

**Eur Urol. 2004 Nov;  
46(5):670-674**

**THE EFFECT OF  
HYPERTHERMIA ON  
MITOMYCIN-C INDUCED  
CYTOTOXICITY IN FOUR  
HUMAN BLADDER CANCER  
CELL LINES**

ANTOINE G. VAN DER HEIJDEN<sup>A</sup>,  
CORNELIUS F.J. JANSEN<sup>A</sup>, GERALD  
VERHAEGH<sup>A</sup>,  
MICHAEL A. O'DONNELL<sup>B</sup>, JACK A.  
SCHALKEN<sup>A</sup>, J. ALFRED WITJES<sup>A,\*</sup>

<sup>a</sup>Department of Urology, University  
Medical Centre Nijmegen, Nijmegen,  
The Netherlands

<sup>b</sup>Department of Urology, University of  
Iowa, College of Medicine, Iowa City,  
IA, USA

Introduction: Hyperthermia and mitomycin-C (MMC) have given very encouraging results in several clinical studies for the treatment of superficial transitional cell carcinoma of the bladder. However, a synergistic effect of hyperthermia and MMC on the decrease of cell proliferation has never been demonstrated accurately in vitro. We investigated the effect of MMC versus MMC combined with hyperthermia on the cytotoxicity in four human bladder cancer cell lines.

Material and Methods: The RT112, RT4, 253J and T24 human bladder cancer cell lines were seeded in 96-well microtiter plates at  $2.0 \times 10^4$  cells per well and were left to attach for 24 hours. The cells were then treated for 60 minutes with MMC concentrations ranging from 0 to 400  $\mu\text{g}/\text{ml}$  at a temperature of  $37^\circ\text{C}$  or  $43^\circ\text{C}$ . After treatment cells were rinsed three times with culture medium and left for 24 hours in the incubator. Dimethyl thiazolyl tetrazolium (MTT) solution was added and after 4 hours of incubation the MTT containing media was aspirated from all wells and 100  $\mu\text{l}$  of dimethyl sulfoxide was added to each well. A spectrum analysis was performed at 595 nm light wavelength.

Results: A decrease of cell proliferation after treatment with increasing concentrations MMC was demonstrated. Hyperthermia has a synergistic effect on the decrease of cell proliferation by different concentrations MMC. In the cells treated without MMC no significant difference in the extent of cell killing at  $37^\circ\text{C}$  and  $43^\circ\text{C}$  was observed.

Furthermore, no difference was observed between cells with a p53 protein mutation (RT112 and T24) or without a p53 protein mutation (253J and RT4).

Conclusion: A clear synergistic effect of MMC and hyperthermia has been demonstrated in four human bladder cancer cell lines.