Animal Models of Mucositis: Implications for Therapy

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Mucositis is a major acute clinical problem in oncology, caused by the cytotoxic effects of cancer chemotherapy and radiotherapy. The condition may affect the mucosa of the entire alimentary tract (AT), causing mouth and throat pain, ulceration, abdominal pain, bloating, vomiting, and diarrhea, depending on the target tissue. Mucositis is extremely common, occurring in approximately 40% of patients following standard doses of chemotherapy and in almost all patients undergoing high-dose chemotherapy with stem-cell transplantation or head-and-neck radiation. This represents a significant clinical and, importantly, economic burden in oncology. The presence of any mucositis during a cycle of cancer therapy significantly increases the risk of dose reduction, the frequency of infections and bleeding, and the length and cost of hospitalization. Reductions in treatment doses lead to reduced survival. Resource utilization by patients during episodes of mucositis is also significantly increased, with the need for nutritional adjuncts including fluid replacement, liquid diets, and total parenteral nutrition. Due to the association with infection, antibiotic therapy is also more common in patients with mucositis. Combined this translates to an incremental cost increase of US $3,500 per cycle of standard-dose chemotherapy with mucositis and US $1,700—$6,000 for radiation-induced oral mucositis depending on the grade.

Despite the severity and prevalence of mucositis, there is currently no broadly effective preventative treatment available. Standard management is currently limited to pain relief, anti-diarrheal medication, and maintenance of good oral hygiene depending on the area of the tract affected. A large problem with determining the appropriate treatment for mucositis is that the mechanisms underpinning the condition are not fully elucidated. The entire AT has the same embryological route of development, with the differences observed being due to the cellular differentiation required in order to conduct specialized functions. It is therefore highly likely that mucositis will be the same throughout the AT, with the specialized differences in local function offering an explanation for why different regions of the tract are more susceptible to “early” mucositis and others are more susceptible to “late” mucositis. The true extent of the complexity of therapy-induced injury is still being realized, and one of the many issues yet to be fully understood involves the timing and sequence of injury events. A further challenge to elucidating the mechanism of mucosal injury is the relative difficulty and invasiveness of obtaining samples from sites within the AT. Therefore, in order to obtain longitudinal data from multiple sites, animal models are necessary. In addition, any new potential antimucotoxic agent must be first subjected to rigorous testing in animal models to prove efficacy and safety before translation to early clinical trials.

Animal models of mucositis have provided extensive information relating to mechanisms of cancer therapy–induced mucosal injury.
has been an increase in the variety and complexity of models over time, from basic survival end points to specialized biomarkers of damage. In line with this, a number of models have become routinely utilized for testing of new antimucotoxic agents. There have been too many experiments to name them all and their authors; however, a brief summary of the history of animal models of mucositis is given in Figure 1. Furthermore, it must be noted that animal models are highly dynamic, moving between injury-inducing agents and combinations of therapies over time. Below, we describe the most frequently published models arranged according to region inspected and damage-inducing agent.

**Oral Mucositis Animal Models**

While there are a number of different animal models in oral mucositis research, two in particular have been studied extensively.

**RADIATION MOUSE MODEL**

Wolfgang Dorr and colleagues have developed a radiation model in mice, which involves irradiating the tongue and snout.9–13 This model first emerged around 199014 to study epithelial repopulation. The mouse model of radiation uses inbred male and female C3H/Neu mice that originally came from the Dresden colony.9 Over the years Dorr and colleagues have refined their radiation method, such that radiation damage to the lower tongue is induced via a combination of two techniques: the first is a percutaneous irradiation of the entire snout, with the second technique requiring local “top-up” radiation of the lower tongue.9 Graded single doses of 25 kV X-rays are delivered as a 3 × 3 mm² area in the center of the lower tongue surface,15 while fractionated radiation is given as 5 × 3 Gy/week for one or two weeks.16,17 This treatment results in mucosal ulceration within the treatment field of the lower tongue surface, corresponding to confluent mucositis, grade 3 of the classification of the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC).

