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Animal models of mucositis: critical tools for advancing pathobiological understanding and identifying therapeutic targets

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Abstract

Purpose of review: Mucositis remains a prevalent, yet poorly managed side effect of anticancer therapies. Mucositis affecting both the oral cavity and gastrointestinal tract predispose to infection and require extensive supportive management, contributing to the growing economic burden associated with cancer care. Animal models remain a critical aspect of mucositis research, providing novel insights into its pathogenesis and revealing therapeutic targets. The current review aims to provide a comprehensive overview of the current animal models used in mucositis research.

Recent findings: A wide variety of animal models of mucositis exist highlighting the highly heterogenous landscape of supportive oncology and the unique cytotoxic mechanisms of different anticancer agents. Golden Syrian hamsters remain the gold-standard species for investigation of oral mucositis induced by single-dose and fractionated radiation as well as chemoradiation. There is no universally accepted gold-standard model for the study of gastrointestinal mucositis, with rats, mice, pigs and dogs all offering unique perspectives on its pathobiology.

Summary: Animal models are a critical aspect of mucositis research, providing unprecedent insight into the pathobiology of mucositis. Introduction of tumour-bearing models, cyclic dosing scheduled, concomitant agents and genetically modified animals have been integral in refining our understanding of mucositis.

Key words: mucositis, animal models, preclinical, pathobiology

1.0 INTRODUCTION

Mucositis remains a poorly managed side effect of almost all anticancer regimens, affecting 40-100% of people undergoing cancer therapy [1]. Although variations exist in its clinical presentation and histological features based on regional specific mechanisms, mucositis is largely underpinned by ulcerative lesions throughout the alimentary tract (mouth to anus). Mucositis affecting the oral cavity is fairly well defined, owing to the ease at which oral mucosa is accessed and the impact of resulting symptoms on people undergoing cancer therapy [2]. Gastrointestinal mucositis instead remains poorly understood [3], reflecting the difficulties in accessing the entire gastrointestinal tract [4] and the region-specific complexities of the gastrointestinal tract.

Despite decades of intensive research efforts, there remains no gold standard prophylactic or therapeutic intervention for mucositis, with the majority of treatments targeted at reducing the burden of symptoms and preventing secondary complications [5]. Given the logistical and ethical obstacles in collecting human biospecimens in supportive oncology, animal models remain heavily relied upon for continued research efforts aimed at understanding the pathobiology of mucositis, as well as assessing novel interventions. However, with mucositis an almost ubiquitous toxicity of cancer therapy, there are a wide variety of preclinical animal models tackling various aspects of mucositis development across a variety of clinical scenarios. These models each provide a unique perspective and highlight the need to select an appropriate model for the specific research question of interest. This, combined with increasingly sophisticated approaches, will see enhanced translation of preclinical findings with tangible impact for supportive oncology.

This review aims to provide a comprehensive overview of the current animal models used for mucositis research, highlighting their specific contribution to the field of supportive oncology and the major advances in the understanding of the pathogenesis of mucositis.

2.0 ANIMAL MODELS OF MUCOSITIS

There are a number of animal models of mucositis that are readily available and easily established in most academic or scientific institutions. The major overarching benefit of developing such models is that although the intricate pathogenesis of mucositis is unclear, the causative factor leading to its development is clear. This homologous nature of mucositis models, defined by the fact that the cause of disease mimics the human condition, greatly increases the validity of these models and their associated findings. This has undoubtedly driven the large and increasing number of animal models used to understand the pathobiology of both oral and gastrointestinal mucositis caused by a variety of anticancer agents.

