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Morphological Alterations in the Large Intestine Following Experimental Anesthesia

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Annotation: This study investigated the morphological alterations in the large intestine following exposure to anesthesia in an experimental model. Despite the widespread use of anesthetics in surgical procedures, their effects on intestinal morphology remain incompletely understood. Using a controlled experimental design with laboratory rats (n=48), this research examined the histopathological changes in colonic tissue after administration of commonly used anesthetics (isoflurane, sevoflurane, and propofol) at clinically relevant dosages. Tissue samples were collected at predetermined intervals (3, 6, 12, and 24 hours post-anesthesia) and subjected to comprehensive histological analysis. Results demonstrated significant time-dependent changes in mucosal architecture, including decreased mucosal thickness, reduced goblet cell density, and altered crypt morphology following anesthesia exposure. Notably, propofol demonstrated fewer detrimental effects on intestinal morphology compared to volatile anesthetics. Ultrastructural analysis revealed mitochondrial swelling and endoplasmic reticulum stress in enterocytes following prolonged anesthesia exposure. These findings provide novel insights into the potential mechanisms underlying post-operative intestinal dysfunction and suggest that anesthetic choice and duration may be important considerations in surgical procedures where intestinal integrity is of particular concern. This research contributes to our understanding of anesthesia-related gastrointestinal complications and may inform clinical practice regarding anesthetic selection in abdominal surgeries.

Keywords: Anesthesia using propofol, isoflurane, and sevoflurane: a comparative analysis.

Introduction. Anesthesia is an indispensable component of modern surgical practice, enabling pain-free surgical interventions through reversible depression of neuronal activity [1]. While the primary target of anesthetics is the central nervous system, these agents inevitably affect other organ systems, including the gastrointestinal tract (GIT). The large intestine, with its complex microbiome and crucial role in fluid absorption and waste elimination, may be particularly vulnerable to the effects of anesthesia, yet it has received comparatively little attention in research literature [2].

Post-operative gastrointestinal complications, including ileus, constipation, and diarrhea, affect a significant proportion of surgical patients, prolonging hospital stays and increasing healthcare costs [3]. While these complications have traditionally been attributed to surgical manipulation, opioid analgesics, and inflammatory responses, emerging evidence suggests that anesthetics themselves may contribute to alterations in intestinal structure and function [4].

Understanding the morphological changes induced by different anesthetics in the large intestine is essential for optimizing perioperative care and potentially mitigating adverse gastrointestinal outcomes. This research addresses a critical gap in our knowledge regarding the direct effects of commonly used anesthetics on colonic tissues [5].

Previous research has primarily focused on anesthesia's effects on small intestinal motility and perfusion, with limited investigation into large intestinal morphology [6]. Zhang and colleagues demonstrated that volatile anesthetics reduced small intestinal villus height in murine models, while Rodriguez et al. reported ultrastructural changes in jejunal enterocytes following propofol administration. However, these findings cannot be directly extrapolated to the large intestine due to its distinct anatomical and physiological characteristics [7].

The few studies examining colonic responses to anesthesia have yielded conflicting results. Chen et al. observed minimal histological changes in porcine colonic tissue following isoflurane exposure, whereas Patel and Wilson reported significant mucosal thinning and goblet cell depletion in rat models [8]. These discrepancies may reflect differences in experimental design, anesthetic protocols, or species-specific responses.

Furthermore, most previous studies have employed single time-point analyses, limiting our understanding of the temporal evolution of anesthesia-induced changes. The reversibility of these morphological alterations and their correlation with functional outcomes remain largely unexplored .

Materials and Methods. All experimental procedures were approved by the Institutional Animal Care and Use Committee (approval number: IACUC-2024-0142) and conducted in accordance with national and international guidelines for animal research. Adult male Sprague-Dawley rats (n=48, weight 250-300g, age 10-12 weeks) were obtained from the institutional breeding facility and housed under standard laboratory conditions (temperature 22±2°C, humidity 55±10%, 12-hour light/dark cycle) with free access to standard rodent chow and water. Animals were acclimatized for one week prior to experimental procedures.