The mouse radiation model accurately assesses the mucosal response to treatment and has been used to test a variety of agents, including keratinocyte growth factor (palifermin),11,15,17–23 sodium selenite,24 and amifostine.25 This model has also been used to investigate the effects of combining targeted therapy, with an epidermal growth factor (EGF) receptor tyrosine kinase inhibitor, and radiation on the mucosal response,26 which is extremely important in the new era of combination therapies. The evolution of this model has also seen it used extensively to investigate the effects of combined chemotherapy and radiotherapy.18,27 In addition, it has enabled detailed studies to be conducted comparing effects of single-dose and fractionated radiotherapy on the head.11,15,16,28,29

**HAMSTER ORAL MUCOSITIS MODEL**

Another model which has significantly advanced the understanding of the mechanisms of oral mucositis is the hamster model, which was first described in 199030 and has been used extensively by Stephen Sonis and colleagues. This model of mucositis uses male golden Syrian hamsters as, unlike other rodents, they have a buccal cheek pouch, which is susceptible to chemotherapy when scratched. Mucositis can be induced by the administration of 5-fluorouracil (5-FU) at 60 mg/kg for three days (days 0, 5, and 10). The buccal pouch mucosa is superficially irritated (mechanically scratched) on days one, two and three, resulting in mucositis in most of the animals.30 The publication of this model revolutionized research into chemotherapy-induced mucositis. However, perhaps more importantly, the development of this model allowed for the current working five-phase hypothesis of mucositis to be developed.1,31–33 Since the publication of this hypothesis, significant advances in mucositis research have occurred.

The hamster model has also been used in single-dose radiation,14–37 fractionated radiation,38,39 and chemoradiation-induced mucositis.39,40 Mucositis in the radiation model is induced by everting the hamster’s left buccal pouch and applying a single focused dose of 40 Gy radiation, while the remainder of the animal is protected by a lead shield. This dose of radiation predictably elicits severe ulcerative mucositis. Clinically detectable mucositis generally occurs in this model by day six, with peak mucositis at about day 14–15. A fractionated dose is given as cumulative dose of 60 Gy radiation, partitioned into eight fractions of 7.5 Gy each on days 0–3 and 7–10, with a three-day rest period between days four and six. The chemoradiation model consists of 35 Gy radiation on day 0 and cisplatin (5 mg/kg) administered on day one to induce oral mucositis.39 Another variation is two doses of 5-FU (60 mg/kg) on days −4 and −2, with 30 Gy radiation on day zero.40

This model has been used to test many agents, including EGF,41 transforming growth factor β (TGFB),42 interleukin-11 (IL-11),43,44 keratinocyte growth factor (KGF, palifer-
mucositis.1,31–33

Gastrointestinal Mucositis Animal Models

Few consistent animal models exist that investigate mucositis in the remainder of the gastrointestinal tract (GIT).

CHEMOTHERAPY MODELS

The dark agouti rat mammary adenocarcinoma model of gastrointestinal mucositis. Dorothy Keefe and colleagues, recognizing that gastrointestinal mucositis was a big problem, developed the dark agouti (DA) rat model of gastrointestinal mucositis in the mid-1990s.66 This model is unique as it is capable of modeling the changes that occur in the human GIT following insult with chemotherapy.34

Using female inbred DA rats for the model of mucositis has allowed the Keefe laboratory to investigate a range of antimucotoxic agents, including palifermin,48,49 velafenparmin,50 probiotics,51 and IL-11,52 and to study the mechanisms of damage induction by a number of cytotoxics, including methotrexate,53,54 irinotecan,55–62 and 5-FU.63,64 The main advantage of the model is the isogenic tumor, which allows simultaneous assessment of tumor protection, a major fear with any potential antimucotoxic under development. All rats are subcutaneously implanted in each flank with mammary adenocarcinoma cells nine days before chemotherapy. There is excellent homogeneity between all animals in tumor growth and response to treatment.

