

Modeling Colitis-Associated Cancer with Azoxymethane (AOM) and Dextran Sulfate Sodium (DSS).

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Overview

- **Keywords:** Medicine, Cancer Biology, Immunology, Physiology, Colitis, Cancer, Dextran Sulfate Sodium (DSS), Azoxymethane (AOM), Inflammation, Animal model, Crohn's Disease.
- **Aim of the study:** Establishment of a new protocol for colitis associated cancer in animal model.
- **Application:** Murine endoscopy, Immunohistochemistry.
- **Animal Model:** Mice
- **Material:** AOM, DSS, cellulose acetate filter, 10% Formalin, 70% Ethanol, 2% agar, 18G gavage needle.

Protocol

Colitis-associated Cancer Induction

1. Set aside cages of sex and age-matched 6-8 week old mice to be used for experimental and control groups. Mice can be individually labeled with tail markings or ear clips.
2. On day 0, record baseline weights and inject each mouse intraperitoneally (IP) with 10 mg/kg of AOM working solution (1 mg/ml in isotonic saline, diluted from 10 mg/ml stock solution in dH₂O kept at -20 deg C). Based on experience, this dose can be adjusted between 7-14 mg/kg and/or repeated early in the experiment.

Caution: AOM is a volatile genotoxic agent and should be handled carefully according to the accompanying MSDS. Dilutions should be prepared in a chemical hood, maintained on ice, and discarded following institution specific protocols.

3. Make a 2.5% (2.5 g/100 ml) DSS solution in distilled water and pass through a 0.22 µm cellulose acetate filter by vacuum. This dose can be adjusted between 1-3.5% depending on mouse strain and environment. Once prepared, DSS solution can be kept refrigerated for up to 1 week.
4. On day 7, supply DSS solution to mice as their drinking water. Approximately 250 ml/cage will be needed every time new DSS is provided for a maximum of 5 mice/cage; however, these are only estimations and will vary depending on the type of water bottles used in your animal facility.

Protocol and Parameters

- To provide a continuous supply of DSS for seven days, DSS solution should be replaced in clean bottles three times (every 2-3 days) during this period. Some investigators measure the amount of DSS consumed prior to replacing with new solution as a measure of exposure.
- On day 14, switch cages back to standard drinking water for two weeks.
- Repeat steps (1.4) - (1.6) on days 28 and 49 to provide a second and third cycle of DSS. A DSS «cycle» consists of one week of DSS in the drinking water followed by 2 weeks of regular (autoclaved) water.

AOM and DSS administration protocol:

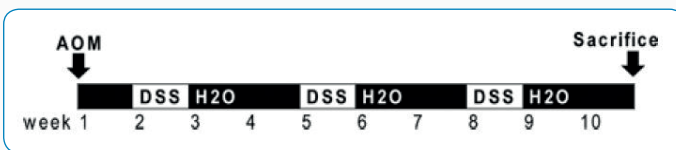


Figure 1. Schematic of AOM and DSS administration.

AOM (10 mg/kg) is injected on day 0. At the beginning of the second week (day 7), 2.5% DSS solution is administered to mice in their drinking water. Seven days of DSS is followed by two weeks of autoclaved water. An additional two cycles of DSS are administered prior to sacrifice.

Results

5-10% mouse weight loss with DSS treatment.

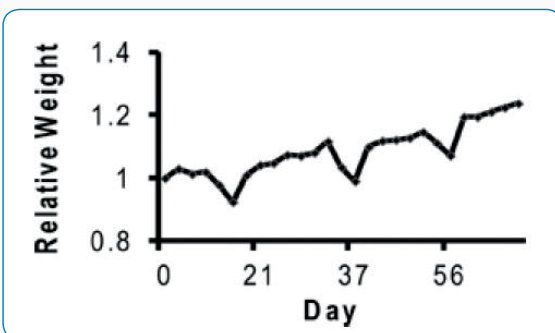
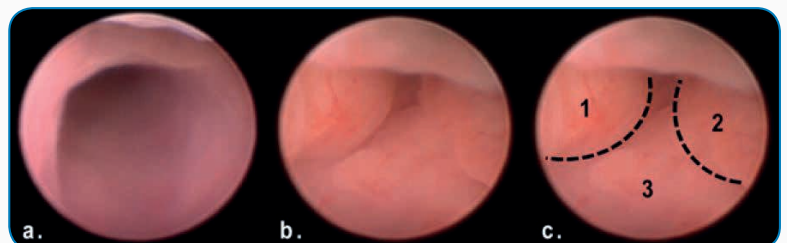


Figure 2. Mouse weight relative to baseline during AOM and DSS administration. Note that in the week following each DSS cycle, mice lose 5-10% of their body weight. Weight loss in this experiment is a surrogate marker for colitis severity.

Formation of multiple polypoid masses obstructing distal colon's lumen after 50 days AOM/DSS treatment.

Figure 3. View of tumors in distal colon via murine endoscopy at day 50 of AOM/DSS treatment. Note the multiple polypoid masses obstructing the lumen of the distal colon (b, c) in comparison to the normal colon (a).



Results

Tumors formation in mouse colon.

