

# Differential Response to DNA Damage May Explain Different Cancer Susceptibility Between Small and Large Intestine

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Although large intestine (LI) cancer is the second-leading cause of cancer-related deaths in the United States, small intestine (SI) cancer is relatively rare. Because oxidative DNA damage is one possible initiator of tumorigenesis, we investigated if the SI is protected against cancer because of a more appropriate response to oxidative DNA damage compared with the LI. Sixty rats were allocated to three treatment groups: 3% dextran sodium sulfate (DSS, a DNA-oxidizing agent) for 48 hrs, withdrawal (DSS for 48 hrs + DSS withdrawal for 48 hrs), or control (no DSS). The SI, compared with the LI, showed greater oxidative DNA damage ( $P < 0.001$ ) as determined using a quantitative immunohistochemical analysis of 8-oxodeoxyguanosine (8-oxodG). The response to the DNA adducts in the SI was greater than in the LI. The increase of TdT-mediated dUTP-biotin nick end labeling (TUNEL)-positive apoptosis after DSS treatment was greater in the SI compared with the LI ( $P < 0.001$ ), and there was a positive correlation ( $P = 0.031$ ) between DNA damage and apoptosis in the SI. Morphologically, DSS caused an extensive loss of crypt structure shown in lower crypt height ( $P = 0.006$ ) and the number of intact crypts ( $P = 0.0001$ ) in the LI, but not in the SI. These data suggest that the SI may be more protected against cancer by having a more dynamic response to oxidative damage that maintains crypt morphology, whereas the response of the LI makes it more susceptible to loss of crypt architecture. These differential responses to oxidative DNA damage may contribute to the difference in cancer susceptibility between these two anatomic sites of the intestine. *Exp Biol Med* 230:464–471, 2005

**Key words:** small intestine; colon; oxidative DNA damage; apoptosis; cell proliferation

## Introduction

Although both the large intestine (LI) and the small intestine (SI) are lined by similar types of epithelial cells with similar crypt-based morphologies, their susceptibilities to cancer are very different. The second-leading cause of death from cancer in the United States is LI cancer, whereas SI cancer is rare (1). In 2004, deaths from LI cancer accounted for 10% of all cancer deaths in the United States, while deaths from SI cancer constituted less than 0.2% (1).

Oxidative DNA damage, one possible initiator of tumorigenesis (2), is produced by oxidative stress generated by reactive oxygen species (ROS; Refs. 3 and 4). If reactive-species generation exceeds the capacity for neutralization by the antioxidant defense system during inflammation, it can generate oxidative stress, which can readily damage DNA (3, 4). Respiration, tobacco use, irradiation (2, 5), or chemical substances (e.g., dextran sodium sulfate [DSS]) can produce ROS (6). A large molecule (40 kD), DSS may be directly toxic to normal epithelial cells, lymphocytes, and the extracellular matrix (7), which results in the induction of inflammation and oxidative stress in rodents (6, 8, 9). In this study, we measured 8-oxodeoxyguanosine (8-oxodG) DNA adduct levels as a biomarker of oxidative DNA damage. The 8-oxodG adduct is one form of oxidative DNA damage that is closely related to tumorigenesis (6, 10). Unless this lesion is repaired and/or removed by apoptosis, it can induce GC → TA transversions during DNA replication, which are found in mutated oncogenes and tumor-suppressor genes (11). The propagation of these mutated cells can lead to the malignant transformation of colonic mucosa.

The purpose of this study was to determine whether the SI, compared with the LI, responds more favorably to oxidative stress. Because intestinal tumors arise from perturbations of homeostasis among cell proliferation and apoptosis (12), we hypothesized that the SI is protected against cancer by a more beneficial response to oxidative DNA damage *via* enhancing apoptosis and decreasing proliferation to suppress oxidative DNA damage. To test this hypothesis, oxidative DNA damage, apoptosis, and cell

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proliferation were assessed within intestinal crypts in the SI and the LI using a DSS-treated rat model.

## Materials and Methods

**Animals and Study Design.** The animal-use protocol was approved by the University Animal Care Committee of Texas A&M University and conformed with guidelines from the National Institutes of Health. Rats were individually housed and maintained in a temperature- and humidity-controlled animal facility. Sixty male, weanling, Sprague-Dawley rats (Harlan Sprague-Dawley, Houston, TX) were acclimated for 4 to 6 days and then assigned to one of three groups: control (no DSS), 3% DSS in drinking water for 48 hrs (DSS), or 3% DSS for 48 hrs and an additional 48 hrs of DSS withdrawal (withdrawal). After acclimation, rats ( $n = 40$ ) were administered 3% w/v DSS (40 kd; ICN Biomedicals, Aurora, OH) in the drinking water for 48 hrs, while controls ( $n = 20$ ) continued to receive normal drinking water (no DSS). Following 48 hrs of DSS treatment, all of the controls and half of the DSS-treated rats ( $n = 20$ ) were euthanized by CO<sub>2</sub> gas. The remaining DSS-treated rats ( $n = 20$ ) were provided normal drinking water for an additional 48 hrs before they were euthanized. The 3% DSS level of administration was chosen based on the results of a preliminary experiment and from a review of the literature (6, 8, 9). The level of DSS was sufficient to induce oxidative DNA damage without severe pathologic inflammation. During the DSS-treatment and removal periods, body weight and water consumption were recorded.