The DA rat model of mucositis has also recently been modified to study fractionated radiotherapy and the occurrence of acute and subacute GIT injury. In a small pilot study, Ann Yeoh and colleagues developed a six-week fractionated radiotherapy course (total 45 Gy/18 fractions/6 weeks treating at radiation dose of 2.5 Gy/fraction).65 They determined that fractionated radiotherapy induced gastrointestinal changes from as early as week one (i.e., 7.5 Gy), with severe injury seen in the small intestine at later time points.65 Furthermore, many of the changes that were induced by fractionated radiotherapy were identical to those induced by chemotherapy,65 adding considerable weight to the current hypothesis of mucositis.5,31–33

Rat mucositis model of natural therapies. The DA rat has also been used to study natural antimucotoxic agents in an adapted mucositis model from the laboratory of Gordon Howarth and colleagues since 1996.66 Intestinal damage and bacterial translocation are induced in this non-tumor-bearing model by three times daily subcutaneous methotrexate.67–70 Work carried out in TGF-α knockout mice has shed some light on the role of the EGF signaling pathway in the repair of methotrexate-induced gut damage.71 The majority of work has been carried out to develop the potential antimucotoxic agent whey-derived growth factor extract,70,72,73 a by-product of cheese production that is in clinical trials. Emu oil is another natural agent under investigation currently.74 Howarth and colleagues have also investigated the mechanisms of damage following abdominal radiation in the DA rat. Radiation enteritis is induced by a single dose of 10 Gy to the whole abdomen. Markers investigated four days later include growth curves, intestinal weight, and mucosal morphology.75–77

RADIOThERAPY MODELS

Radiation therapy for head and neck cancers results in 30%-60% of patients developing oral mucositis, while pelvic radiotherapy can lead to acute radiation damage to the anorectal region in up to 75% of patients.78 These symptoms can be severe enough to interrupt the planned course of treatment in around 10% of patients.79 Chronic injury is less common but has extreme long-term quality-of-life implications, with sometimes life-threatening complications.

Mouse crypt survival model. Chris Potten and colleagues have utilized and adapted the clonogen survival assay80 for extensive experiments to determine aspects of sensitivity, timing, and dose dependence in the radiation response of intestinal crypt cells, particularly stem cells. Specifically, for the model, mice are killed four days after whole-body irradiation (or, in some experiments, chemotherapy), and the terminal ileum is fixed in Carnoy’s fixative and prepared as gut bundles, where the intestine is cut into 10 segments and bundled together with micropore tape. Paraffin sections are stained with hematoxylin and eosin, and microcolonies are recorded by counting the surviving crypts per circumference of the 10 different transverse sections. Fifteen representative longitudinal sections of crypts from each mouse also have the width measured at the midpoint along the crypt to enable the data to be corrected for any variations in the size of the crypts between experimental groups.81 Crypt survival curves are determined using computer-based curve-fitting programs. These results are supplemented by assays which specifically detect apoptosis and proliferation.

In 1983, the first intervention experiment for preventing radiation-induced gastrointestinal damage using streptomycin sulfate was conducted and showed a modest improvement in mortality but no significant improvement in survival of clonogens.82 Next came investigating the effect of circadian rhythm on sensitivity to irradiation, which found that mornings were associated with a higher apoptotic response to treatment.83 Similar results have been found in patients treated with morning vs. evening pelvic radiotherapy.84 Further agents tested include KGF,85 TGF-β3,86,87 IL-11,88,89 and teduglutide.90 Ferrel et al85 performed an experiment with the microcolony assay which provided evidence of the therapeutic potential of KGF (palifermin) for mucositis that translated into clinical trials. Palifermin remains the only Food and Drug Administration–approved drug for prevention and treatment of mucositis.

