al.*, 2018b). Notably, higher levels of pro-inflammatory SIR factor, PGE2 (prostaglandin E2), and its precursor enzyme cyclooxygenase-2 (COX2) has been observed in mouse GI mucosa after high-LET radiation exposure (Cheema *et al.*, 2014) and higher PGE2 is also known to increase GI-cancer incidence in humans (Eberhart *et al.*, 1994; Kargman *et al.*, 1995; Sano *et al.*, 1995).

Aspirin (acetylsalicylic acid or ASA) is a well-studied drug with long-history of human use that effectively reduces PGE2 levels via inhibition of COX (both COX-1 and 2) enzymes and its chemo-preventive potential against GI-cancer has been noted in both human epidemiological and animal studies (Drew *et al.*, 2016). Low-dose daily aspirin use in long-term human clinical trials was found to be safe and effective in reducing chronic systemic inflammation and mortality due to GI cancer (Ishikawa *et al.*, 2014; Morris *et al.*, 2009). Moreover, the association between increasing dose and duration of aspirin use with a reduction in human GI-cancer incidence has also been observed (Bosetti *et al.*, 2020). In this study, we evaluated the GI-cancer preventive effects of human equivalent doses of aspirin (75, 150, and 300 mg/day) against high-LET radiation in $Apc^{1638N/+}$, a well-characterized

murine model of human GI-cancer. These mice develop 3–5 tumors throughout the GI tract and mimic sporadic human colorectal cancers where the loss of functional *Apc* is prevalent in ~80% of the tumors (Bakker *et al.*, 2013; Suman *et al.*, 2017). The overall purpose of this study was to evaluate the dose-dependent effects of dietary aspirin on high-LET radiation-induced GI-tumorigenesis and serum PGE2 levels in $Apc^{1638N/+}$ mice.

2. Materials and Methods

2.1 Mouse colony, study groups and radiation exposure

Mice were bred and genotyped, as reported earlier (Suman *et al.*, 2017) and male $Apc^{1638N/+}$ mice (n=20/group) were randomly assigned to the experimental groups, as described in Table-1. Aspirin supplemented diet in applicable groups was initiated at 4 weeks of age and continued till the end of the study (Fig. 1). At seven weeks of age, all experimental mice including the control group were shipped to Brookhaven National Laboratory (BNL) and after one week of acclimation, mice were exposed to ^{28}Si-ion (10 or 50 cGy dose; 69 keV/μm; 300 MeV/n) at the NASA Space Radiation Laboratory (NSRL) in BNL, as described previously (Suman *et al.*, 2017). All animals were group-housed in a well-ventilated cage with easy access to standard or aspirin supplemented diet and water. Humidity (~50%), temperature (22 °C), and 12-h dark-light cycle were kept consistent and the health of all animals was regularly observed as per our approved institutional animal protocol at GU and NSRL/BNL.

2.2 Aspirin dose calculation and administration regimen

The physician recommended human dose of aspirin range from 75 to 325 mg/day, where 75 mg represents the low-dose aspirin and the 325 mg represents adult aspirin dose. We calculated mouse aspirin dose equivalent to human aspirin dose based on established dose calculation guidelines (Reagan-Shaw *et al.*, 2008; Bachmanov *et al.*, 2002). Briefly, to calculate human equivalent dose in mouse, we used mean human daily dose for 60 kg adult i.e., 1.25 mg/kg/day and mouse and human Km factor of 3 and 37, respectively. Finally, we used 61.5 mg, 123 mg, or 246 mg of aspirin per kg of diet so that mice will have the human equivalent of daily 75, 150, or 300 mg aspirin. Aspirin formulated diet was obtained from ENVIGO (www.envigo.com) in a color-coded form for each different aspirin dose. No difference in food consumption and body weight among control and aspirin diet groups were noted during the course of the study.

