SPRING 2012

IN FOCUS

ITR Newsletter

YOUR DEDICATED PARTNER IN DRUG SAFETY

ITR & PHARMACODYNAMIC EXPLORATION

ITR Laboratories Inc. is now developing various animal models and specific biomarkers for assisting Sponsors in drawing a broader and more accurate picture of the pharmacological activity and safety of their drug-candidates.

TWO EXAMPLES OF *IN VIVO* EFFICACY MODELS

I. Orthotopic model for bladder carcinoma (C57/BL6 mice)

Introduction

Epithelial carcinoma of bladder represents over 75% of all the types of bladder carcinomas described in humans. At ITR we have developed, in C57/BL6 mice, an allogeneic orthotopic model for urinary bladder epithelial carcinoma using the MB-49 bladder carcinoma cells of murine origin.
This model appears suitable for pharmacology studies assessing the antitumor activity of test articles against bladder epithelial carcinomas.

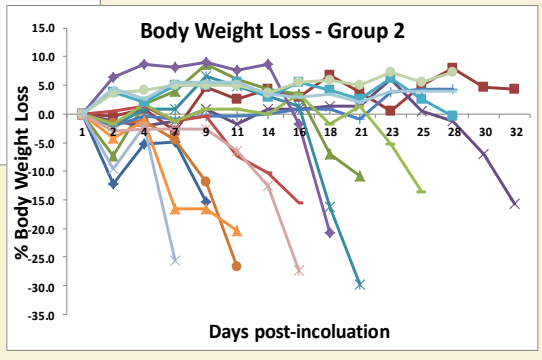
Methodology

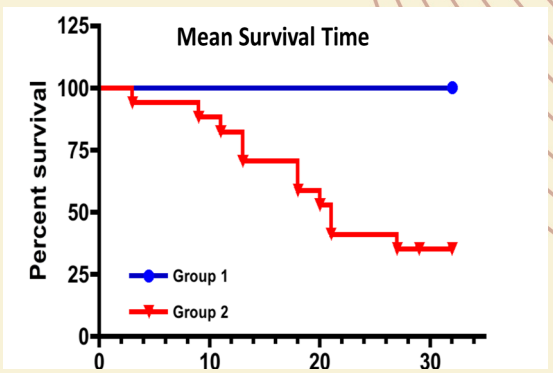
During the procedures, mice from all groups were anesthetised using isoflurane gas and they were catheterized through the urethra into the urinary bladder using an Angiocath 24G 0.75. To facilitate adhesion of cancer cells on the epithelium of bladder, the urinary bladder of female C57/BL6 mice was conditioned by instilling 1 mg/ml of poly-L-Lysine for 30 minutes. Immediately after bladder conditioning, mice, were instilled either with the culture medium (Group Sham/Control) (n=3) or with MB-49 cells ($2x10^6$ cells in 0.1 ml) (Group 2, n=17, in 3 replicates). The inoculate was retained in the bladder for approximately 90 min and then animals were allowed to recover from anaesthesia.

During the in-life portion of the study animals were weighed 3 times a week and they were checked for clinical signs and mortality at least once a day. Animals were euthanized either at the end of the study or when they lost at least 10% of the original body weight or/and showed important clinical signs (e.g. hematouria, decreased activity, severe dehydration …). Formation of solid carcinoma within the bladder was assessed macroscopically (gross examination) and microscopically. Upon completion of the gross pathology examination, the bladder was collected and fixed with neutral buffered 10% formalin to determine precise location of the mass. Tissues were prepared for microscopic examination by embedding in paraffin wax, sectioning and staining with haematoxylin and eosin.

Results

Body Weight and Survival: Animals from the sham/control group, remained healthy (stable body weight, absence of clinical signs) and they survived until termination of the study. In contrast, seven to ten days following tumour cell inoculation, the body weight of 11 out of 17 mice from Group 2 showed significant decrease (10-30%) always accompanied by severe clinical signs (hematuria, activity decrease, thin skin condition, dehydration). These animals were euthanized for humane reasons. The mean survival time was calculated according to the Kaplan Meyer method and for mice inoculated with tumor cells the mean time was evaluated to 21 days. No significant difference was observed between the 3 replicates.