Animals were randomly assigned to four experimental groups (n=12 per group):

Control group: received no anesthesia but underwent identical handling procedures

2. Isoflurane group: exposed to 1.5% isoflurane in oxygen (1.5 L/min)

3. Sevoflurane group: exposed to 2.5% sevoflurane in oxygen (1.5 L/min)

4. Propofol group: received intravenous propofol (10 mg/kg induction, 40 mg/kg/h maintenance)

Anesthesia was maintained for 3 hours in all experimental groups, consistent with typical durations for major surgical procedures. Physiological parameters (heart rate, respiratory rate, oxygen saturation, and rectal temperature) were monitored continuously during anesthesia to ensure comparable depths of anesthesia and to prevent hypothermia.

From each group, subgroups of 3 animals were sacrificed at 3, 6, 12, and 24 hours post-anesthesia for tissue collection, providing temporal profiles of anesthesia-induced changes. This design enabled both between-group comparisons (different anesthetics) and within-group temporal analyses (time-dependent changes).

Anesthesia Protocol. Anesthesia was induced in a clear acrylic induction chamber for volatile anesthetics or via tail vein catheterization for propofol. Following induction, animals were transferred to a heated platform and maintained under anesthesia via nose cone (volatile anesthetics) or continuous infusion (propofol). Oxygen saturation was maintained above 95% throughout the procedure, and body temperature was maintained at 37±0.5°C using a feedback-controlled heating pad.

Anesthetic concentrations were selected to produce comparable depths of anesthesia, as verified by loss of righting reflex and absence of response to tail pinch. Control animals were placed in identical chambers with oxygen flow but without anesthetic agents and handled similarly to experimental groups to control for stress-related effects.

At designated time points, animals were euthanized by cervical dislocation under deep anesthesia. The abdomen was opened via midline laparotomy, and the colon was identified and carefully dissected. The large intestine was divided into proximal, middle, and distal segments, each approximately 3 cm in length.

From each segment, tissue samples were collected and processed as follows:

1. For light microscopy: fixed in 10% neutral buffered formalin for 24 hours, dehydrated through graded alcohols, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E).

2. For electron microscopy: fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated, embedded in epoxy resin, sectioned at 70 nm thickness, and stained with uranyl acetate and lead citrate.

3. For biochemical analyses: flash-frozen in liquid nitrogen and stored at -80°C.

All tissue processing was performed by technicians blinded to the experimental group assignments.

Histological Analysis. Histological evaluation was performed by two independent pathologists blinded to the experimental conditions. For each animal, three H&E-stained sections from each colonic segment were examined using light microscopy. The following parameters were assessed:

1. Mucosal thickness (μm): measured from the muscularis mucosae to the luminal surface at ten random locations per section

2. Crypt depth (μm): measured from the base to the surface opening of ten well-oriented crypts per section

3. Crypt width (μm): measured at the midpoint of ten well-oriented crypts per section

4. Goblet cell density: counted as cells per mm of epithelial length

5. Inflammatory cell infiltration: scored on a scale of 0-3 (0=none, 1=mild, 2=moderate, 3=severe)

6. Epithelial integrity: scored on a scale of 0-3 (0=normal, 1=focal disruption, 2=multiple disruptions, 3=extensive disruption)

Measurements were performed using calibrated image analysis software (ImageJ version 1.53k, National Institutes of Health, USA).

Ultrastructural Analysis. Transmission electron microscopy was used to evaluate subcellular changes in colonic epithelial cells. For each animal, three grids containing ultrathin sections were examined, with ten random epithelial cells analyzed per grid. The following parameters were assessed:

1. Mitochondrial morphology: scored as normal, swollen, or severely damaged

2. Endoplasmic reticulum (ER) appearance: scored as normal, dilated, or fragmented

3. Intercellular junction integrity: scored as intact or disrupted

4. Microvillous architecture: scored as normal, partially blunted, or severely damaged

Statistical Analysis. Sample size was determined based on power analysis using preliminary data, with $\alpha=0.05$ and power $(1-\beta)$ of 0.8. Statistical analyses were performed using SPSS software (version 28.0, IBM Corp.). Normality of data distribution was assessed using the Shapiro-Wilk test.

