Comparison of Plastic and Metal Oral Gavage Needles in C57BL/6 Mice

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Poster Board #: P145

Introduction

Oral administration is one of the most common routes of dosage, with oral gavage being the most frequently and widely performed oral dosing technique in biomedical research.[1] In rodents, gavage needles are required for accurate dosing due to their small body size. Metal ball-tipped gavage needles have been historically used and preferred by researchers, but their use has been shown to cause complications including trauma and aspiration pneumonia.[1,2,3,4] Recently, newer polypropylene plastic needles have been introduced as an animal welfare refinement.[1] While these needles have been observed to cause fewer complications, evidence of the benefits compared to metal gavage needles is lacking. In this study, we evaluated the clinical and histopathologic effects of daily oral gavage using either metal ball-tipped gavage needles or flexible polypropylene plastic gavage needles in C57Bl/6 mice.

Methods

Animals: Thirty male C57BL/6NCrl mice (8-9 weeks old, 25-35g) were used for this study. Animals were randomized into three groups: Metal gavage needles (*n*=10), plastic gavage needles (*n*=10), restraint-only, no gavage (*n*=10). Animals were maintained in an AAALAC-accredited facility and all study work was performed under an IACUC-approved animal protocol.

Study Design: Animals were dosed with 10mL/kg drinking water at approximately the same time of day, daily, for five days. Clinical observations (Table 1) were performed by a blinded individual (Author: J. Herrod) twice daily at 15 minutes post-dose and again 4 hours post-dose. Body weights were collected on d0, d5, and d7 of the study. On day 7 animals were euthanized and select tissues were collected for histopathology.

Table 1

Clinical Observation Ethogram	
Posture	0 - normal, 1- hunched
Breathing	0- normal, 1- abnormal
Activity	0- normal, 1- decreased, 2- moribund
Eyes	0- normal, 1- mild squint, 2- moderate squint, 3- dull, obviously squinted
Fur	0- normal, 1- mildly ruffled, 2- moderately ruffled, 3- dull, ungroomed, piloerection

Gavage Procedure: Animals were dosed by the same experienced researcher throughout the study (Author: J. Maye). The animals were gently restrained, their necks were extended, and the gavage needle either metal (Popper and Sons, 20G/37mm) or plastic (Instech, 20G/38mm) was gently inserted to the level of the cardiac sphincter before administering the dose. The restraint-only group was restrained for 15 seconds and then returned to their cage.

Histology: The esophagus, trachea, and lungs were collected from animals, embedded in paraffin blocks, processed onto slides, and stained with hematoxylin-eosin. A blinded veterinary pathologist reviewed all tissues (Author: I. Kim) and assigned a histological grade based on inflammation (Table 2).

Statistical Analysis: Significance was set at p≤ 0.05. All data analysis was performed using GraphPad Prism 9.

Results

Figure 1

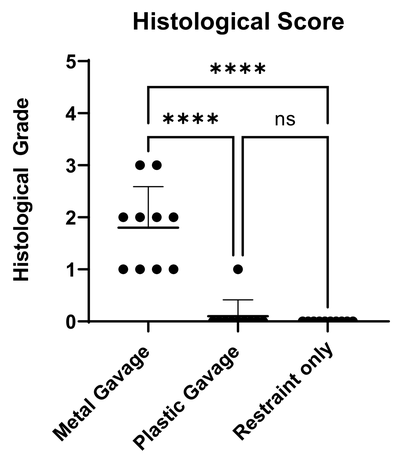

Table 2

Histology Grading Scheme	
0	No inflammation
1	Minimal inflammation
2	Slight inflammation
3	Moderate inflammation
4	Marked inflammation
5	Severe inflammation

Figure 1: Histological grade was significantly different between mice dosed with a metal gavage needle (Kruskal-Wallis; *p*= < 0.001) compared to mice dosed with a plastic gavage needle or only restrained. There were no significant differences (Kruskal-Wallis; *p*= >0.99) between mice who were dosed with plastic gavage needles and mice who were only restrained.

Histological Images

Figure 2

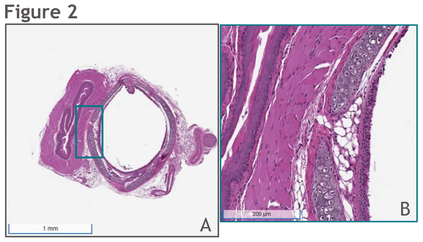

Figure 2: All mice in the restraint-only gavage group had no significant findings noted in the esophagus, trachea, and lungs (2A, 2B).