2.3 Serum prostaglandin E2 (PGE2) measurements

Animals were euthanized using an asphyxiation chamber attached to a carbon dioxide cylinder with a flow rate of 30 to 70 % chamber volume per minute. Blood was collected through cardiac puncture, and BD Microtainer SST™ serum separator tubes were used to isolate serum, as per the manufacture's recommendation. Finally, serum samples were aliquoted and flash-frozen in liquid nitrogen and later stored at −80 °C until PGE2 ELISA assay. Serum samples (n=6/group) were diluted (1:10) in assay buffer and relative changes in PGE2 levels were measured using a competitive ELISA kit, as per manufacturer's instructions (Cat # ab133021, Abcam, Cambridge, MA, USA). Optical density (O.D.) was recorded at 405 nm with a multi-well plate reader (Molecular Devices, CA, USA) and was

normalized using blank samples. Finally, the percentage change in PGE2 levels over control samples was calculated. This ELISA assay has the sensitivity to detect 13.4 pg/ml of PGE2 and is recommended for detection of species independent PGE2 levels in the range of 39.1 to 2500 pg/ml.

2.4 Tumor quantification

GI tumor number, size (>2 mm^2), and clusters (>5 adjoining tumors) were quantified, as described previously (Suman *et al.*, 2017). Briefly, after euthanasia small and large intestines were surgically obtained, cleaned with phosphate-buffered saline, and opened longitudinally to expose the GI tumors. Finally, tumors were observed and counted by individuals blinded to the experimental groups using Leica MZ6 dissecting microscope scope.

2.5 Statistics and data analysis

All statistical analyses were performed in GraphPad Prism v6.0a (La Jolla, CA). Initially, data normality was analyzed using Shapiro-Wilk test and $p<0.05$ indicated a non-normal distribution. Therefore, non-parametric one-way ANOVA (Kruskal-Wallis test) followed by post-hoc Dunn's multiple comparison test was performed to detect any significant difference between the groups. All values presented as a bar graph for each group show means and error bars are presented as SEM. Statistically significant differences among groups were considered when the p-value was ≤ 0.05.

3. Results

3.1 High-LET radiation exposure causes a dose-dependent increase in GI-tumorigenesis

Exposure to both 10 cGy and 50 cGy ^{28}Si-ions led to significantly higher GI tumorigenesis at 150 days post-exposure, relative to the control group (Fig. 2). A dose-dependent increase in intestinal and colonic tumors were observed, where 10 cGy (intestinal tumor 5.9 ± 0.28; colon tumor 0.18 ± 0.08; $p<0.05$) and 50 cGy (intestinal tumor 7.95 ± 0.47; colon tumor 0.23 ± 0.11; $p<0.05$) led to a statistically significant increase in intestinal (Fig. 2A) and colonic tumor counts (Fig. 2B), relative to control (intestinal tumor 3.85 ± 0.28; and no colon tumor). Moreover, a statistically significant increase in mean tumor cluster (Fig. 2C) and mean count of large tumors (Fig. 2D) were observed among control (cluster 0.045 ± 0.045, large tumor 1.63 ± 0.21), 10 cGy (cluster 0.11 ± 0.07, large tumor 2.73 ± 0.24; $p<0.05$ relative to control) and 50 cGy (cluster 0.30 ± 0.12, large tumor 4.23 ± 0.58; $p<0.05$ relative to control) animals. The highest GI tumorigenesis was observed after 50 cGy of ^{28}Si-ion, where mean tumor count (Fig. 2A) and intestinal clusters (Fig. 2D) both were significantly higher than the 10 cGy dose.

3.2 Effect of dietary aspirin on serum prostaglandin levels

Dietary aspirin use was associated with marked reductions in serum level of PGE2 at all tested doses and the highest effect was observed with the 300 mg daily dose. A dose-dependent reduction (% to control) in serum PGE2 was observed at 75 mg/day (88.48 ± 1.84; $p<0.05$); 150 mg/day (75.49 ± 1.52; $p<0.05$) and 300 mg/day (69.44 ± 2.55; $p<0.05$) aspirin doses (Fig. 3A), while a dose-dependent increase in high-LET IR-induced serum PGE2 was observed in ^{28}Si-ion exposed mice, compared to the control group (Fig.

3B). A significant reduction in radiation-induced serum PGE2 levels was also observed in all ASA treated groups, however, the 150 and 300 mg human equivalent ASA dose was highly effective in reducing ^{28}Si-induced PGE2 levels, relative to 75 mg dose (Fig. 3C–D). Therefore, our results clearly showed that aspirin is effective in reducing basal as well as high-LET radiation-induced PGE2 levels.