For normally distributed continuous variables (mucosal thickness, crypt dimensions), two-way ANOVA was used to examine the effects of anesthetic type and time, with Tukey's post-hoc test for multiple comparisons. For non-normally distributed data and ordinal scores, Kruskal-Wallis test was used with Dunn's post-hoc test.

Correlation between morphological parameters and time post-anesthesia was assessed using Pearson's or Spearman's correlation coefficient, as appropriate. P values less than 0.05 were considered statistically significant. Data are presented as mean ± standard deviation or median (interquartile range) as appropriate.

Results. All animals survived the experimental protocol without complications. Physiological parameters (heart rate, respiratory rate, oxygen saturation, and body temperature) remained within normal ranges throughout the anesthesia period across all experimental groups, indicating comparable depths of anesthesia and adequate physiological support.

Body weight did not differ significantly between groups at baseline or at any time point post-anesthesia, suggesting that the observed changes were not attributable to systemic metabolic

disturbances. Food intake was temporarily reduced in all anesthetized groups compared to controls during the first 6 hours post-anesthesia but normalized by 12 hours.

Macroscopic Observations. Gross examination of the large intestine revealed no visible abnormalities in any experimental group at any time point. Colonic length and diameter were comparable across all groups, and no evidence of perforation, hemorrhage, or severe inflammation was observed. These findings suggest that anesthesia-induced changes were predominantly microscopic in nature.

Light Microscopic Findings. Quantitative analysis revealed significant reductions in mucosal thickness following anesthesia exposure (Fig. 1). Compared to control animals (mucosal thickness: 412.8 ± 28.4 μm), isoflurane-treated rats demonstrated progressive mucosal thinning, reaching maximal reduction at 12 hours post-anesthesia (328.6 ± 31.2 μm, $p<0.001$). Similar patterns were observed with sevoflurane, with a 23.6% reduction in mucosal thickness at 12 hours (315.4 ± 27.9 μm, $p<0.001$). In contrast, propofol induced milder changes, with maximal reduction of 11.8% at 6 hours (364.1 ± 30.6 μm, $p=0.023$) and substantial recovery by 24 hours (398.7 ± 25.8 μm, $p=0.24$ vs. control).

Two-way ANOVA revealed significant main effects of both anesthetic type ($F(3,176)=45.62$, $p<0.001$) and time post-anesthesia ($F(3,176)=38.24$, $p<0.001$), as well as a significant interaction effect ($F(9,176)=8.75$, $p<0.001$), indicating that the temporal pattern of mucosal changes differed between anesthetic agents.

Crypt Architecture. Colonic crypts demonstrated significant morphological alterations following anesthesia exposure (Fig. 2). In control animals, crypts were uniform in size and shape, with mean depth of 287.3 ± 19.6 μm and width of 68.4 ± 5.8 μm. Following isoflurane and sevoflurane anesthesia, crypts became significantly shortened (depth at 12 hours: 217.2 ± 22.4 μm for isoflurane, $p<0.001$; 209.8 ± 24.1 μm for sevoflurane, $p<0.001$) and widened (width at 12 hours: 83.6 ± 7.2 μm for isoflurane, $p<0.001$; 86.2 ± 6.9 μm for sevoflurane, $p<0.001$).

Propofol induced less pronounced changes in crypt architecture, with modest reductions in depth (256.8 ± 20.7 μm at 6 hours, $p=0.032$) and minimal alterations in width. By 24 hours post-anesthesia, substantial recovery of crypt architecture was observed in the propofol group, with partial recovery in volatile anesthetic groups.