2.1 Small animal models of mucositis

2.1.1 Oral mucositis

In contrast to the clinical scenario, the investigation of oral mucositis using animal models is inherently challenging. This is primarily driven by the clear disparities in oral anatomy and physiology in rodents and humans [6], reflecting different dietary habits of each species (e.g. omnivores, herbivores, carnivores). Rodents exhibit a thin keratinised epithelium with low epithelial extensions, both of which minimise transport across the mucosa and reduce its sensitivity to overt

injury [6]. Unlike humans and other primates, rodents do not express glycogen-rich content in the cytoplasm of epithelial cells and exhibit higher antigen presenting cell (APC) density within the epithelium and lamina propria, indicating a local immune capacity overpowers an adaptive immune response [6]. Furthermore, rodents have been characterised to have low numbers of mast cell, only present in deeper tissue layers, suggesting lower communicative signaling between the apical surface of the mucosa and immune cells of the underlying tissue [6]. Salivary function has also been shown to differ between rodents and humans, with rodents not actively concentrating compounds (e.g. nitrate) in the saliva [7]. Collectively, these disparities affect the sensitivity of the oral mucosa in rodents to allergens, toxins and other pathogens and reduces the clinical presentation of mucositis-type lesions. In fact, it has been demonstrated that in rodents, the presentation of frank ulceration of the oral cavity is scarce with only histological evidence of reduced epithelial thickness indicative of mucositis development in many models [8]. This is further impacted by the relative difficultly in accessing the oral cavity of rodents without anaesthesia.

In order to overcome these obstacles, the ***oral cheek pouch in hamsters*** has provided a unique opportunity to study oral mucositis [9]. Using this model, clinically-relevant mucositis has been induced using both chemotherapy [9] and radiotherapy [10], in which multiple doses are given on 3 separate days in addition to a physical insult used to replicate mechanical injury to the oral mucosa (e.g. eating, teeth rubbing). The hamster check pouch model is advantageous for a number of reasons [11]. Firstly, the hamster cheek consists of a renewing squamous epithelium, which is in many ways similar to the human mucosa. The cheek pouch is also large, facilitating examination and application of topical therapeutics. Furthermore, the oral bacterial flora is considered to parallel that of humans, dominated by gram-positive microbes [11].

The publication of this model revolutionised research approaches to mucositis development and prevention, with this model being instrumental in defining the universally accepted 5 phase pathobiological model of mucositis [12]. This model has been used in almost all iterations of cytotoxic therapy, including single dose radiation [13-15], fractionated radiation [10, 16] and combined chemoradiation [16, 17]; each developing clinically relevant features of oral mucositis. For these reasons, it has been used countless times for the assessment of anti-mucositis agents, including epidermal growth factor (EGF) [18], transforming growth factor beta (TGF-β) [19], interleukin-11 (IL-11) [20], keratinocyte growth factor-1 (KGF-1, or palifermin) [13] and velafermin [10]. Importantly, palifermin is now recommended by the Multinational Association for Supportive Care in Cancer (MASCC) for the prevention of mucositis in specific oncological cohorts [5], demonstrating the integral part preclinical models play in mucositis management.

Importantly, this model built upon the previously used ***mouse model of radiation-induced mucositis*** developed by Wolfgang Dorr and colleagues in the early 1990s [21, 22]. This model was one of the earliest models of mucositis, originally designed to study epithelial repopulation. The model is based upon an initial course of radiation to the snout of the mouse, given as a fractionated dose of 5 X 3 Gy/week for 1-2 weeks, followed by an additional top up dose localised to the lower tongue. This results in mucosal ulceration consistent with the clinical assessment criteria of the Radiation Therapy Oncology Group, and has therefore been used to study numerous interventions [21, 23, 24].

2.1.2 Gastrointestinal mucositis

Immunological responses both locally and systematically, as well as the interaction with the resident microflora of the host, are key factors in the pathobiology of gastrointestinal mucositis [25]. Unfortunately, they too differ amongst species [26-28]. When considering this in combination with the highly heterogenous landscape of supportive oncology, the difficulties in translating preclinical findings for mucositis interventions are not surprising. In saying this however, animal models have proven invaluable in shaping our understanding of the mechanisms that contribute to gastrointestinal mucositis and the identification of novel modifiable targets. This has certainly been the case over the past decade, with increasingly more sophisticated methods used to assess gastrointestinal function and carefully manipulate mechanisms of interest. These advances are now setting a precedent for more clinically translatable models, with clear overlap with the clinical scenario, thus aiding and accelerating the development of mucositis interventions.