3.3 Aspirin administration has no effect on GI-tumorigenesis in Apc$^{1638N/+}$ mice

The intestinal tumor count in *Apc*$^{1638N/+}$ mice (3.85 ± 0.28) was not altered in the ASA-treated group and no statistical difference was observed between 75 mg/day (4.15 ± 0.26), 150 mg/day (4.2 ± 0.32), and 300 mg/day (4.25 ± 0.23) aspirin groups (Fig. 4A). The mean colonic tumor count (Fig. 4B) and the number of intestinal tumor clusters (Fig. 4C) were rare in all experimental groups. Similar to tumorigenesis, no significant difference was observed in count of large (>2mm^2) size tumors between control (1.63 ± 0.21) and aspirin 75 mg/day (1.48 ± 0.25), 150 mg/day (1.72 ± 0.25) and 300 mg/day (1.62 ± 0.30) aspirin groups (Fig. 4D). Altogether, our results show no effect of dietary aspirin on GI tumorigenesis in the 75–300 mg dose range.

3.4 Dietary aspirin does not confer protection against high-LET radiation-induced GI-tumorigenesis in Apc$^{1638N/+}$ mice

Aspirin administration did not show a significant reduction in high-LET IR-induced radiogenic GI tumorigenesis as well as on the number of tumor clusters and size (Fig. 5). In control, IR-exposed, aspirin only, and aspirin + IR-exposed groups the distribution of tumors throughout GI-tract was similar. The mean number of radiogenic GI tumors at 10 cGy (5.90 ± 0.28) was not significantly affected in aspirin-treated mice i.e. 75 mg/day (5.75±0.31), 150 mg/day (5.95±0.34), and 300 mg/day (5.30±0.29) (Fig. 5A–C). In addition, the mean number of radiogenic GI tumors at 50 cGy (7.95 ± 0.47) was also not affected among aspirin-treated mice i.e., 75 mg (7.35±0.55), 150 mg (7.05±0.58), and 300 mg (7.40±0.43) (Fig. 5D–F). Similar to tumor count, no statistical difference was observed in the number of large tumors and clusters between ^{28}Si exposed and aspirin + ^{28}Si exposed groups at all aspirin doses (Fig. A-F). Altogether, we demonstrate no significant effect of aspirin on high LET IR-induced GI tumorigenesis in male *Apc*$^{1638N/+}$mice at the 75–300 mg/day dose range.

4. Discussion

Anti-inflammatory and GI-cancer preventive effects of aspirin are attributed to its COX inhibitory activity (Stolfi *et al.*, 2013; Wang & DuBois, 2013; Sostres *et al.*, 2014), whereas high-LET radiation-induced persistent inflammation and higher GI-cancer incidence are in part ascribed to higher intestinal COX2 expression and accumulation of its pro-inflammatory product PGE2 (Cheema *et al.*, 2014; Kumar *et al.*, 2018b). This study demonstrates that aspirin was able to reduce radiation-induced serum PGE2. However, aspirin had no effects on either on background or on IR-induced GI-tumorigenesis.

Aspirin dose and duration of its use are often associated with its efficacy and side effects in humans (Ishikawa *et al.*, 2014; Morris *et al.*, 2009; Bosetti *et al.*, 2020). We used a

spectrum of human equivalent doses of aspirin i.e., low (75 mg/day), intermediate (150 mg/day), and high (300 mg/day) to test its efficacy against spontaneous as well as ^{28}Si-induced GI-tumorigenesis. Aspirin did not alter ^{28}Si-induced tumor count, cluster, or size in the irradiated animals; however, this study is limited to continuous aspirin dosing and one time-point. In concurrence, other reports on aspirin's effect on GI-tumorigenesis in *Apc*$^{1638N/+}$ mice also displayed no appreciable prevention from GI-tumorigenesis (Williamson *et al.*, 1999). The aspirin's GI-cancer prevention studies using other similar mouse models of CRC i.e. *Apc*$^{Min/+}$ mice and also in human clinical trials have also shown mixed results (Reuter *et al.*, 2002; Chiu *et al.*, 2000; Barnes & Lee, 1998; McNeil *et al.*, 2021; Drew *et al.*, 2020; Burn *et al.*, 2020; Qiao *et al.*, 2018). Since the duration of aspirin use for effective GI-cancer prevention is also not established in mouse or human studies (Bosetti *et al.*, 2020) therefore further studies might be required to optimize aspirin timing and duration to achieve desired chemoprevention.

