Combination of Mitomycin C and Low Dose Suramin Increases Survival in an Optimized Orthotopic Model of Urinary Bladder Carcinoma in Mice

J. Tremblay, C. Glover, L. Kolodziejski, D. Lacroix, S. Thebaud, J. Godin-Ethier, A Nelson and V. Dimitriadou
ITR Laboratories Canada Inc., Montreal, QC, Canada

Abstract

Epithelial carcinoma of urinary bladder represents over 70% of all the types of bladder carcinomas described in humans. Therapies are often managed by retrograde routes and are not always followed by a cure. Here, we report efficacy data with Mitomycin C combined to tisk dose Suramin, known to potentiate the activity of Mitomycin C against chemoresistant prostate cancer in urinary bladder tumours in mice. Thirty-neutered female (C57BL/6J x DBA/2J) mice were castrated and MB-49 bladder cancer cells of nude origin (10^6 cells) were injected into the urinary bladder (Group 3) following conditioning with Polylysine. Group 1 and 2 animals were treated with tumor cells, Group 3 animals were treated by intravesical injection of a 5:1 mixture of Mitomycin C and Suramin sodium salt starting 3 to 7 days following tumor cell inoculation. The median survival time for the non-treated Group 5 mice was 20 days, and that of the treated Group 3, significantly prolonged to 36.5 days. Microscopic examination of hematoxylin and eosin stained sections confirmed the presence of primary tumors in both Group 2 and 5/10 Group 3 animals. As compared to Group 1 and 2, Group 3 animals showed significant basal membrane thickening occasionally associated with dystrophic urothelial hyperplasia and decreased vascularisation that are compatible with the mechanism of action of Suramin. In conclusion, this model and the route of administration appear suitable for assessing the antitumor activity of test agents against bladder epithelial carcinomas. In addition, the combination between Mitomycin C and Suramin appears to result in a significantly beneficial effect for this type of tumor.

Introduction

The objective was to optimize and characterize the orthotopic model of MB-49 bladder carcinoma in syngeneic mice. Pharmacologically, this model resembles that of human urothelial carcinomas. Here, we report efficacy data with Mitomycin C combined to tisk dose Suramin, known to potentiate the activity of Mitomycin C against chemoresistant prostate cancer in urinary bladder tumours in mice.

Material and Methods

Step 1: Optimization of the model in function of inoculon dose (0.5 x 10^6 MB-49 cells: Fig. 1)

Step 2: Characterization of the sensitivity of the model in the presence of Mitomycin C and Suramin sodium salts.

Results

Body Weight and Survival

Animals from the sham/control group (Group 1) remained healthy (stable body weight, absence of clinical signs) and survived until termination of the study (Day 40). The physical condition of non-treated mice of Group 2 started deteriorating within the first week following tumor cell inoculation, losing weight and showing severe clinical signs (hematuria, activity decrease, thin skin condition, dehydration). Consequently, 9 out of 10 mice from this group were gradually euthanized for humane reasons and the median survival time, calculated according to the Kaplan Meyer method, was 20 days. In contrast, at Day 20, 80% of the animals of Group 3 treated with Mitomycin C and Suramin were still alive and the median survival time was significantly prolonged to 36.5 days. At termination on Day 40, 30% of treated animals of Group 3 had survived. Note that, two mice from this group died accidentally during anesthesia.

Histopathology

Microscopic examination of the urinary bladders confirmed the presence of primary tumors in 5/10 animals of the Group 2 and in 5/10 animals of the Group 5 but none in Group 1 (Fig. 3). Histologically, unencapsulated, densely cellular, non-papillary carcinomas, focally effaced the urothelium (Fig. 3) and progressively infiltrated the subepithelial connective tissue and spread into the inner layers. Within some carcinomas areas of necrosis, hemorrhage, atrophy and inflammation (Fig. 4) were noted. Interestingly, bladder tumors of majority of the Group 3 animals showed significant basal membrane thickening (Fig. 5), occasionally associated with dystrophic urothelial hyperplasia and decreased vascularisation that are compatible with the mechanism of action of Suramin (inhibition of cell tumor-mediated degradation of the subepithelial basal membrane and extracellular matrix).

Immunohistochemistry

Urinary bladder carcinoma (Group 2) showing strong expression of VEGF protein in the tumor cells (Fig. 6).

Conclusion

In conclusion, this model and the route of administration used, appear suitable for assessing the antitumor activity of test agents against bladder epithelial carcinomas. In addition, the combination between Mitomycin C and Suramin appears to result in a significantly beneficial effect for this type of tumor.