Both rats and mice have been used to study gastrointestinal mucositis caused by a variety of anticancer agents. Each model is unique to its host institution, however follows a fairly generic framework in which radiation or chemotherapy are delivered as a single dose or repeated exposures. Each method has advantages, with a single dose model enabling an unobstructed view of the time-course mucositis development [29]. Repeat exposure models certainly reflect the clinical scenario more adequately, however mechanistic interpretation is clouded by the confounding variables associated with innate and adaptive immunity and overlap between healing and insult [11].

2.1.2.1 Chemotherapy-induced gastrointestinal mucositis

Methotrexate (MTX), 5-Fluorouracil (FU) and irinotecan remain the most commonly studied chemotherapeutic drugs in preclinical models of mucositis, owing to their high rated of gastrointestinal mucositis seen clinically. The ***MTX-induced mucositis*** model demonstrates a predictable, self-limiting mucositis time course. Using a single dose of 45 mg/kg – 60 mg/kg (intravenously), MTX induces clinically-relevant symptoms in male albino Wistar rats, including moderate diarrhoea, reduced food/water intake and weight loss, which peak at day 4 [30]. To date, this model has primarily been used to test a range of anti-mucositis interventions and nutritional strategies [30-34], as well as develop and validate the use of plasma citrulline as a biomarker [35]. Slight variations exist in this model, for example using an intraperitoneal dose of 20 μg/kg MTX (in Sprague Dawley rats) which, despite the extremely low dose, resulted in significant weight loss and histopathological features consistent with the clinic [36]. This model has primarily been used by Sukhotnik and colleagues to study enterocyte turnover [37], growth factors (e.g. glutamine, L-arginine and TGF-α) [38-40], nutritional supplements [41-43] and Wnt/β-catenin signaling in mucositis development [36]. Unfortunately, these are yet to be translated to clinical practice guidelines.

The model of MTX mucositis has also been adapted for multiple chemotherapy dosing cycles, reflective of the clinic, with 1.5-7 mg/kg delivered on three consecutive days (subcutaneously)[44, 45]. This model induces clinically comparable symptoms of diarrhoea, reduced food intake and weight loss, peaking between days 6-8 (after first MTX dose). Preclinical results using this model have shown promise for anti-inflammatory agent, Olmesartan [45], and have highlighted overlapping mechanisms for mucositis and associated cachexia/anorexia [46].

Models of ***5-FU*** induced mucositis are also prevalent within the literature, however significant variation exists in the dosing schedules used, with doses ranging from 25 mg/kg to 450 mg/kg. In

dark agouti rats, 150 mg/kg has been shown to induce intestinal injury and clinically relevant endpoints [47]. This model has been extensively used by Howarth et al. to study nutraceuticals designed to prevent mucositis [47-53], however there has been negligible translation of largely positive results. This group has also been the first to implement colonoscopic analysis of mucosal architecture in a model of colitis induced colorectal cancer [54], introducing a promising new method of mucositis assessment.

Cyclic models of 5-FU induced mucositis also exist, with 3-5 doses of 5-FU at ranges between 25-450 mg/kg. To date, two dose finding studies have been conducted to optimize this method in both male BALB/C and C57Bl6 mice [55, 56]. Unsurprisingly, weight loss and diarrhoea increased dose-dependently, with a concomitant increase in mortality. TUNEL and western blot demonstrated apoptosis in both the ileum and colon following 5-FU. In both studies five doses of 50-100 mg/kg (intraperitoneally) was optimal to induced clinically-relevant mucositis without unacceptable mortality. This contrast other models in which 5 cycles of 30 mg/kg 5-FU are administered to BALB/C mice; an approach recently used to investigate the benefits of probiotic supplementation [57, 58], Rebamipide (enteroprotective agent)[59], IL-1Ra [60], 5-HT_3 antagonists [61, 62] and minocycline [63]. These studies have together demonstrated the key roles and therapeutic potential of the microbiome, inflammatory signaling and oxidative stress in the development of 5-FU mucositis.