In another *Apc*-based GI-cancer model i.e., *Apc*$^{Min/+}$ mice up to 50% chemoprevention was achieved but with a bolus aspirin dose (Barnes & Lee, 1998), which is many-fold higher than the adult human dose. However, in *Apc*$^{Min/+}$ mice it was noted that *in-utero* aspirin use is more effective compared to aspirin use after weaning (Perkins *et al.*, 2003; Rohwer *et al.*, 2020). Unlike *Apc*$^{Min/+}$, the mutation in *Apc*$^{1638N/+}$ is localized outside the β-catenin interaction domain therefore WT and truncated protein forms could suppress oncogenic β-catenin, therefore might reflect mutation-specific differential aspirin responses. Moreover, GI-mucosal physiology of *Apc*$^{1638N/+}$ mice is very similar to WT mice as WT *Apc* allele is often maintained even in the GI-polyps derived from *Apc*$^{1638N/+}$ mice, and loss of the WT allele is not detected until the development of frank tumors (Haigis *et al.* 2002; Wang et al. 2002). Additionally, ~50% decrease in WT APC protein expression in *Apc*$^{1638N/+}$ mice, indicates a primary role of *Apc* haploinsufficiency as a plausible mechanism of tumorigenesis in this model (Wang et al. 2002), rather than inactivating mutation.

A significant reduction in serum levels of PGE2 was noted at all tested aspirin dose. In concurrence, aspirin mediated effective reductions in mucosal as well as serum PGE2 levels with reduced sign of systemic inflammation have been observed both in mouse and human studies (Morris *et al.*, 1985; Cryer *et al.*, 1990; Montrose *et al.*, 2015; Liu *et al.*, 2013; Boutaud *et al.*, 2016; Kumar *et al.*, 2018a). PGE2 is known to exert pleiotropic effect on many of the hallmarks of cancer (Greenhough *et al.*, 2009) including activation of the β-Catenin signaling and mechanisms involved in tumor promotion, maintenance and progression (Buchanan & DuBois, 2006). Studies using *Ptgs-2* (gene coding for COX-2) knock-out mice have indicated resistance to colorectal carcinogenesis (Chulada *et al.*, 2000), while *Ptgs-2* transgenic mice have revealed that COX-2/PGE 2 pathway activation alone is insufficient to induce tumorigenesis (Oshima *et al.*, 1996). Therefore, activation of COX/PGE2 pathway in the tumor promotion process is more defined rather than its role in tumor initiation process.

Greater expression of PGE2 have shown a good correlation with larger tumor size in both human and mouse model studies (Kettunen *et al.*, 2003; Yang *et al.*, 1998). While no correlation for aspirin use and tumor size is available for comparison, and we did not observe any significant reduction in tumor size in aspirin treated mice. Aspirin mediated

reductions in COX/PGE2 is important anti-cancer mechanism, however mechanisms independent of COX/PGE2 might also be important in ^{28}Si-induced GI-tumorigenesis; as no appreciable effect of aspirin on ^{28}Si-induced GI-tumorigenesis and size was noted in *Apc*$^{1638N/+}$ mice. While higher COX2/PGE2 level was observed after high-LET IR (Cheema *et al.*, 2014), but other pro-inflammatory/proliferative factors (IGF1, IL-8 and IL-6) (Kumar *et al.*, 2018b; Kumar *et al.*, 2019; Suman *et al.*, 2016a) have also been found to accumulate in GI-tissues after high-LET IR exposure and might function as alternate signaling to drive high-LET IR-induced GI-tumorigenesis, rendering aspirin mediated reductions in PGE2 levels ineffective. Therefore, we postulate that aspirin at levels used in this study is reducing COX-related inflammation but other pro-inflammatory and pro-proliferative responses independent of COX/PGE2 are also being elicited after high-LET exposure to promote GI-tumorigenesis in *Apc*$^{1638N/+}$ mice. However, further studies in the WT or *Apc* gene independent model of GI-cancer using various aspirin doses and duration against high-LET radiation are required for validation and to rule out any genotype-specific bias that may prevail in this study.