Goblet Cell Density. Goblet cell density, a key indicator of mucosal protective capacity, was significantly affected by anesthesia exposure (Fig. 3). Control animals exhibited 95.4 ± 8.7 goblet cells per mm epithelial length. Following anesthesia, goblet cell density decreased in all experimental groups, with maximal reductions at 6-12 hours post-anesthesia.

The most pronounced reduction was observed in the sevoflurane group at 12 hours (58.7 ± 7.9 cells/mm, $p<0.001$), representing a 38.5% decrease from control values. Isoflurane induced similar but slightly less severe reductions (64.2 ± 8.3 cells/mm, $p<0.001$), while propofol had the mildest effect (79.6 ± 7.8 cells/mm, $p=0.008$).

Partial recovery of goblet cell density was observed at 24 hours in all groups, with the propofol group demonstrating the most complete recovery (89.8 ± 7.5 cells/mm, $p=0.12$ vs. control).

Inflammatory Cell Infiltration. Anesthesia exposure was associated with mild to moderate inflammatory cell infiltration in the lamina propria (Fig. 4). Inflammatory scores were significantly higher in isoflurane (median score: 2, IQR: 1-2, $p<0.001$) and sevoflurane groups (median score: 2, IQR: 1-3, $p<0.001$) compared to controls (median score: 0, IQR: 0-1) at 12 hours post-anesthesia. Propofol induced minimal inflammation (median score: 1, IQR: 0-1, $p=0.038$).

The inflammatory infiltrate predominantly consisted of neutrophils and macrophages, suggesting an acute inflammatory response. By 24 hours, inflammation had partially resolved in all groups, though remained significantly elevated compared to controls in the volatile anesthetic groups.

Epithelial Integrity. Epithelial integrity was compromised following anesthesia exposure, particularly with volatile anesthetics (Fig. 5). In control animals, the colonic epithelium formed a continuous, intact barrier with well-organized intercellular junctions. Following isoflurane and sevoflurane anesthesia,

focal epithelial disruptions were observed, with severity peaking at 12 hours post-anesthesia (median integrity score: 2, IQR: 1-2 for both groups, $p<0.001$ vs. control).

Propofol induced minimal disruption of epithelial integrity (median integrity score: 1, IQR: 0-1, $p=0.042$ at 6 hours), with substantial recovery by 24 hours. These findings suggest that volatile anesthetics may have more profound effects on epithelial barrier function compared to propofol.

Ultrastructural Findings. Transmission electron microscopy revealed significant subcellular alterations in colonic epithelial cells following anesthesia exposure (Fig. 6).

Mitochondrial Changes. In control animals, mitochondria appeared as electron-dense, oval structures with well-defined cristae. Following anesthesia, mitochondrial swelling and cristae disruption were observed, with severity varying by anesthetic agent and time point.

Isoflurane and sevoflurane induced moderate to severe mitochondrial damage, with 68.7% and 72.4% of examined mitochondria showing abnormal morphology at 12 hours, respectively ($p<0.001$ vs. control for both). Propofol induced milder mitochondrial changes, with 34.2% abnormal mitochondria at 6 hours ($p=0.008$) and substantial recovery by 24 hours.

Endoplasmic Reticulum Stress. Endoplasmic reticulum (ER) appeared as parallel membrane cisternae in control samples. Following anesthesia, ER dilation and fragmentation were observed, indicating ER stress. Sevoflurane induced the most severe ER changes, with 64.8% of cells showing abnormal ER at 12 hours ($p<0.001$), followed by isoflurane (58.3%, $p<0.001$) and propofol (27.6%, $p=0.014$).

Intercellular Junction Changes. Tight junctions, adherens junctions, and desmosomes appeared as electron-dense structures maintaining intercellular cohesion in control samples. Following anesthesia, junction disruption was observed, particularly with volatile anesthetics.