***In Vivo* Measurement of Oxidative DNA Damage.** After the rats were euthanized, the SI and the LI were immediately resected. The first 1 cm of the duodenum and the last 1 cm of the most distal colon after removal of the rectum were taken. These segments were chosen because the distal part of the colon (i.e., the descending colon) is where colon cancer occurs most frequently in humans, and the proximal duodenum is where SI adenocarcinoma occurs most prevalently, according to the National Cancer Institute (13). Oxidative DNA damage was measured by quantitative immunohistochemistry using a monoclonal anti-8-oxodG antibody (Trevigen, Gaithersburg, MD), as previously described (14). The specificity of 8-oxodG has been validated by competitive enzyme-linked immunoabsorbent assay (15). Omission of primary antibody was used as a negative control.

Slides from rats treated with 6% DSS (i.e., a higher level than used in this study) were run as positive controls. To check the reproducibility of 8-oxodG staining, tissue sections from the same animal were tested several times over several days in preliminary experiments. The range of staining intensity and pattern was very similar among tissue sections. The tissue slides from the animal were used as standards, and standard tissue sections were also run with actual samples for quality control. At least 20 crypt columns per animal, in both the SI and the LI, were randomly chosen for quantitative analysis in control (no DSS) and 48-hr DSS

treatment groups. Because there was a significant reduction in the number of intact crypts in the distal colons of rats in the withdrawal group, a total of 480 intact crypts were analyzed (rather than 20 intact crypts per animal). In the SI of the withdrawal group, at least 20 crypt columns per animal were quantified. Staining intensity was assessed by cell position within the crypt using NIH Image software (Scion Corporation, Frederick, MD). The conditions, brightness, filter, and light exposure were adjusted to optimize the image brightness and contrast. For accurate and consistent results, the settings remained constant for all images in both the SI and the LI once established. Each nucleus on one side of a crypt column (starting from the lowest cell in the base of the crypt) was outlined, and the staining intensity was measured. The background staining intensity was subtracted from the staining intensity of the target cells.

***In Vivo* Apoptosis Measurement.** The apoptosis assay used (Intergen, Purchase, NY) was based on the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL; Refs, 16, 17). Positive control slides were treated with DNase I (Ambion, Austin, TX) at 37°C. Negative control slides were incubated without the TdT enzyme. Crypt height in number of cells and the number and location of apoptotic cells with crypts of SI and LI were recorded. The apoptotic index was 100 times the mean number of apoptotic cells per crypt column divided by the mean of the total number of cells per crypt column.

***In Vivo* Measurement of Cell Proliferation.** Cell proliferation was determined using Ki-67 (BD Biosciences, San Diego, CA), as previously described (18). Paraformaldehyde-fixed, paraffin-embedded tissue sections were deparaffinized, and then antigen was retrieved by microwave treatment in 0.1 M sodium citrate buffer (pH 6.0). Monoclonal anti-Ki-67 antibody (BD Biosciences) was used as the primary antibody, and omission of primary antibody was used as a negative control. The number and position of labeled cells in the crypts of the SI and the LI were recorded. Only strongly labeled cells were counted. The proliferation index (PI) was calculated as 100 times the number of labeled cells per crypt column divided by the total number of cells per crypt column. Proliferative zone was calculated as 100 times the position of the highest-labeled cell divided by the number of cells per crypt column.

**Statistical Analyses.** Oxidative DNA damage, apoptosis, and cell proliferation data were analyzed using one-way ANOVA to determine the effect of treatment in the SI and the LI. If the  $P$  values for the interaction were  $<0.05$ , means of the treatment groups were then separated using Fisher's protected least significant difference test. The comparisons between the SI and the LI, with respect to oxidative DNA damage, apoptosis, and cell proliferation, were analyzed only within the crypt because there are no villi in the LI. Comparisons of DNA damage, apoptosis, and

cell proliferation in the SI and the LI were evaluated using paired *t* tests. Comparisons among the bottom, middle, and top third of crypts in each SI and LI on DNA damage, apoptosis, and cell proliferation were analyzed using a least significant difference multiple comparison test. The correlations between oxidative DNA damage and apoptosis or cell proliferation were tested using Pearson's correlations, and statistical significance was assessed using Fisher's distribution, with calculations done using PROC CORR in SAS (SAS Institute, Cary, NC; Ref. 19).