5. Conclusions

In summary, this study suggests that aspirin has no preventive effect against high-LET radiation-induced GI tumorigenesis in *Apc*$^{1638N/+}$ mice. Although an effective reduction in serum PGE2 level was noted in aspirin treated group, however, deemed insufficient to reduce high-LET radiation-induced tumorigenesis due to its limited role in the tumor initiation process (Oshima *et al.*, 1996). A multitude of molecular alterations caused by high-LET IR exposure such as, increase in chronic oxidative stress, persistent DNA damage and accumulation of pro-oncogenic SIR factors (Datta *et al.*, 2012; Cheema *et al.*, 2014; Datta *et al.*, 2016; Suman *et al.*, 2018; Kumar *et al.*, 2018b; Kumar *et al.*, 2019; Suman *et al.*, 2020) might undermine aspirin's efficacy in preventing radiation-induced GI tumorigenesis. However, reductions in ^{28}Si-induced PGE2 levels with aspirin use is encouraging and warrants further experimentation to optimize aspirin's preventive efficacy in a drug combination setting to simultaneously target alternate tumorigenic pathways induced after high-LET IR exposure.

Acknowledgments

We are grateful to Dr. Peter Guida, Dr. Adam Rusek, and all the support staff at the NASA Space Radiation Laboratory (NSRL) for providing excellent support to conduct ^{28}Si-ion exposures. We are also thankful to the Georgetown University (GU) and Brookhaven National Lab (BNL) animal facility members for their help in the management and transportation of our mouse colony.

6. Funding

This study is supported in part by NASA grant NNX09AU95G.

Abbreviations

ASA	Acetyl salicylic acid (Aspirin)
LET	Linear energy transfer

GCR	Galactic cosmic radiation
NSAID	Non-steroidal anti-inflammatory drug
Apc	Adenomatous polyposis coli gene
REID	Risk of radiation exposure-induced death

8. References

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Highlights

- High-LET ionizing radiation (IR) causes a dose-dependent increase in GI-tumorigenesis.
- Dietary aspirin reduces serum PGE2 levels in a dose-dependent manner.
- Dietary aspirin reduces high-LET IR-induced serum PGE2 levels.
- Dietary aspirin has no preventive effect against high-LET IR-induced GI-tumorigenesis.

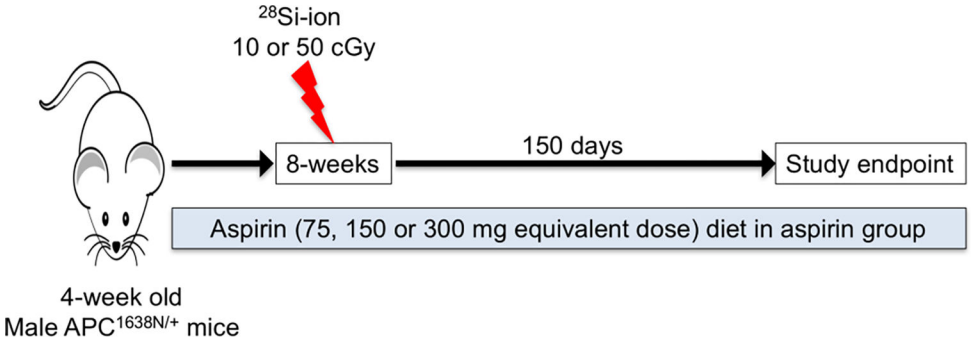

Figure 1.

Schematic summary of the experimental approach to test chemo-preventive efficacy of dietary aspirin on high-LET radiation-induced GI-tumorigenesis.