Similar protocols have also been used for ***irinotecan***, in both rats and mice. Irinotecan is associated with an early onset cholinergic diarrhoea and a late onset diarrhoea resulting from mucosal injury [29, 64]. Despite this, only some models routinely administer atropine (subcutaneously) with irinotecan. Animal models of irinotecan-induced mucositis were certainly the catalyst for understanding how the microbiome contributes to mucositis development, with bacterial β-glucuronidase integral in the metabolic processing of SN-38G (the inactive form of irinotecan) [65-67]. Dosing ranges vary significantly between studies, reflecting the variety of rodent strains used, but generally fall between 75-300 mg/ml, with peak mucositis occurring between days 3-5. Importantly, irinotecan must be administered in an acidic sorbitol lactic acid buffer for appropriate activation, with control animals receiving appropriate parallel dosing.

In addition to advancing our understanding of bacterial β-glucuronidase, models of irinotecan-induced mucositis have also been critical in shaping our understanding of intestinal barrier function in permitting mucositis development [68], and the interaction between innate immune receptors (e.g. toll-like receptors (TLR)) and mucositis severity [69, 70]. A number of interventions have also been studied in these models including St John's Wort [71-74], probiotic yeasts [75] and antioxidant agents targeting ROS production (e.g. fullerol) [76]. These studies have undoubtedly contributed to the current state of knowledge regarding irinotecan-induced mucositis, resulting in clear clinical strategies to prevent adverse toxicity. Of particular interest is the current FDA regulation requiring pharmacogenetic profiling of prospective patients for mutations in the UGT1A1 enzyme pathway [77, 78]. More recently, it has been demonstrated that irinotecan-induced gastrointestinal injury occurs simultaneously with markers of neuroinflammation, furthering our appreciation for the gut-brain axis in supportive oncology [69].

The last major class of chemotherapy used more commonly in preclinical models of gut dysfunction is ***oxaliplatin***. This platinum-based chemotherapy is not typically associated with frank ulceration throughout the gastrointestinal tract, but is associated with severe gut dysfunction and peripheral neuropathy, suggesting alternative neural mechanisms are at play [79]. Nurgali and colleagues developed a preclinical model of oxaliplatin-induced gut dysfunction, in which six 3

mg/kg doses (over 2 weeks) induces weight loss, nausea (pica) and constipation in BALB/C mice [80]. Using this model, it has been demonstrated that oxaliplatin is associated with loss of enteric neurons, increasing the proportion of neuronal NO synthase-immunoreactive neurons and levels of mitochondrial superoxide and cytochrome c in the myenteric plexus [79-82]. Subsequent studies from this group have also demonstrated changes in TLR-expressing cells, microbiota composition and high-mobility group box 1 expression consistent with reduced transit time and gastrointestinal motility. These studies have lead the way for motility-based assessment in gastrointestinal mucositis; a mechanism that has otherwise received very little attention.

Although these agents represent the majority of animal models dedicated to mucositis research, there remain a number of other studies focused on other anticancer agents. Doxorubicin-induced mucositis has been studies in dark agouti rats [83] and BALB/C mice [84, 85], as well as zebrafish [86]. Dosing ranges from 4-20 mg/kg, and is typically administered via two intraperitoneal injections on subsequent days (or separated by a few days). Variations of this model have shown key roles for TLR2/9 signaling, epithelial and mesenchymal gene signaling and sodium glucose transport mechanisms, Administration of doxorubicin in zebrafish offers a novel platform for high throughout analysis and simple genomic modification specifically tailored for toxicology studies [86]. For example, a green fluorescent kidney [Tg(wt1b:GFP)] and a red fluorescent skin transgenic zebrafish line [Tg(*k18:dsred*)] have been reported to evaluate the toxic effects on kidney and skin [87, 88].