## Results

**Daily Water Consumption and Body Weight Gain.** Water consumption ( $10.42 \pm 0.27$  g daily) and body weight gain ( $1.85 \pm 0.36$  g daily) were significantly reduced in DSS rats compared with control rats ( $P < 0.05$ ;  $13.42 \pm 0.56$  g daily and  $5.88 \pm 0.33$  g daily, respectively). Although 48-hr DSS administration caused loose stools, a common symptom of experimental and clinical ulcerative colitis, there were no symptoms of severe inflammation (e.g., severe diarrhea, bloody stools). In withdrawal rats, water consumption ( $15.13 \pm 0.50$  g daily) and body weight gain ( $7.65 \pm 0.47$  g daily) were higher than in the control and DSS groups.

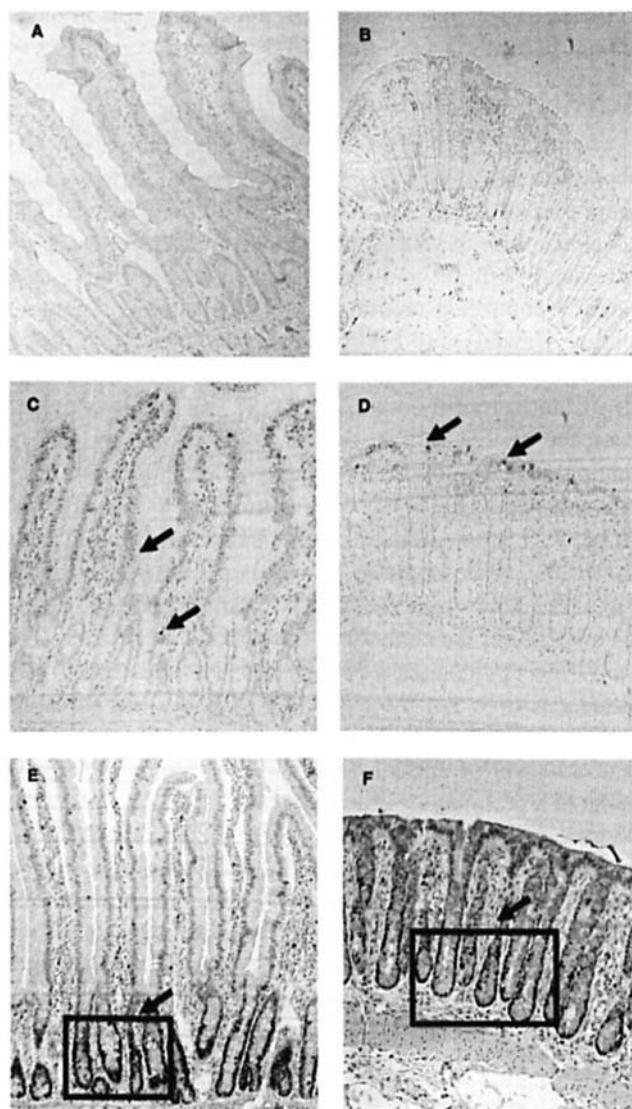
**Distribution of Oxidative DNA Damage, Apoptosis, and Proliferation.** In the SI, oxidative DNA damage was higher in the villi than in the crypts (Fig. 1A). Within the SI crypts, DNA damage was greatest in the lower and top parts of the crypts than in the middle third of the crypts in all treatments ( $P < 0.05$ ; Table 1). In the LI, oxidative DNA damage was greatest in the lower part of the crypts in control rats. In contrast, oxidative DNA damage was greatest in the upper part of the crypts in DSS-treated rats (Table 1).

In the SI, apoptosis was much higher in the villi compared with the crypts (Fig. 1C). Within the crypts, the apoptotic index tended to be higher in the upper part of the SI crypt in control rats. In the LI, apoptotic cells were evenly distributed within a crypt in control rats. In the DSS and withdrawal groups, the major localization for apoptosis ( $P < 0.05$ ) was the upper part of the crypts in both the SI (Fig. 1C) and the LI (Table 1 and Fig. 1D).

Cell proliferation was very rare on the villi of the SI (Fig. 1E). Within the crypts, cell proliferation was primarily localized in the lower part of the crypts regardless of treatment in both the SI ( $P < 0.05$ ; Fig. 1E) and the LI ( $P < 0.05$ ; Table 1 and Fig. 1F).

**Oxidative DNA Damage and Relationship With Apoptosis.** The basal level of oxidative DNA damage (8-oxodG) was greater in the SI compared with the LI ( $P < 0.001$ ) (Fig. 2). Oxidative DNA damage was increased by DSS in both the SI ( $P = 0.05$ ) and the LI ( $P = 0.001$ ), but the increase of DNA damage was greater in the LI (167%) than in the SI (59%) (Fig. 2).