Fractionated radiotherapy model of acute and chronic mucosal injury. Martin Hauer-Jensen and colleagues first described an animal model of accelerated fractionation on radiation injury of the rat small intestine in the late 1980s.91 The model was primarily designed to allow investigation of both acute damage at two weeks and chronic radiation damage at 26 weeks
after completion of treatment. The animal model involves transposing a segment of the small intestine and fixing it to the scrotum following bilateral orchidectomy. The 4 cm intestinal loop can then be subjected to repeated irradiation at varying time points and doses. By using this model of chronic radiation, Hauer-Jensen and colleagues have been able to demonstrate that the changes occurring in the acute phase of damage are associated with the severity of damage seen long term. The most common dose regimens that have been investigated include 2.8 Gy in normal and accelerated fractionation schedules up to a total of 56 Gy and 4.2 or 5.6 Gy fractions to a total dose between 33.6 and 67.2 Gy. In this model, relatively large doses can be delivered with few systemic problems, the only common adverse effect being local skin irritation. Examples of agents used in the various animal models of mucositis described are shown in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>INTERVENTION</th>
<th>MODEL SPECIES</th>
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<tbody>
<tr>
<td>KGF (palifermin)</td>
<td>Mouse, rat, hamster</td>
</tr>
<tr>
<td>FGF-20 (velafermin)</td>
<td>Mouse, rat, hamster</td>
</tr>
<tr>
<td>IL-11</td>
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<td>EGF</td>
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<tr>
<td>TGF-β</td>
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<td>TGF-α</td>
<td>Rat</td>
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<tr>
<td>WDGFE</td>
<td>Rat</td>
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<tr>
<td>Probiotics</td>
<td>Rat</td>
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<tr>
<td>Sodium selenite</td>
<td>Mouse</td>
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<td>Amifostine</td>
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<td>ITF</td>
<td>Mouse</td>
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<tr>
<td>Glutamine</td>
<td>Mouse, rat</td>
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KGF, keratinocyte growth factor; FGF, fibroblast growth factor; IL, interleukin; EGF, epidermal growth factor; TGF, transforming growth factor; WDGFE, whey-derived growth factor extract; ITF, intestinal trefoil factor.

Successes and Failures of Translated Agents

Antimucotoxic agents progress from discovery to animal models to clinical trials with varying degrees of success. Just to name a few, these include VSL#3, IL-11, low-level lasers, amifostine, glutamine, and various herb-based agents. While many are still under further investigation, a number of agents have failed at the clinical trial level by not adequately reaching clinical end points. One example is velafermin, a recombinant human FGF-20 that did not reach its primary end point (reduced incidence of severe oral mucositis) in its phase II dose-confirmatory trial of hematologic cancer patients receiving autologous stem-cell transplantation. Success in animal models does not always translate to the clinic, which may be due to a variety of issues, of which a few are outlined below.

**DIFFICULTIES OF ANIMAL MODELS IN MUCOSITIS RESEARCH**

While animal models undoubtedly have benefits, they also have difficulties and limitations. The Sonis hamster model has the confounding issue of wound healing. Hamsters have cheek pouches, and mucositis can be induced by either chemotherapy or radiotherapy. However, following administration of the chemotherapy, the cheek pouch needs to be “mechanically” scratched or irritated in order to induce ulcerated lesions. In humans, however, the oral mucosa does not need to be superficially irritated in order to induce mucositis, so this model is not exactly the same as the clinical setting. Additionally, superficial irritation may result in wound-healing mechanisms being initiated. Dose and scheduling issues are also important and cannot be overlooked. The doses used in rats do not automatically translate to humans: There may be species differences in susceptibility to different agents, and the traditional milligram per kilogram dosing of rodents is not often used in humans, where we tend to use (for reasons that are not always logical) body surface area dosing. Despite similarities, animal models are never identical to humans, and there will always be issues with translation from animal to human research. This does not, however, devalue animal research; it just adds an appropriate note of caution.
An added difficulty with animal models has been introduced with the development of monoclonal antibodies for treatment of human disease. Fully humanized monoclonal antibodies may not be active in animal models, and toxicities may not develop until translation occurs to the human situation.