2.1.2.2 Multimodal models

Despite polypharmacy being routine clinical practice, the majority of models studying mucositis continue to be investigated in single drug injection models. Recently, there has been increasing research efforts diverted to developing multi-drug models of mucositis to facilitate and promote clinical translation. In 2016, Pereira and colleagues successfully induced mucositis in C57BL/6 mice via intraperitoneal injection of irinotecan (30-45 mg/kg) and 5-FU (25-50 mg/kg) [89]. They reported that the optimal dose concentration was 45 mg/kg and 37.5 mg/kg respectively (delivered on 4 consecutive days), with significant diarrhoea, body weight loss, intestinal damage, inflammatory cell infiltrate and cytokine production. Importantly, the dual treatment strategy induced mucositis to a greater extent to agents delivered in isolation, highlighting the critical need to develop more clinical relevant models of mucositis. Similarly, 5-FU and oxaliplatin have been studied in combination, reflecting their combined use in colorectal cancer, with evidence indicating therapeutic potential of IL-1R agonism [90] and probiotics [91]

2.1.2.3 Radiation-induced gastrointestinal mucositis

While a number of models exist for chemotherapy-induced gastrointestinal mucositis, radiation models are less common. This is in stark contradiction to the clear impacts radiation has on intestinal function, both acutely and chronically, with many survivors suffering from rectal bleeding, faecal and mucous leaking, excessive gas and uncontrolled defecation years after treatment [92]. Paralleling the complex regimens for pelvic malignancies, in which daily irradiation occurs for several weeks is logistically cumbersome to model preclinically [93]. Much of our knowledge stems from models of total body irradiation with a limited number of high dose fractions given, with many animals not surviving past a few weeks [94]. This limits the study of long term gut dysfunction.

To overcome these limitations, a new model has been developed by Bull and colleagues, in which C57BL/6 mice are exposed to small-field radiation restricted to 1.5cm of the colorectum using a linear accelerator [95]. Each mouse receives 6-8 Gy, twice daily in two, three or four fractions. Validation of their model identified acute cell death in the colorectum, with associate crypt degeneration and immune cell infiltrate. Angiogenesis was elevated, paralleling clinical findings, with fibrosis observed 4 months after irradiation. This model allows for the longitudinal analysis of the mechanisms contributing to both acute and chronic toxicity resulting from pelvic irradiation, offering a more suitable platform for the study of interventions and development of biomarkers.

2.1.2.4 Tumour-bearing models

It is clear that animal models of mucositis have improved our collective understanding of its pathobiology, leading to updated pathobiological models and in some cases, changes to clinical practice guidelines. However, in many respects they fail to adequately represent the entire clinical scenario. In contrast, tumour-bearing models offer a novel opportunity to assess mucositis in the presence of a neoplasm, and its associated effects on intestinal physiology and systemic signaling. They also offer an opportunity to mimic the immunological state of an oncology patient, who in many cases demonstrated compromised immunity and neutropenia. The presence of neutropenia in an individual with mucositis is clinically and mechanistically important, placing them at an increased risk of infectious complications and likely impacting on the severity of mucositis. As such, tumour-bearing models offer an opportunity to more accurately mimic the clinical scenario.

The ***Dark Agouti Mammary Adenocarcinoma (DAMA) rat model of mucositis*** is the most widely used tumour-bearing model in mucositis [29, 96]. Developed by Keefe and colleagues in the mid-1990's, this model overcomes the limitations of many models in that it simultaneously assesses mucositis and tumour cytotoxicity. Female DA rats are inoculated with an isogenic mammary adenocarcinoma, ~7-10 days prior to chemotherapy administration. Gibson et al, reported that mucositis was more severe in tumour-bearing animals, highlighting an important aspect of its pathobiology that would otherwise be overlooked in non-tumour bearing models [29].

