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

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Abstract

Epithelial carcinoma of urinary bladder represents over 75% of all the types of bladder carcinomas described in humans. These tumors are often managed by transurethral surgical resection, followed by chemotherapy, mainly Mitomycin C. Here, we report efficacy data with Mitomycin C combined to low dose Suramin, known to potentiate the effect of various chemotherapy agents, in an optimized orthotopic model of urinary bladder carcinoma in mice. Thirty-anesthetized female mice (C57/BL6, n=10/group) were catheterized and MB-49 bladder cancer cells of murine origin (1x106 cells/ml) were instilled into the urinary bladder (Group 2 and 3) following conditioning with poly-L-lysine. Group 1 animals were not instilled with tumor cells. Group 3 animals were treated by intravesical instillation of a 0.1 ml mixture of Mitomycin C and Suramin sodium salt starting 5 to 7 days following tumor cell inoculation. The median survival time for the non-treated Group 2 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 9/10 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 atypical 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 items 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. A previous study showed that inoculation of these tumor cells into the bladder resulted in formation of solid epithelial tumors with a mean survival time of 21 days. In this study as a first step, we determined the optimal density of tumor cells adhering to the urothelium following orthotopic inoculation and in a second step we assessed the sensitivity of model towards a combination of Mitomycin C (MMC) and Suramin sodium salts.

Material and Methods

Step 1 Optimization of the model in function of inoculate density (0.5-2 x10^6 MB-49 cells; Fig. 1)
Step 2 Characterization of the sensitivity of the model in the presence of Mitomycin C and Suramin sodium salts

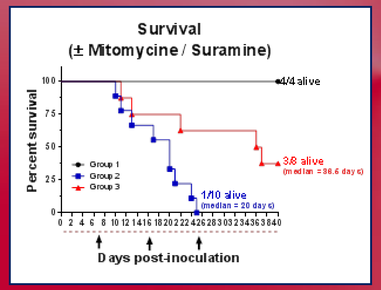
Group Number	Bladder Conditioning (50 µl)	Duration of Bladder Conditioning	Density of MB-49 cells	Mitomycin C / Suramin Treatment	Animals
1	Poly-L-Lysine	30 min	-	0	4
2	Poly-L-Lysine	30 min	1x10^6	0	10
3	Poly-L-Lysine	30 min	1x10^6	Mitomycin C* + Suramin sodium salt**	10

* Mitomycin C (5 mg/kg; 1 mg/ml); ** Suramin sodium salt (10 mg/kg; 2mg/ml)

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.



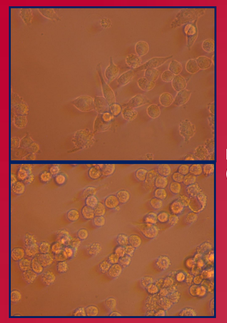


Fig. 1: MB-49 cells (10.0x10^6 cells/Ml)

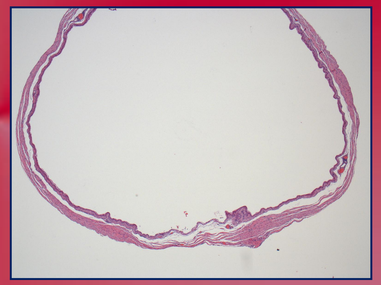


Fig. 2: Urinary bladder (Control)

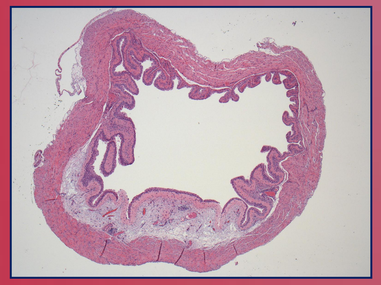


Fig. 5: Urinary bladder (Group 3)

Histopathology

Microscopic examination of the urinary bladders confirmed the presence of primary tumors in 9/10 animals of the Group 2 and in 5/10 animals of the Group 3 but none in Group 1 (Fig. 2). Histologically, unencapsulated, densely cellular, non-papillary, pleomorphic 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, edema and/or inflammation (Fig. 4) were noted. Interestingly, bladder tissues of majority of the Group 3 animals showed significant basal membrane thickening (Fig. 5), occasionally associated with atypical 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).

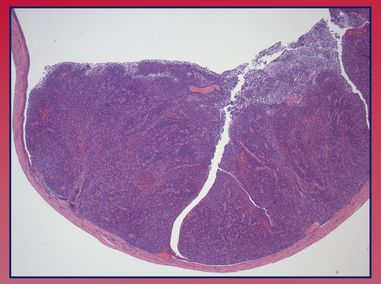


Fig. 3: Urinary bladder carcinoma (Group 2)

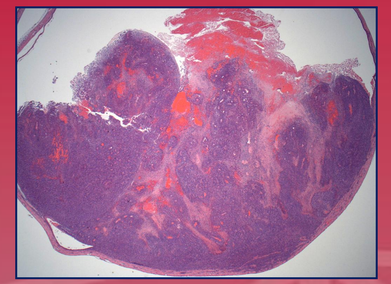


Fig. 4: Urinary bladder carcinoma (Group 2)

Immunohistochemistry

Urinary bladder carcinoma (Group 2 and 3) showing strong expression of VEGF protein in the tumor cells (Fig. 6).

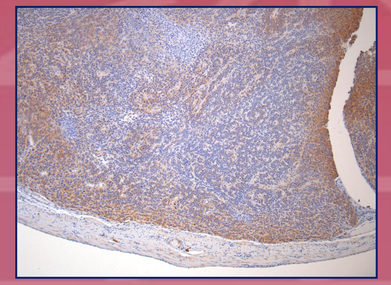


Fig. 6: Urinary bladder carcinoma (Group 2)

Conclusion

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