Apoptosis was greater in the SI than the LI in the



**Figure 1.** Representative image of oxidative DNA damage (8-oxodG), apoptosis, and cell proliferation in the SI and the LI of a DSS-treated animal. The DNA damage, apoptosis, and proliferation were greater in the SI (A, C, and E, respectively) compared with the LI (B, D, and F, respectively). In the SI, DNA damage and apoptosis were higher (A and C), but proliferation (E) was much lower in the villi than in the crypts. Within the crypts, the majority of apoptosis was localized in the upper part of the crypts in both the SI and the LI (C and D) of the DSS group. In contrast, proliferation was predominantly located in the lower part of the crypts in both tissues (E and F). Arrows and boxes represent stained target cells.

control ( $P = 0.004$ ) and DSS treatment groups ( $P = 0.001$ ). Treatment with DSS resulted in a 100% increase of the apoptotic index in the SI ( $P = 0.05$ ). In contrast, there was no significant increase of apoptosis (40%) after DSS treatment in the LI (Fig. 3A).

The relationship between oxidative DNA damage and apoptosis was tested, and there was a positive correlation between oxidative DNA damage and apoptosis in the SI. As oxidative DNA damage increased in SI crypts, apoptosis also increased ( $r = 0.46$ ;  $P = 0.031$ ; Fig. 3B). In contrast, there was no such relationship found in the LI (Fig. 3B).

**Table 1.** Oxidative DNA Damage, Apoptosis, and Proliferation in the Bottom, Middle, and Top Third of the Crypts<sup>a</sup>

|                                 | SI                   |                    |                      |      | LI                 |                    |                    |      |
|---------------------------------|----------------------|--------------------|----------------------|------|--------------------|--------------------|--------------------|------|
|                                 | Bottom               | Middle             | Top third            | SEMD | Bottom             | Middle             | Top third          | SEMD |
| DNA damage (staining intensity) |                      |                    |                      |      |                    |                    |                    |      |
| Control                         | 17.72 <sup>a**</sup> | 14.96 <sup>b</sup> | 18.36 <sup>a</sup>   | 0.99 | 7.32 <sup>a</sup>  | 5.21 <sup>b</sup>  | 5.57 <sup>b</sup>  | 0.47 |
| DSS                             | 28.26 <sup>a</sup>   | 25.04 <sup>b</sup> | 26.28 <sup>a,b</sup> | 0.99 | 16.59 <sup>a</sup> | 14.50 <sup>b</sup> | 17.82 <sup>c</sup> | 0.56 |
| Withdrawal                      | 23.90 <sup>a</sup>   | 20.22 <sup>b</sup> | 23.21 <sup>a</sup>   | 0.89 | 10.30 <sup>a</sup> | 8.96 <sup>a</sup>  | 12.92 <sup>b</sup> | 0.78 |
| Apoptosis (%)                   |                      |                    |                      |      |                    |                    |                    |      |
| Control                         | 0.39                 | 0.37               | 0.76                 | 0.30 | 0.09               | 0.07               | 0.08               | 0.05 |
| DSS                             | 0.53 <sup>a</sup>    | 0.93 <sup>a</sup>  | 1.75 <sup>b</sup>    | 0.34 | 0.02 <sup>a</sup>  | 0.19 <sup>b</sup>  | 0.22 <sup>b</sup>  | 0.10 |
| Withdrawal                      | 0.43 <sup>a</sup>    | 0.42 <sup>a</sup>  | 1.00 <sup>b</sup>    | 0.27 | 0.77 <sup>a</sup>  | 1.07 <sup>a</sup>  | 3.56 <sup>b</sup>  | 1.01 |
| Proliferation (%)               |                      |                    |                      |      |                    |                    |                    |      |
| Control                         | 74.90 <sup>a</sup>   | 66.65 <sup>b</sup> | 20.37 <sup>c</sup>   | 3.15 | 53.79 <sup>a</sup> | 20.03 <sup>b</sup> | 0.43 <sup>c</sup>  | 3.50 |
| DSS                             | 77.73 <sup>a</sup>   | 64.25 <sup>b</sup> | 14.15 <sup>c</sup>   | 2.83 | 47.77 <sup>a</sup> | 23.91 <sup>b</sup> | 1.93 <sup>c</sup>  | 5.17 |
| Withdrawal                      | 85.45 <sup>a</sup>   | 74.94 <sup>b</sup> | 23.40 <sup>c</sup>   | 2.36 | 41.53 <sup>a</sup> | 25.31 <sup>b</sup> | 4.10 <sup>c</sup>  | 5.94 |

<sup>a</sup> SEMD, standard error of the mean difference. Different letter superscripts are different among bottom, middle, and top third of the crypt each tissue at  $P < 0.05$ .

**Cell Proliferation.** The PI was greater in the SI compared with the LI in the control ( $P < 0.001$ ) and DSS treatment groups ( $P < 0.001$ ; Fig. 4). However, there was no DSS treatment effect on the PI (Fig. 4) or proliferative zone (data not shown).