This model has been used extensively over the past few decades to assess the efficacy of various anti-mucositis agents including palifermin/velafermin [97-99], IL-11 [100], probiotics [101] and naloxone [102]. These studies, and those without an intervention, have been critical in identification of novel mechanisms including altered barrier function [68], aberrant extracellular matrix (ECM) signaling [103, 104], enteric glia dysfunction [105] and mucin production [106]. The model has now been modified to study fractionated radiotherapy, with homologous clinical and subclinical features [107]. Most recently, this model was used to characterise ECM dysfunction and microvasculature changes associated with radiation-induced gut injury, a novel aspect of acute mucositis [108, 109]. Despite its prevalent use, this model lacks translatability for chemotherapeutic agents used to treat other solid tumours, and as such, greater research efforts should be diverted to developing a wider range of tumour bearing models. Recently, Mi et al., published a colorectal cancer model, in which dimethyl hydrazine was administered for 10 weeks to Sprague Dawley rats, followed by inoculation with SW480 cells. This model was successfully used to assess simultaneously assess probiotic efficacy in preventing mucositis without compromising chemoefficacy [91].

The introduction of a tumour-bearing mucositis model has been instrumental in advancing our understanding of how tumour burden affects mucositis development, with studies showing pro-inflammatory cytokines released by the tumour not only serve to amplify mucositis, but also affect

other parameters intimately involved in mucositis development [110]. For example, it was demonstrated that IL-1β and IL-6, produced by tumour tissue, not only affect food uptake but also energy expenditure leading to cancer cachexia [111]. However implanting tumours into rodents is challenging, often requiring immunosuppression, altering body weight and influencing drug metabolism [110, 112]. As such, while use of a tumour-bearing model of mucositis is important in late-stage drug development, non-tumour bearing models remain an important tool in fundamental mucositis research.

2.1.2.5 Emerging models for next generation anticancer agents

With the increasing use of non-cytotoxic anticancer agent such as targeted therapies, immunotherapies and monoclonal antibodies, the need to adequately understand their unique mucositis phenotype and underlying mechanisms is critical. Until recently, much of our understanding of the toxicities associated with these therapies has been limited to clinical observation. In 2014, the first ***rat model of tyrosine-kinase inhibitor (TKI)-induced diarrhoea*** was developed by Bowen and colleagues, using the agent lapatinib [113, 114]. This model utilises a four-week schedule of daily oral lapatinib (50-100 mg/kg) treatment to induce mild-moderate diarrhoea in male albino Wistar rats. This schedule achieves an intermittent and repeated presentation of diarrhoea, paralleling the clinic. Of particular interest is the lack of microscopic or macroscopic changes in the jejunum and colon of these rats, highlighting stark differences in the pathobiology of TKI-induced diarrhoea compared to that of 'classical' mucositis. This contradicts findings from previous studies in which mice exposed to gefitinib and elotinib TKIs [115, 116] demonstrated marked abnormalities in intestinal morphology, and thus highlights species-dependent variation in response to. Despite these variations, all models reported positive effects on intestinal morphology or symptomology following co-treatment with the intestinal growth factor glucagon-like peptide-2.

More recently, Van Sebille et al. developed a comparable model of ***TKI-induced mucositis using dacomitinib***. 7.5 mg/kg of dacomitinib, administer daily via oral gavage for 21 days was sufficient to induce moderate diarrhoea and associated weight loss [117]. In contrast to lapatinib, severe ileal injury was observed, along with changes in MCP-1 expression and intestinal permeability; novel preclinical findings for dacomitinib associated toxicity. This model has subsequently been used to investigate crofelemer, aimed at targeting excessive secretory mechanisms that lead to diarrhoea [118].

2.1.2.2 Sophisticated manipulation in small animal models of mucositis

More recently, increasingly sophisticated methods have been used to study mucositis pathogenesis including genetic modification, manipulation of the microbiome and elegant targeting of inflammation. ***Knockout studies*** focusing on toll-like receptors have been most popular of recent, with studies focusing on cytokines (e.g. IL-4), mucin proteins, trefoil factor, p53, p21 and IL-1R, iNOS, IL-10, TLR4, TLR2 and TLR9, informed by immunogenomic analyses and preclinical findings [55, 69, 85, 119-125]. For example, germ-line deletion of TLR4 [69] and MyD88 [122] were shown to be protective against irinotecan-induced gastrointestinal mucositis. Importantly, these effects appear to be drug- and receptor specific, with TLR2 deletion shown to improve irinotecan-induced mucositis, yet exacerbate MTX-induced mucositis [120]. This highlights the importance of translating findings from animal models in a specific and informed manner.