Difficulties also arise in the DA rat model of mucositis. Unlike the hamster, in the rat visible oral mucositis does not occur due to the highly keratinized nature of the epithelium (D. Wilson and D. Keefe, personal communication) which makes it difficult to successfully investigate oral mucositis. Furthermore, higher doses of chemotherapy are required to induce mucosal injury in animal models, due to the resilience of the rat AT. Another difference is the presence of squamous epithelium in the rat stomach, which can lead to reduction in oral intake when KGF, a stimulator of epithelial growth, is used. Rats do not have an emetogenic reflex, and since some vomiting is a manifestation of mucosal injury, this is a disadvantage. However, it is possible to use pica as an indirect marker for nausea.109

The route of chemotherapy administration has important implications for drug metabolism. In the DA rat model of mucositis, intravenous administration of chemotherapeutic drugs is extremely difficult, with administration into the tail vein being made especially difficult due to the skin pigmentation. As a result, mucositis induced by drugs administered via this route is not routinely investigated. Although all chemotherapeutic drugs cause damage, the mechanisms by which they do this may be different.

Other contributing factors also cause difficulties in animal research; including: stresses in the animals from isolation due to experimental procedures, the need to anesthetize animals on a regular basis and the effect that this has on mucosal homeostasis, and the efficacy of any investigatory drugs on tumor load. Toxicities associated with cancer treatment include those that are localized or regional (ulcers, xerostomia, abdominal pain, malabsorption) and those that are more generalized systemic (fatigue, lack of appetite, nausea, cognitive impairment).112 The recent realization of concurrent tissue-based and systemic toxicities has resulted in the new paradigm of toxicity clustering.113 Interestingly, the proof-of-principle testing for this new way of thinking was carried out in cancer patients.113 Translational research in the laboratory using animal testing is now occurring to examine in greater detail some of the initial findings. Looking at multiple toxicities in combination will add new knowledge in the area as well as uncover new challenges in applying the models.

The final issue in animal models is strain and sex differences. The route of chemotherapy administration is important for example, with the ICMJE Form for Disclosure of Potential Conflicts of Interest and none reported.

Future Use of Animal Models in Mucositis Research

With the constant application of drug screening and agent testing for potential cancer treatments and supportive agents to use in the clinic, the animal model of mucositis will continue to be a highly valuable tool. We are currently in an age where the biotechnology and pharmaceutical industry is progressing rapidly, offering exceptional new drugs for development. However, proper rigorous preclinical testing in appropriate and truly representative models needs to be carried out with each new antimucotoxic treatment to ensure that innovative treatment approaches are not introduced before the technology or its understanding has matured sufficiently to extract maximal benefit. Industry is only now beginning to realize the importance of toxicity testing of their agents. With the hype of targeted therapies, toxicity was not expected, so almost no testing was completed in some cases. Now, more agents are being tested earlier in development and with input from toxicity specialists.

Impact

Over the next 20 years, due to the aging population, the global incidence of cancer will greatly increase and, with it, mucositis. There will also be an increase in consumerism in medicine, with better-informed and assertive patients seeking out novel therapies. For these reasons, the continued development of clinically useful therapies for mucositis is essential. However, the combination of complex factors including technological success, society’s willingness to pay, and future health care–delivery systems will undoubtedly influence how preclinical models are designed and implemented.118

Summary

Gastrointestinal mucositis is an extremely common side effect following cancer chemotherapy and radiotherapy, occurring in a large percentage of patients. Alleviating mucositis using antimucotoxics may lead to increased maximum tolerated doses of chemotherapy or radiotherapy and improve the quality of life for cancer patients both during and after treatment. Research needs to continue to focus on developing antimucotoxics that are both effective and safe. Identification of therapeutic targets and development of these novel agents requires well-validated and clinically relevant animal models to be employed. The future will see more antimucotoxic agents reach clinical trials in a broader range of therapeutic settings, in particular targeted therapies and multimodality regimens.

Acknowledgments: J. M. B. is supported by an NMHRC Postdoctoral Training Fellowship; D. M. K. K. is the Cancer Council South Australia chair in Oncological Medicine.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.
References


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