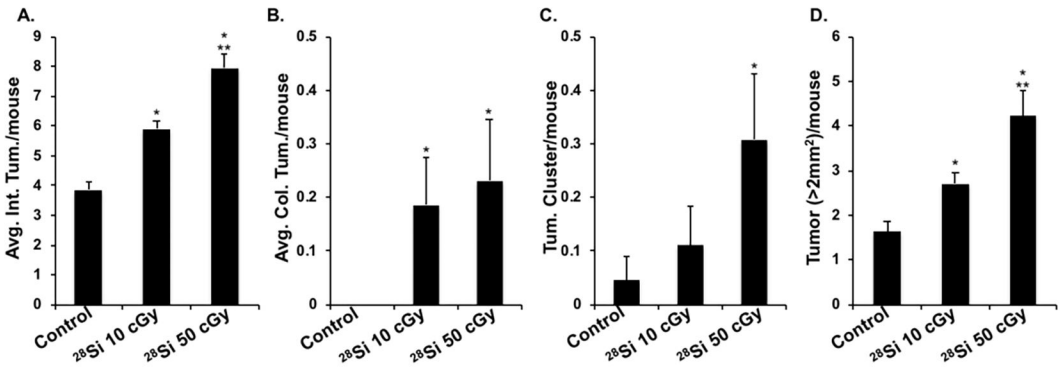

Figure 2.

High-LET IR-induced GI-tumorigenesis in *Apc*$^{1638N/+}$ mice. A) Mean intestinal tumor per mouse. B) Mean colonic tumor/mouse. C) Mean tumor clusters/mouse. D) Mean large size tumor/mouse in control, and ^{28}Si-ion (10 and 50 cGy) irradiated animals. Bars represent mean ± SEM and * indicates $p<0.05$ compared to control whereas ** indicates $p<0.05$ between irradiated groups.

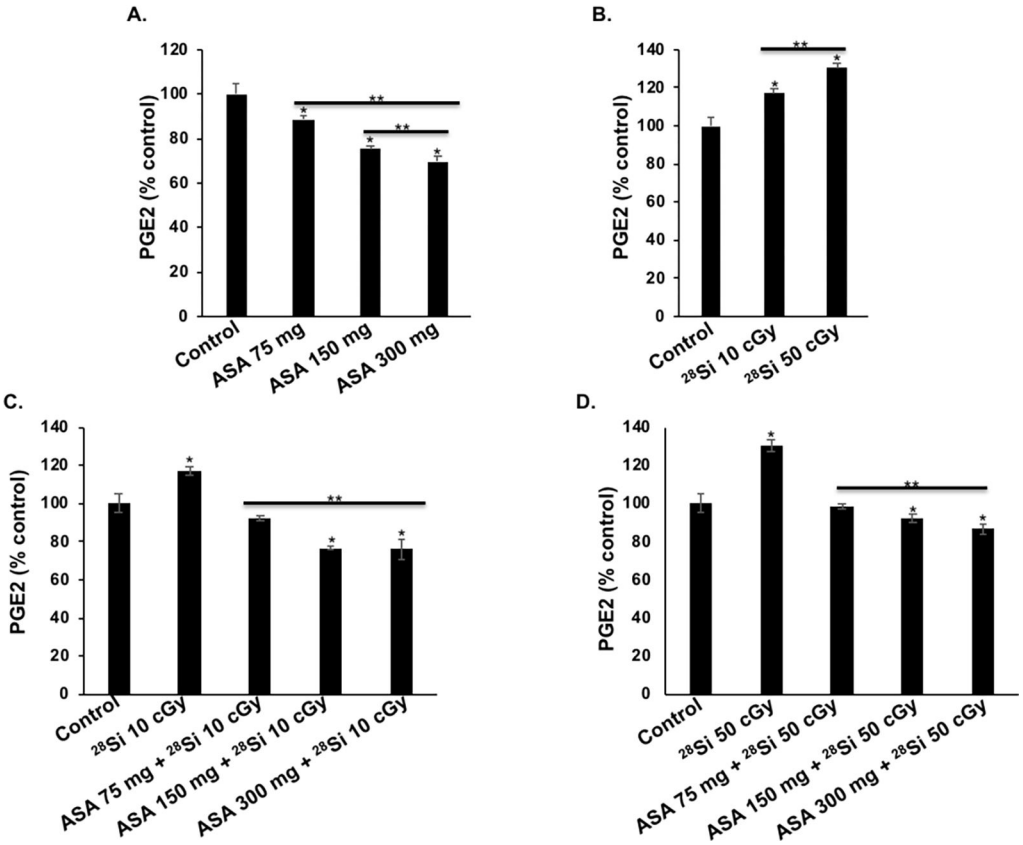

Figure 3.