This is also the case for microbiome-related findings in mucositis. This has undoubtedly been the biggest area of growth for gastrointestinal mucositis, with countless studies now indicating changes in the bacterial composition of animals (and humans) exposed to anticancer agents [126]. Studies aimed at dissecting the causative relationship between the microbiome and mucositis are scarce and somewhat contradictory. Evidence for a direct contribution of the intestinal microbes was demonstrated in ***germ-free mice*** which were protected against irinotecan-induced mucositis, but lost protection when colonised with a diverse microbiome [65]. This is in stark contrast to studies that utilise ***antibiotic-induced microbiome depleted (AIMD) rodents***, which are typically more susceptible to mucositis development.

Inflammatory mechanisms have always been central to mucositis development, demonstrated in some of the earliest animal models. Although based on a sound scientific rationale, targeting of inflammation has been largely underwhelming, with limited clinical translation. More recently however, work using transgenic mice expressing nuclear protein Smad7 in keratinocytes has suggests antagonising TGF-1 and NFkB may be a useful approach in preventing oral mucositis caused by radiotherapy [127]. Similarly, mouse models of chemotherapy-induced mucositis have led to more sophisticated understanding of the immune contributors to mucositis pathogenesis, with blockade of CXCL4 and CXCR3 protecting intestinal tissue from chemotoxicity [128, 129].

2.2 Large animal models of mucositis

Although rodents are primarily used for the preclinical study of mucositis, large animal models offer a unique perspective and unparalleled investigation of specific mechanisms of mucositis. Large animals offer greater flexibility in the procedures able to be performed given their size, and are considered to have greater genetic overlap with humans [130]. This is particularly the case for the gastrointestinal metabolome, which is critical when assessing host-microbe/immune interactions [131]. In many models, large animals develop both oral and gastrointestinal manifestations of mucositis However, these models come at a cost, with housing/husbandry expenses and the cost of consumables significantly higher than that of rodents [110].

The use of ***dogs*** is scarce in mucositis research, with studies primarily opting for this species when investigating nausea and vomiting associated with mucosal injury [132, 133]. A more commonly used large animal is the pig, given its superior reputation in biomedical research based on higher genetic, anatomical and physiological homology with humans. ***Pigs*** are also able to receive the complex and clinically relevant supportive care interventions including antibiotics, anti-emetics and analgesics enhancing translational potential [134]. Models of bone marrow transplantation and chemotherapy-induced mucositis have both been developed using minipigs, with both doxorubicin [135] and 5-FU [136] resulting in clinically-comparable symptoms (e.g. diarrhoea, weight loss, sepsis, mortality).

Young pigs have also been used to study mucositis induced by non-myeloablative doxorubicin [135, 137], a common conditioning agent used in paediatric leukaemia, developing both clinically and histologically appropriate manifestations of mucositis. This offers a novel platform to study the unique mechanisms of paediatric mucositis and supportive care interventions aimed at childhood cancer. Importantly however, when very young piglets are used, not all features are apparent with no changes in proinflammatory cytokines, tight junction proteins and digestive enzymes, possibly reflecting the immature and more tolerant state of the infant intestine [110]. These features become

more evident when more intense myeloablative regimens are used (busulfan and cyclophosphamide), however this is accompanied by excessive toxicity and high mortality [134].

3.0 CHALLENGES IN ANIMAL MODELS OF MUCOSITIS

Although animal models have been instrumental in advancing our understanding of mucositis, their applicability to the clinic is limited for several reasons and translation must therefore be performed with caution. Firstly, one of the most troublesome symptom of mucositis, particularly affecting the oral cavity, is pain; an inherent difficult parameter measure in both humans and animals. In the case of animal models, pain is a universally challenging parameter to define and objectively quantify [110]. The facial grimace scale has been developed to assess pain-like behaviors in animals [138, 139], and is preferred over more laborious techniques based on stimulus-evoked responses (e.g. von Frey or Hargreaves tests). However, grimace criteria are inherently subjective and require extensive training. Furthermore, the impact of handling on the manifestation of these criteria remains unclear, and as such, studies employing these techniques should consider automated processes such as the Rodent Face Finder [140].