Figure 3

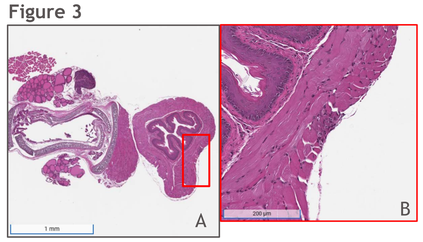

Figure 3: Nine of ten mice in the plastic gavage group had no significant findings noted in the esophagus, trachea, and lungs. One mouse had minimal inflammation in the esophagus (3A, 3B)

Figure 4A

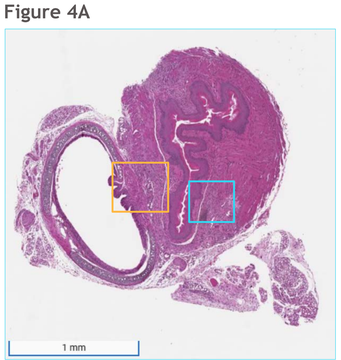

Figure 4B

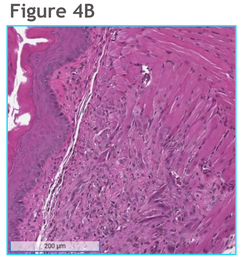

Figure 4C

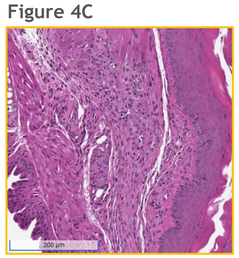

Figure 4: All mice in the metal gavage group showed minimal to moderate mixed-cell inflammation (4A, 4B) with degeneration of skeletal muscle (4A, 4C) in the esophagus. No significant findings were noted in the trachea and lungs.

Figure 5

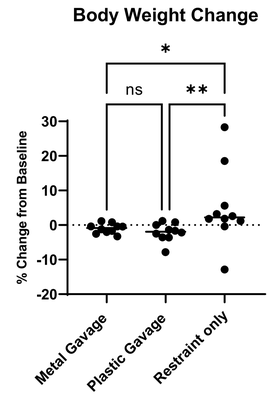

Figure 5: Animals in the restraint-only group gained significantly more weight than animals in the metal or plastic gavage groups throughout the study. (Kruskal-Wallis; *p*= 0.003)

Figure 6

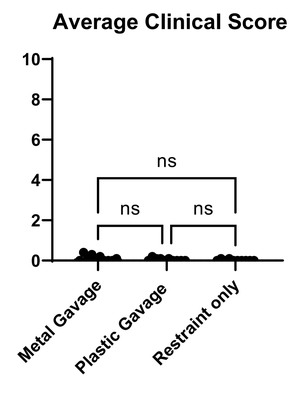

Figure 6: There were no significant differences in clinical observation scores between groups at any time point. (Kruskal-Wallis; *p*= 0.2)

Conclusion

Oral administration of compounds is an essential component of many biomedical research studies. In rodents, the most common experimental technique to administer these substances is directly into the stomach via gastric intubation with a gavage needle.[1] Our results suggest that metal ball-tipped gavage needles cause esophageal inflammation and necrosis in C57Bl/6 mice undergoing daily repeat oral dosing. When designing studies researchers who require oral gavage dosing in mice should consider using flexible polypropylene gavage needles to improve animal welfare. Furthermore, the potential for systemic inflammation and the impact on data should be considered when selecting which type of gavage needle to use. Overall, we conclude that flexible polypropylene gavage needles offer an animal welfare refinement when compared to stainless steel ball-tipped gavage needles.

References

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Acknowledgments and Disclosures

The authors thank Dr. Kristin Matthews for her input on drafting and editing the poster, Ms. Karen Granaldi for her assistance in processing and embedding tissues, and the Animal Care and Husbandry Staff at Bristol Myers Squibb- Lawrenceville for their exemplary care of the animals throughout this study.

All authors are, or have been, employees of Bristol Myers Squibb.

Presented at the Association of Laboratory Animal Science 74th National Meeting, 10/24/2023, Salt Lake City, UT

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