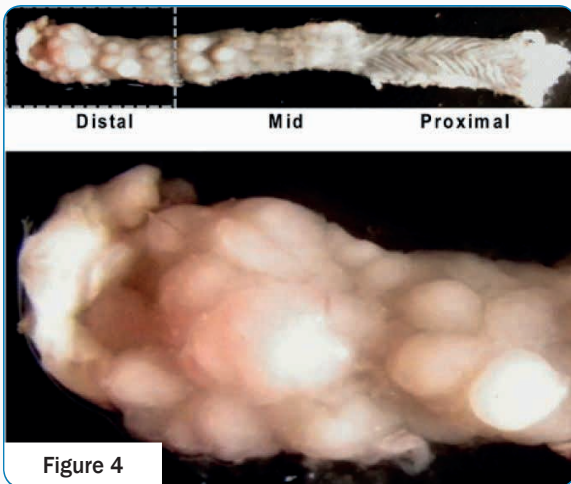


Figure 4. Longitudinally opened mouse colon illustrating gross appearance of tumors. Note the higher tumor burden in the distal colon/rectum (left upper image), and the characteristic rugated texture of the proximal colon (right upper image) with little tumor growth. A close up view of the distal colon shows numerous tumors of varying sizes (below).

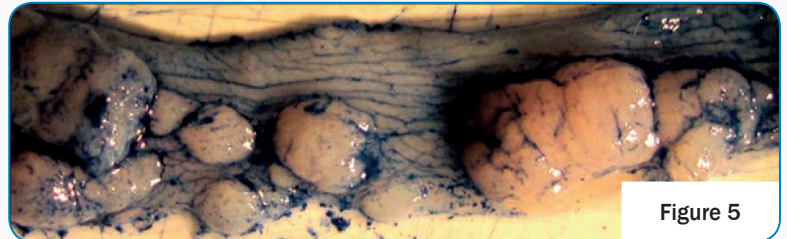


Figure 5. Tumors highlighted by application of Alcian blue stain. Note how the dye emphasizes the normal texture of the colon as well as the borders of each individual tumor. Such staining can be helpful in the precise measurement of tumor areas by ruler or digital measurement.

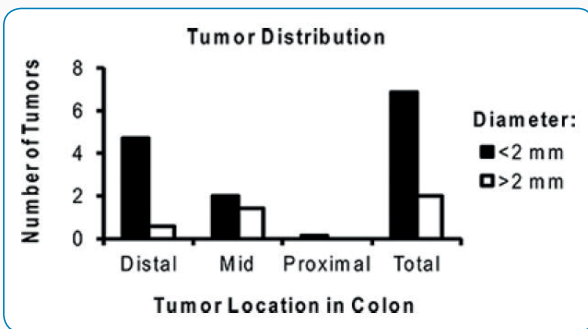
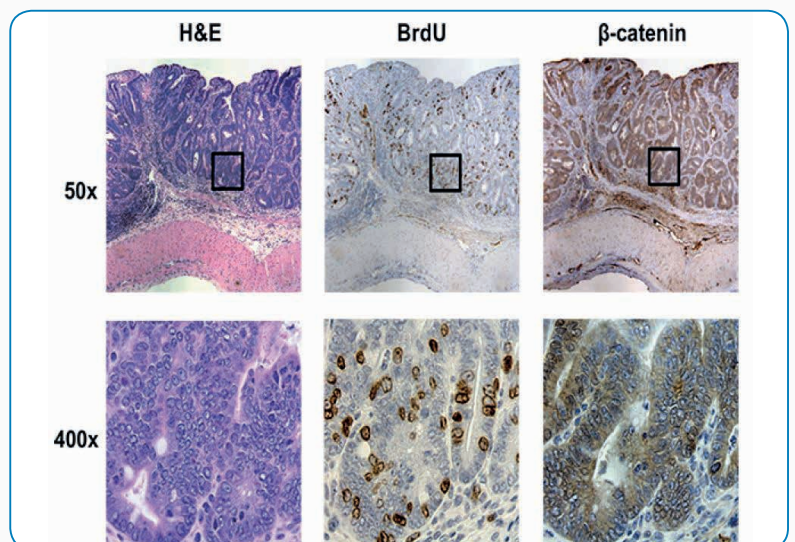


Figure 6. Representative distribution of the average number of tumors per mouse treated with AOM/DSS. Note the majority of tumors are located in the distal colon and are <2 mm in size.

Representative histology of a tumor resulting from AOM/DSS administration in the distal colon.

Figure 7. Representative histology of a tumor resulting from AOM/DSS administration in the distal colon. H&E, BrdU, and β -catenin stained slides at 50X (Top panel) and 400X (Bottom panel) respectively demonstrate dysplastic changes similar to human adenocarcinomas of the colon.



Conclusion

This study shows an efficient, detailed protocol for colitis associated cancer induction by combining DSS and AOM treatment.

► **The combination of DSS with AOM has gained popularity for it's:**

- **Reproducibility**
- **Potency**
- **Ease of use**

► **This method has been proved to be:**

- **Fast and Efficient**

Tumor development in other animal models generally requires several months. Mice injected with AOM and subsequently treated with DSS develop adequate tumors in **as little as 7-10 weeks**.

AOM and DSS can be administrated to mice of **any genetic background** (Knock out, transgenic, etc) without cross-breeding to a specific tumorigenic strain.

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Description	Pack Size	Cat. No
DEXTRAN SULFATE Sodium Salt (DSS)	50 g	0216011050
	100g	0216011080
	500 g	0216011090
Azoxymethane	25 mg	02180139.3
	100 mg	02180139.1

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