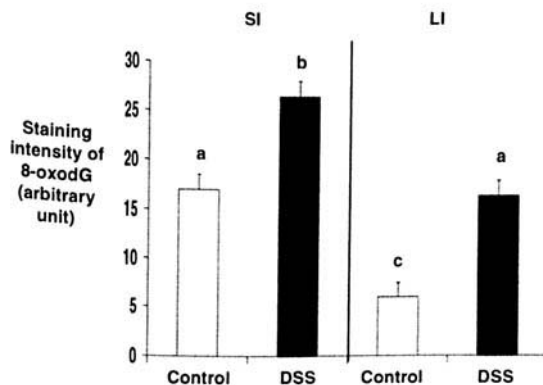
**Crypt Morphology.** In the withdrawal group, crypt morphology was altered in the LI as a consequence of DSS treatment. Crypts were distorted and shorter (Fig. 5B) compared with crypts from control rats (Fig. 5A). Furthermore, when treated with DSS, only crypt remnants were found in some areas of the LI (Fig. 5C), and there were some areas where no intact crypts remained (Fig. 5D). This occurred in the LI, but not in the SI. There was no apparent change in morphology in the SI. To evaluate the effect of DSS on crypt morphology, crypt height in number of cells and number of intact crypts per 300  $\mu$ m were counted. Crypt height in the number of cells ( $P = 0.006$ ; Fig. 6A) and the number of intact crypts per 300  $\mu$ m ( $P = 0.0001$ ; Fig. 6B) was lower in the LI of withdrawal group, whereas there was

no change in crypt height or the number of intact crypts in the SI.

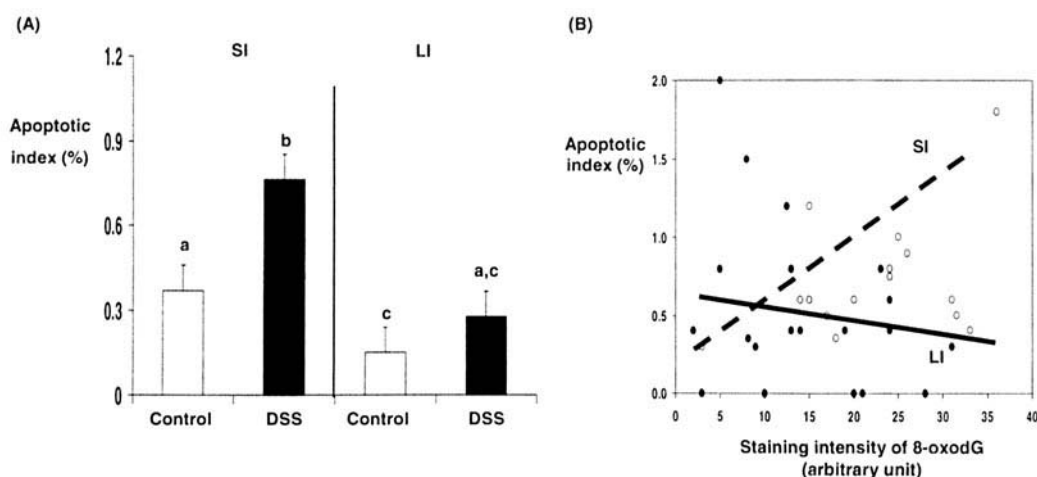
In the withdrawal group, oxidative DNA damage was still higher in the SI compared with the LI ( $P < 0.001$ ; Fig. 7A). The damage in the withdrawal group (Fig. 7A) was less than that in the DSS group (Fig. 2) for both the SI ( $P < 0.001$ ) and the LI ( $P = 0.017$ ). In the SI, oxidative DNA damage was lowered to levels similar to those in control rats ( $P < 0.05$ ). In contrast, damage (Fig. 7A) did not decline to control levels (Fig. 2) in the LI ( $P = 0.05$ ). The apoptotic index was greater in the LI compared with the SI ( $P < 0.001$ ; Fig. 7B), which contributed to the shortening of crypt height in a number of cells in the LI of the withdrawal group, as previously described. The PI was greater in the SI than in the LI ( $P < 0.05$ ; Fig. 7C). The PI increased in the SI of the withdrawal group (Fig. 7C) compared with the DSS group ( $P = 0.029$ ; Fig. 4), but there was no significant change in the PI in the LI of the withdrawal group compared with the DSS group.

## Discussion

The objective of this study was to investigate possible mechanisms that contribute to lower cancer incidence in the SI compared with the LI. The literature suggests that the SI has lower levels of methylation-induced DNA damage (20) and higher methyltransferase DNA repair enzyme activity (21), which may result in a lower incidence of cancer. In the present study, we extended these observations to oxidative DNA damage. The 8-oxodG DNA adduct level was quantitatively measured as an indicator of oxidative DNA damage. Elevated levels of this adduct have been detected in colonic tumors (3). Surprisingly, the SI had greater nuclear oxidative DNA damage compared with the LI, which is the opposite of our hypothesis. However, the degree of the increase in DNA damage after DSS treatment was much higher in the LI (50% more than control) than in the SI. The effect of DSS may be weaker in the SI because the DSS was



**Figure 2.** Oxidative DNA damage (8-oxodG) was greater in the SI than in the LI of control rats ( $P < 0.001$ ). After DSS treatment, DNA damage increased in both the SI ( $P = 0.05$ ) and the LI ( $P = 0.001$ ), but the degree of increase was greater in the LI compared with the SI ( $P = 0.05$ ). Values are means  $\pm$  SE. Bars without a common letter differ.  $n = 10$  per treatment within each tissue.