The functional assessment of mucositis also remains challenging in animals models. Although not strictly related to mucositis, rodents do not have an emetogenic reflex and thus the relationship between mucosal toxicity and nausea/vomiting relies on the indirect marker of pica (ingestion of bedding)[141]. Similarly, although rodents develop diarrhoea in many models of mucositis, assessing the severity of diarrhoea relies on the use of semi-quantitative grading systems which are subject to observer subjectivity and bias, and thus requires appropriate blinding. This is further confounded by the lack of universal accepted and validated biomarker, although plasma citrulline now holds promise for mucositis affecting the small intestine [142, 143].

Another issue relating to mucositis research using animals are sex and strain differences in metabolic enzyme profiles, particularly the CYP family. It is critical that any new model be carefully considered to ensure the species chosen displays the correct metabolic capacity for the drug of interest, and that variations in drug clearance (and thus toxicity) be adequately considered. A further disparity between humans and rodents is the composition of the microbiome, having the potential to impact gastrointestinal physiology, and disease phenotypes [144, 145]. Ley et al, in 2005, demonstrated that 85% of murine gut microbiota are not detected in humans [146]. However, this disparity is only observed at the genus level, with humans and rodents both comprising a majority of Bacteroidetes and Firmicutes phyla [145-148], which may still provide a broader gastrointestinal consistency in terms of function. Options to overcome these limitations include the use and colonization of gnotobiotic mice with a desired colony (such as human microbiota) for specific investigations into the host-microbe immune response associated with mucositis. The inherent variability that is seen in laboratory rodents (resulting from husbandry conditions) may, however, be of value in predicting overall patterns that would occur in humans (also displaying inherent individual variability) under similar disease or treatment conditions [149].

Rodent models to investigate the host-microbe interactions that occur, especially over a time course, are essential to elucidate some of the key immunological factors that drive the pathogenesis of mucositis, despite their limitations. A recent *in vitro* study by Vanlancker et al., (2017) showed that neither 5-FU nor SN-38 (active metabolite of irinotecan) have a direct effect on the microbiota itself, suggesting microbial disturbances are likely to be the result of the host response to these agents [150]. Investigation of these microbial disturbances with a host response

in rodent models is therefore still a useful tool in terms of translation, allowing key mechanistic pathways to be determined, and human equivalents investigated.

4.0 CONCLUSION

Animal research remains a critical aspect in supportive oncology, driving our continued mechanistic understanding of mucositis development and providing an invaluable platform for the assessment of new anti-mucositis agents. To date, animal models have been integral in establishing the 5-phase model of mucositis, and are now becoming increasingly important in defining the unique toxicities associated with newer anticancer agents. Given the highly heterogenous nature of supportive oncology, it is likely that traditional mucositis models will continue to be used to study new interventions, along with increasingly more sophisticated models based on genomic manipulation, careful modification of the microbiome and humanised strains. This will hopefully provide a new wave of data regarding mucositis development and a better understanding of the toxicities of next generation cancer therapies.

5.0 KEY POINTS

1. Animal models of mucositis represent clear homology with the clinical setting

2. Golden Syrian hamsters remain the gold-standard model for oral mucositis

3. Gastrointestinal mucositis is readily induced in a variety of animals, including rodents, pigs and dogs via intraperitoneal and intravenous administration

4. Cyclic and multimodal dosing strategies (including radiation, chemotherapy and targeted/immunotherapies) in tumour-bearing animals are encouraged to parallel the clinical scenario

5. Challenges remain in objectively assessing mucositis severity; plasma citrulline shows promise as a clinically translatable biomarker of small intestinal injury

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