Effects of dietary aspirin (ASA) on serum prostaglandin E2 (PGE2) levels in *Apc*$^{1638N/+}$ mice (n=6/group). A) Percentage change in PGE2 after human daily dose equivalent of 75 mg, 150 mg, and 300 mg ASA. B) Percentage change in PGE2 after whole body ^{28}Si-ion exposure. C) Percentage change in PGE2 in 10 cGy ^{28}Si-ion exposed mice and effect of dietary ASA. D) Percentage change in PGE2 in 50 cGy ^{28}Si-ion exposed mice and effect of dietary ASA. Bars represent mean ± SEM and * indicates $p<0.05$ compared to control whereas ** indicates $p<0.05$ between irradiated groups.

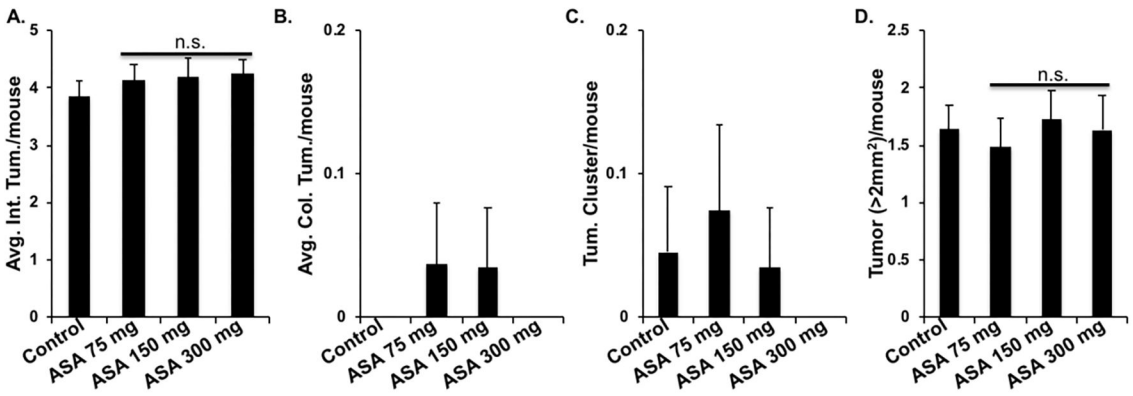

Figure 4.

Effect of dietary aspirin (ASA) on GI tumorigenesis in *Apc*$^{1638N/+}$ mice (n=20/group). A) Intestinal tumor count/mouse. B) Colonic tumor count/mouse. C) Mean tumor clusters/mouse. D) Mean large size tumor/mouse in control, ASA 75, 150, and 300 groups. Bars represent mean ± SEM and n.s. indicates no statistically significant difference among experimental groups.