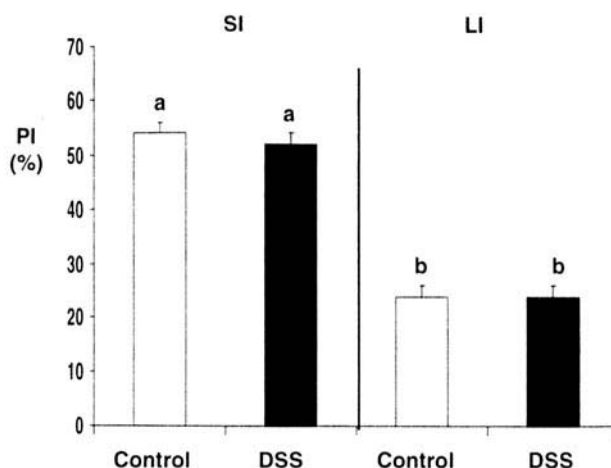
**Figure 3.** (A) The apoptotic index was greater in the SI than in the LI in control ( $P = 0.004$ ) and DSS rats ( $P = 0.001$ ). After DSS treatment, the apoptotic index increased in the SI ( $P = 0.05$ ), but not in the LI. Values are means  $\pm$  SE. Bars without a common letter differ.  $n = 10$  per treatment within each tissue. (B) As oxidative DNA damage increased in the SI ( $\circ$ , broken line), apoptosis also increased ( $r = 0.46$ ;  $P = 0.031$ ). In contrast, increased oxidative DNA damage was not associated with an increase in apoptosis of the LI ( $\bullet$ , solid line).  $r$ , correlation coefficient.  $n = 20$  per tissue.

diluted with food and water. The DSS will become more concentrated in the LI. After DSS withdrawal, SI oxidative DNA damage decreased to control levels, whereas damage in the LI did not decrease to control levels. Therefore, the LI appears more vulnerable to oxidative stress. However, this still does not explain the lower incidence of cancer in the SI because the SI had higher overall oxidative DNA damage. The discrepancy between oxidative DNA damage and cancer incidence led us to develop the new hypothesis that the response to oxidative DNA damage may be more important than the adduct levels.

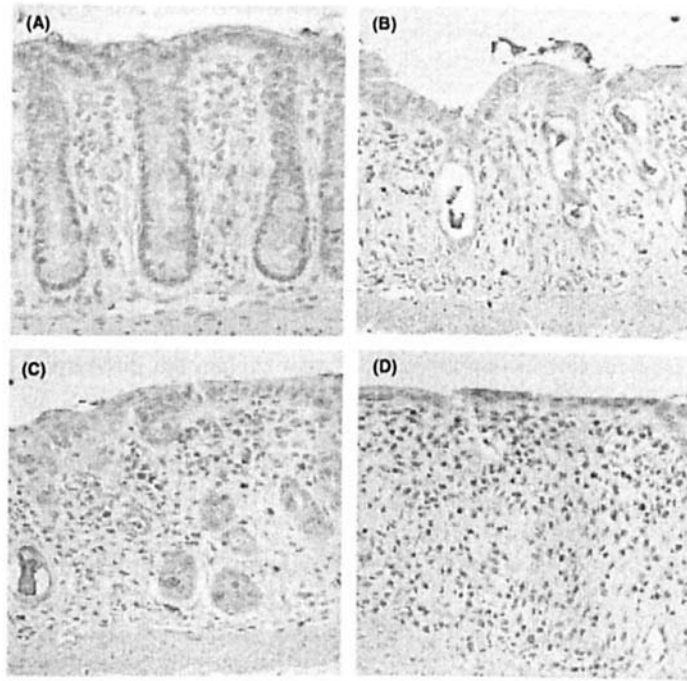
Because intestinal tumors arise from perturbations of the homeostasis among cell proliferation and apoptosis (12), apoptosis and proliferation were determined as responses to DSS-induced oxidative DNA damage. Apoptosis plays a fundamental role in tissue homeostasis by eliminating unwanted or damaged cells, suggesting its involvement in cancer therapy and preventing carcinogenesis (22). Mounting data show that inhibiting this process may be a critical event in developing tumors (22, 23). In this study, spontaneous and damage-induced apoptosis were greater in the SI than in the LI, except for in the withdrawal groups. Moreover, the increase of apoptosis was positively correlated with the level of DNA damage in the SI, suggesting that, in the SI, enhanced apoptosis is targeted to oxidative DNA damage. In the LI, there was no such relationship between damage and apoptosis. Because there was no treatment effect on cell proliferation in either the SI or the LI, apoptosis was one of the key responses to oxidative DNA damage.