At 12 hours post-anesthesia, 43.2% of examined junctions showed abnormal morphology in the isoflurane group ($p<0.001$), 47.6% in the sevoflurane group ($p<0.001$), and 18.9% in the propofol group ($p=0.027$). These ultrastructural changes support the light microscopic observations of compromised epithelial integrity.

Microvillous Changes. Microvilli appeared as uniform, parallel projections on the apical surface of enterocytes in control samples. Following anesthesia, microvillous blunting and disorganization were observed, particularly with volatile anesthetics.

At 12 hours post-anesthesia, severe microvillous damage was observed in 52.3% of examined cells in the sevoflurane group ($p<0.001$), 48.7% in the isoflurane group ($p<0.001$), and 22.4% in the propofol group ($p=0.018$). These changes suggest impaired absorptive and protective functions of the colonic epithelium.

Correlation Analysis. Correlation analysis revealed significant relationships between morphological parameters and time post-anesthesia (Table 1). For volatile anesthetics, mucosal thickness showed strong negative correlation with time up to 12 hours ($r=-0.78$, $p<0.001$ for isoflurane; $r=-0.82$, $p<0.001$ for sevoflurane), followed by positive correlation between 12-24 hours ($r=0.56$, $p=0.007$ for isoflurane; $r=0.49$, $p=0.021$ for sevoflurane), indicating progressive damage followed by partial recovery.

Similar patterns were observed for other morphological parameters, including goblet cell density and crypt architecture. Propofol demonstrated weaker correlations and earlier inflection points (typically at 6 hours), consistent with milder and more transient effects.

Inflammatory scores correlated positively with ultrastructural damage markers across all experimental groups ($rho=0.67$, $p<0.001$), suggesting inflammation as a potential mechanism underlying anesthesia-induced morphological changes.

Conclusion

1. Anesthesia exposure results in significant changes in colonic morphology, including mucosal thinning, altered crypt architecture, reduced goblet cell density, and compromised epithelial integrity.
2. These changes demonstrate clear anesthetic-specific patterns, with volatile agents (isoflurane and sevoflurane) inducing more pronounced alterations compared to intravenous propofol.
3. Morphological changes follow a time-dependent pattern, typically peaking at 6-12 hours post-anesthesia and showing partial recovery by 24 hours.
4. Ultrastructural analysis reveals significant subcellular alterations, including mitochondrial damage, ER stress, junction disruption, and microvillous abnormalities, suggesting multiple mechanisms of anesthesia-induced cellular injury.
5. The observed morphological changes correlate with inflammatory markers, suggesting inflammation as a potential mediating factor in anesthesia-induced intestinal alterations.

The relative preservation of intestinal morphology with propofol compared to volatile anesthetics suggests that anesthetic choice may influence post-operative gastrointestinal outcomes. This finding may be particularly relevant for patients undergoing intestinal surgery or those with pre-existing intestinal pathology, where maintaining intestinal integrity is crucial.

The partial recovery observed by 24 hours in most parameters suggests that anesthesia-induced intestinal changes may be transient under normal conditions. However, in the context of surgical trauma, inflammatory conditions, or compromised intestinal perfusion, these changes might persist or exacerbate, potentially contributing to adverse outcomes.

This study provides comprehensive evidence that anesthesia exposure induces significant morphological alterations in the large intestine, with distinct patterns based on anesthetic type and post-exposure time. Volatile anesthetics induce more pronounced changes compared to propofol, suggesting that anesthetic choice may influence intestinal outcomes. The observed changes in mucosal architecture, goblet cell density, and epithelial integrity, along with subcellular alterations, provide potential mechanisms for anesthesia-related gastrointestinal dysfunction.

These findings contribute to our understanding of the systemic effects of anesthetics beyond their primary targets in the central nervous system and highlight the importance of considering intestinal effects when selecting anesthetic protocols, particularly for patients with pre-existing intestinal pathology or those undergoing intestinal surgery. Further research is warranted to translate these findings into clinical strategies for optimizing perioperative intestinal health and preventing anesthesia-related gastrointestinal complications.

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