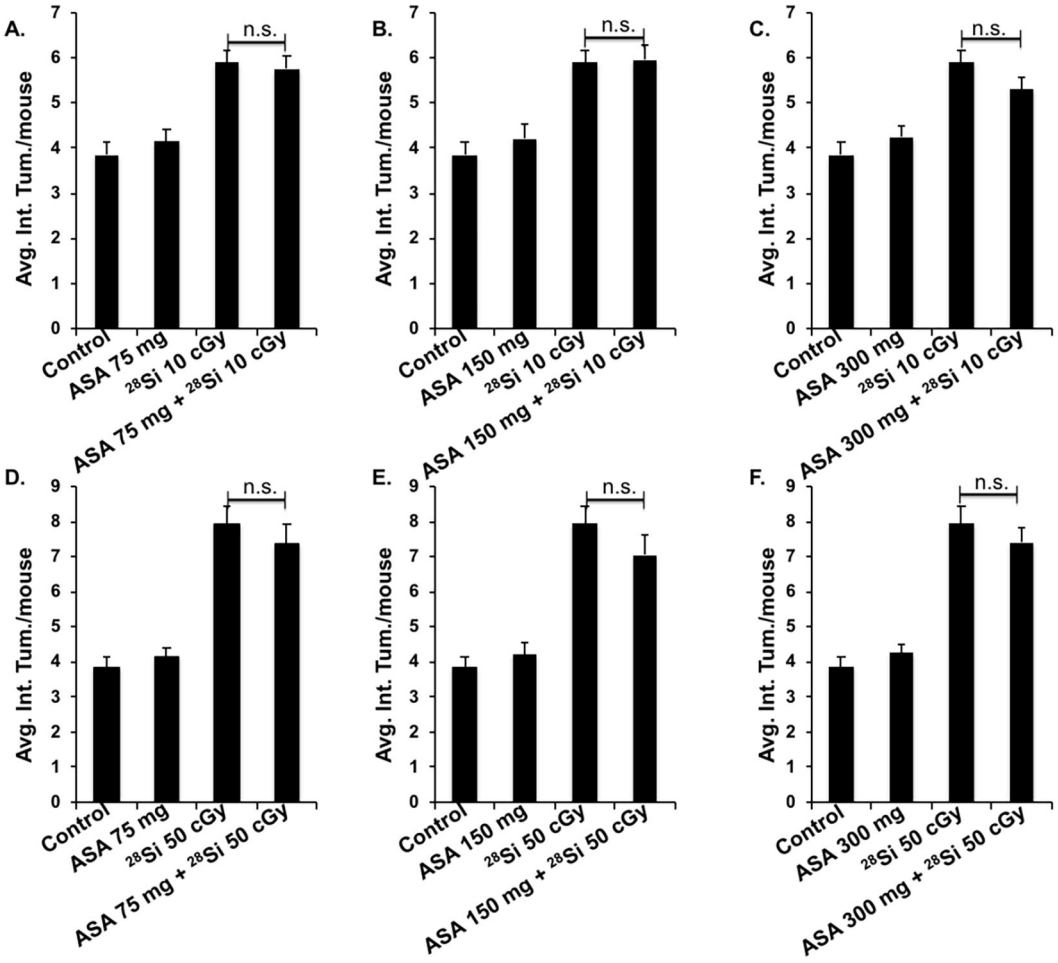

Figure 5.

Effect of aspirin on high-LET IR-induced GI tumorigenesis in *Apc*$^{1638N/+}$ mice (n=20/group). A) Intestinal tumor count/mouse in control, ASA 75 mg, ^{28}Si-ion (10 cGy), and ASA 75 + ^{28}Si-ion exposed groups. B) Intestinal tumor number/mouse in control, ASA 150 mg, ^{28}Si-ion (10 cGy), and ASA 150 + ^{28}Si-ion exposed groups. C) Intestinal tumor number/mouse in control, ASA 300 mg, ^{28}Si-ion (10 cGy), and ASA 300 + ^{28}Si-ion exposed groups. D) Intestinal tumor number/mouse in control, ASA 75 mg, ^{28}Si-ion (50 cGy), and ASA 75 + ^{28}Si-ion exposed groups. B) Intestinal tumor number/mouse in control, ASA 150 mg, ^{28}Si-ion (50 cGy), and ASA 150 + 50 cGy ^{28}Si-ion exposed groups. C) Intestinal tumor number/mouse in control, ASA 300 mg, ^{28}Si-ion (50 cGy), and ASA 300 + ^{28}Si-ion exposed groups. All bars represent mean ± SEM and n.s. indicates no statistically significant difference.

Table 1

Description of experimental groups. Mice were randomly assigned to 12 groups with either standard diet, three aspirin groups, or two IR groups as noted below.

IR\Diet	Standard diet	Aspirin 75 mg/day	Aspirin 150 mg/day	Aspirin 300 mg/day
Sham	Control	ASA 75	ASA 150	ASA 300
^{28}Si 10 cGy	10 cGy	ASA 75 + 10 cGy	ASA 150 + 10 cGy	ASA 300 + 10 cGy
^{28}Si 50 cGy	50 cGy	ASA 75 + 50 cGy	ASA 150 + 50 cGy	ASA 300 + 50 cGy