In the LI, oxidative DNA damage increased after DSS treatment, but there was no significant effect on apoptosis or proliferation. Instead, crypt morphology was obviously changed, with a massive increase of apoptosis in the withdrawal group. As shown in Figure 5, crypts are

shortened and distorted, and there are some areas of the LI where there are only remnants, without the presence of crypts. This finding is consistent with other studies using the LI from DSS-treated rodents (8, 24, 25). One important thing is that this alteration occurred only in the LI, not in the SI. Although crypt height and number of intact crypts were maintained after DSS treatment in the SI, crypts were shortened and there was a greater reduction in the number of intact crypts in LI. The reduction in number of intact crypts and crypt height was caused by a massive increase of apoptosis in the LI of the withdrawal group, without a corresponding increase in cell proliferation. Therefore, DSS causes the extensive loss of colonic crypt architecture, which results in a total remodeling of colonic epithelial mucosa. From the current experiment, it is not possible to



**Figure 4.** The PI was greater in the SI compared with the LI of control ( $P < 0.001$ ) and treatment groups ( $P < 0.001$ ). Treatment with DSS did not change the PI in either the SI or the LI. Values are means  $\pm$  SE. Bars without a common letter differ.  $n = 10$  per treatment within each tissue.



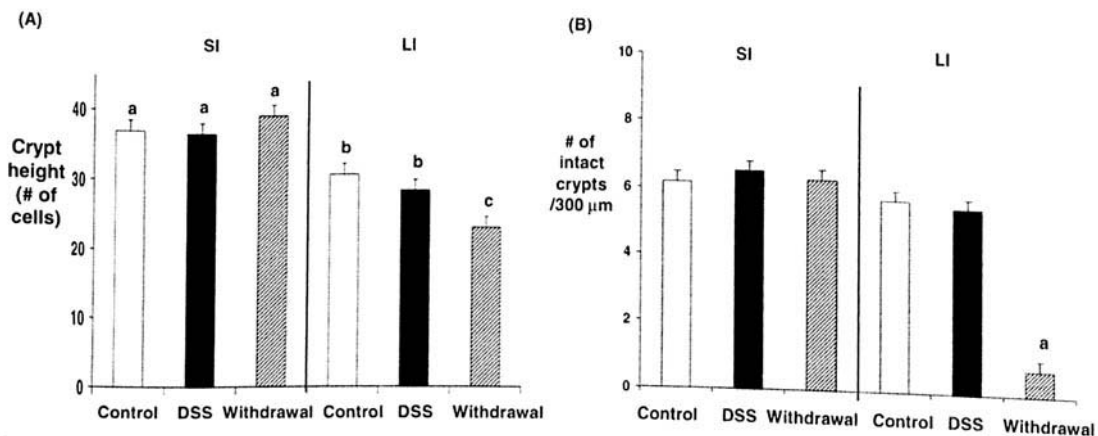
**Figure 5.** Morphologic change after DSS treatment in the LI. (A) Normal colonic crypts became distorted (B) after DSS treatment. (C) In some areas, only crypt remnants were found. (D) Further, there were some areas where there were no more intact crypts.

determine whether the remodeling would result in a physiologically normal colonic crypt.

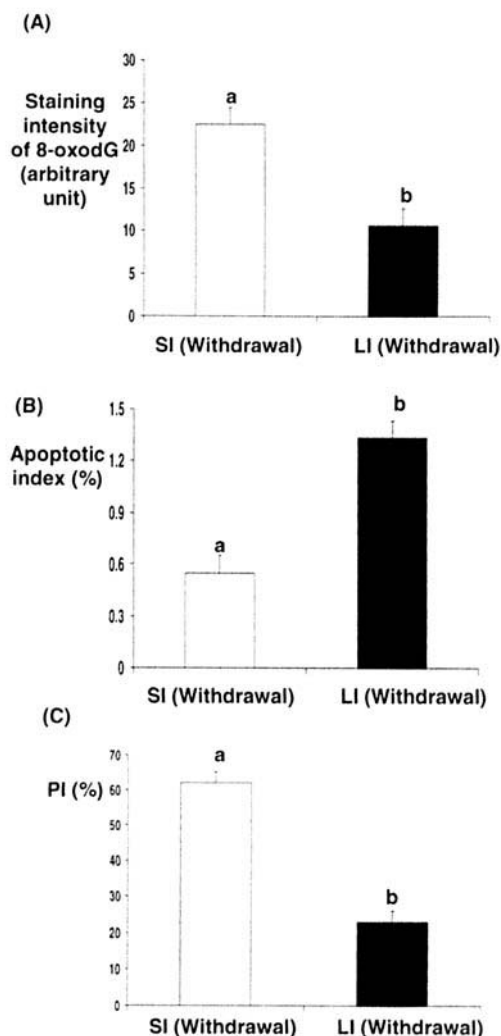
In general, the SI has a higher turnover rate compared with the LI, which accounts for part of the more rapid recovery from oxidative DNA damage in the SI than that observed in the LI. In addition, a significant increase of apoptosis in the SI with DSS treatment may lead to a more beneficial effect by targeted removal of DNA damage (Fig. 3B). The SI appeared to respond to DNA damage by apoptotic removal, whereas an increase in DNA damage in the LI was not correlated with an increase in the apoptotic response. There was a much higher PI in the SI of the withdrawal group than in the LI of the withdrawal group (Fig. 7C). In fact, cell proliferation increased in the SI, but