Histopathology Microscopic examination of the bladders confirmed the presence of transitional cell carcinoma in 12 out 17 mice from Group 2 but none in mice from Group 1. Carcinoma cells were attached on bladder's epithelium that was disrupted allowing progressive infiltration of the malignant cells into the mucosa (stage I) and spreading into the muscle layer (stage II) Carcinomas appeared heterogeneous as they were composed by more than 1 type of cell population, differing in shape, size and intracellular morphology. Some of the carcinomas appeared vascularized with evidence of local hemorrhage. Carcinomas were often associated with minimal to mild oedema and/or inflammation of the submucosa/tunica muscularis.

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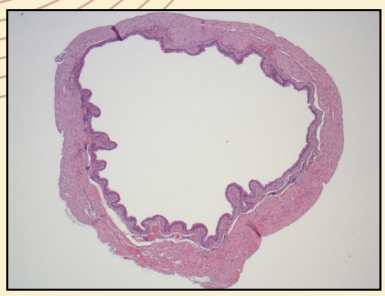
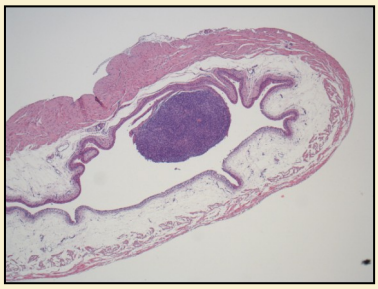
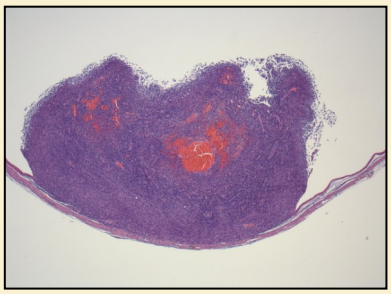


Group 1 : Control / sham (x40)



Group 2: MB-49 carcinoma (x40) (stage I)



Group 2: MB-49 carcinoma (x40) (stage II)

II. Wound healing

Introduction

Over the last few years there has been an increase in the number of requests for targeted wound healing models. ITR has worked on a number of models that include incision and excision wound types and the assessment of positive effects of a material on wound healing, scar formation and/or infection control. However, the converse may also be true where an assessment is made to ensure that there are no adverse effects on normal healing following administration of a drug. It is also necessary to assess the irritancy and toxicity potential of materials that are being used to aid wound healing. There is no doubt that animal models are important in allowing these positive and negative effects to be studied. However, it must be understood that such models are not perfect at predicting human reactions because it is not possible to make allowances for all the human variables such as age, nutritional state, physiology and other environmental issues in any one animal model. Often more than one model is required.

Methodologies

Animals used in such models may be selected because of their "biological responsiveness" or ease of husbandry. The mini pig has become a well used species because of the similarity of its skin to that of man. The rabbit is often selected as it has sensitive skin whereas that of rats is more robust.

Wound types are generally selected to mimic the clinical situation. As such, targeting a surgical incision wound in man would be similarly produced in the animal model including the use of sutures where applicable. An excision wound may be used for situations were, clinically, a large volume of skin tissue has been removed. The volume of tissue removed can be varied from simple stripping of the epidermis to full depth removal of epidermis and dermis. Wounds can be humanely produced either surgically, by using heat/cold or utilizing chemicals.

Wound assessment

Assessment of the progression of healing may be limited to the use of a modified Draize scoring system along with close inspection of the wound. Other tools that may be used for excision wounds may include Clinimetric measurements such as simple measurements, wound tracing, scaled photographs or planimetrics. Although not available at ITR, computerized systems are also available to allow a three dimensional image to be prepared. Each of these methodologies allows the progression to healing of the wound to be tracked through time. A mix of the above techniques can be used to enhance the data collected.

For planimetric measurements ITR has used transparent sheets with a $1mm^2$ grid pattern which is placed over the excision wound at appropriate time points and a careful trace of the wound edges are then drawn. These sheets may then be scanned and using the zoom feature the number of complete squares is added up. A scale area calculation can then be made allowing a comparison of test and control results.

Conclusion

Wound healing models are an important tool to allow the affect of novel materials on surgical or non surgical wounds to be studied whether the treatment is expected to decrease scarring following surgery or simply to speed up the normal process of reconstruction of damaged skin. Given the challenges associated with different human populations with respect to health status, nutrition and age new therapies are certainly required.

Assessment of wound healing is generally subjective as well as difficult to interpret. It does, however, give an indication during the in-life phase of the study relating to differences in the treated vs control groups.

ITR CANADA will be present at the SOT from March 11-15, 2012 in San Francisco, at booth 900.