not in the LI, of the withdrawal group compared with the control and DSS groups. The continued or delayed apoptotic response to DNA damage in the LI resulted in the much-altered morphology of the LI observed during the withdrawal period. There were fewer crypts, and those crypts that remained contained fewer cells than did the crypts at the end of the DSS treatment. Because the apoptotic index is defined as the number of apoptotic cells per crypt column divided by the total number of cells in the crypt column, a higher level of apoptosis coupled with a much lower number of cells in the crypt column may account for the higher apoptotic index observed in the LI of the withdrawal group.

One of the differences between the SI and the LI is that the LI contains a large population of microflora. It has been



**Figure 6.** (A) Crypt height decreased ( $P = 0.006$ ) in the LI of the withdrawal group, but there was no treatment effect on crypt height in the SI. Crypt height was higher ( $P = 0.0001$ ) in the SI compared with the LI, regardless of treatment. (B) The number of intact crypts per 300  $\mu\text{m}$  in the withdrawal group decreased in the LI ( $P = 0.0001$ ). Values are means  $\pm$  SE. Bars without a common letter differ.  $n = 10$  per treatment within each tissue.



**Figure 7.** Oxidative DNA damage, apoptosis, and proliferation in the withdrawal group. (A) Oxidative DNA damage (8-oxodG) was greater in the SI compared with the LI ( $P < 0.001$ ). (B) The apoptotic index was greater in the LI compared with the SI ( $P < 0.001$ ) due to the shortening of the crypt height in a number of cells in the LI. (C) The PI was greater in the SI compared with the LI ( $P < 0.001$ ). Values are means  $\pm$  SE. Bars without a common letter differ.  $n = 10$  per tissue.

reported that DSS changes the microflora population in rat colitis (26), and antibiotic administration suppresses the histologic alterations in acute DSS colitis (27). According to these reports, intestinal microflora may play a role in the induction of oxidative DNA damage and colitis. This suggests that the observed LI histologic alterations in the current study may be due to microbial modification of DSS. However, DSS-induced colitis also occurs in germ-free mouse intestine (28). Therefore, whether or not microflora are required for the induction of colitis is not clearly established.

Repair enzymes and/or antioxidant enzymes that suppress oxidative DNA damage may be other candidates for the differential cancer susceptibility between the SI and the LI. We previously showed, using reverse transcription-polymerase chain reaction, that mRNA expression of the oxoguanine glycosylase 1 repair enzyme for 8-oxodG was lower in the SI than in the LI with DSS treatment (29). We

also showed that antioxidant enzyme activity (e.g., catalase, superoxide dismutase, glutathione S-transferase) was lower in the SI than in the LI (30). Higher oxidative DNA damage in the SI of the present study may be partly attributed to the lower antioxidant enzyme activity and repair enzyme expression shown in our previous studies.

As shown in this study, DSS affects oxidative DNA damage, apoptosis, and cell kinetics. In addition, DSS causes other responses (i.e., changes in the expression of NF- $\kappa$ B, cyclooxygenase-2, and nitric oxide synthase; peroxynitrite formation; Refs. 31–33). These responses may explain the differential cancer susceptibility in the SI and the LI.

Both the SI and the LI are prone to respond differentially to oxidative DNA damage, which may result in the observed differential cancer incidence. In this study, the SI had greater overall apoptosis and cell proliferation compared with the LI. Furthermore, apoptosis was elevated to a greater extent in cells with elevated levels of oxidative DNA damage in the SI. Cell proliferation increased to compensate for the cell loss by apoptosis in the SI of the withdrawal group. In the LI, the increase of apoptosis was not accompanied by an increase of cell proliferation in the withdrawal group, which led to a destruction of colonic crypt structure. From these results, we conclude that the SI may be more protected against cancer by having a more dynamic response/adaptation to oxidative stress that maintains crypt morphology, whereas the LI response makes it more susceptible to loss of crypt architecture. These differential responses to oxidative DNA damage may contribute to the difference in cancer susceptibility between the two anatomical sites of the intestine.

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