567 - NEW IMMUNOTHERAPY FOR NON MUSCLE INVASIVE BLADDER CANCER (NMIBC): EFFECTS OF IMMUNOMODULATOR P-MAPA

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INTRODUCTION AND OBJECTIVES

Immunotherapy represents one of the approaches for the treatment of cancer. Compounds which are able to act as toll-like receptors (TLRs) agonists may represent promising candidates to be developed as medicines against cancer. Bacillus Calmette-Guerin (BCG) is used as a therapeutic tool for some cancer types, including the urothelial cancer. However, BCG use is limited in Non-muscle invasive bladder cancer (NMIBC) by treatment failure, adverse effects and intolerance that occurs in over two-thirds of all patients and consist largely of irritative voiding symptoms including haematuria, dysuria and urgency. P-MAPA is an acronym for Protein Aggregate Magnesium-Ammonium Phospholinoleate-Palmitoleate Anhydride, a proteinaceous aggregate of ammonium and magnesium phospholinoleate-palmitoleate anhydride, with immunomodulatory properties produced by fermentation from *Aspergillus oryzae*, under development by Farmabrasilis, a non-profit research network. Also, P-MAPA shows significant *in vivo* antitumor effects. Thus, the aims of the hereby study were to characterize effects of the P-MAPA on TLRs *in vitro* and *in vivo*, as well as to verify its potential as adjuvant therapy for NMIBC. For its purpose, the efficacy of P-MAPA was compared versus BCG in the NMIBC animal model.

MATERIALS AND METHODS

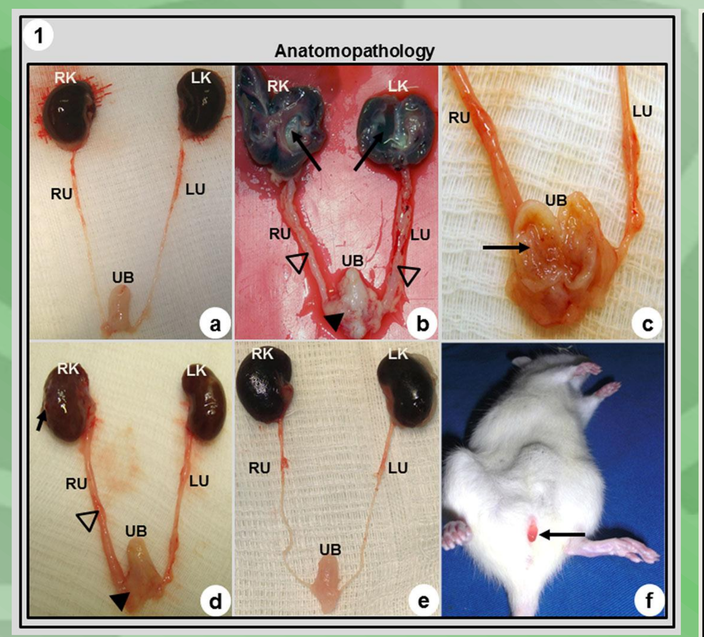
Thirty female Fisher 344, 7 week old, rats were anesthetized and received 1.5 mg/kg dose of n-methyl-n-nitrosourea (MNU), intravesically every other week for 7 weeks. After MNU treatment, the 30 rats were divided into 3 groups: The MNU group received 0.30 ml dose of 0.9% physiological saline for 8 weeks; The BCG group received 10^6 CFU (40 mg) dose of BCG for 8 weeks; The P-MAPA group received 5 mg/kg dose of P-MAPA for 8 weeks. After 15 weeks, all bladders were collected for immunological and Western Blotting analysis for TLR 2, TLR 4, p53, Ki-67 (cellular proliferation) and apoptosis detection. The activity of P-MAPA on TLRs was assayed in vitro through NF-κB activation in HEK293 cells expressing a given TLR.

RESULTS

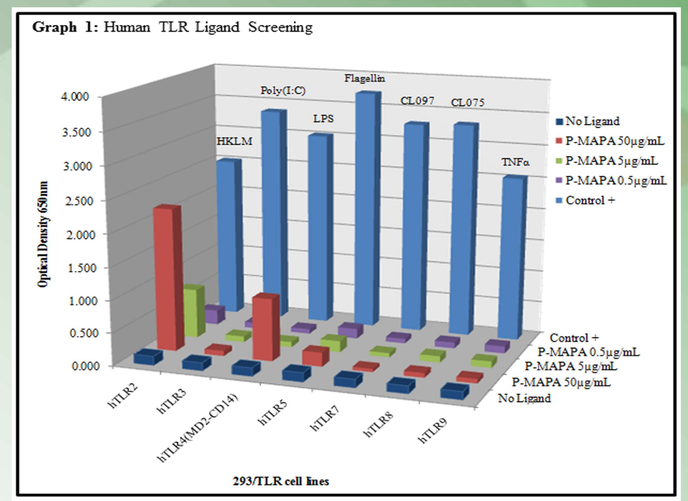
Table 1: Toxicological and Biochemical Biomarkers for Control, MNU, BCG and P-MAPA groups.

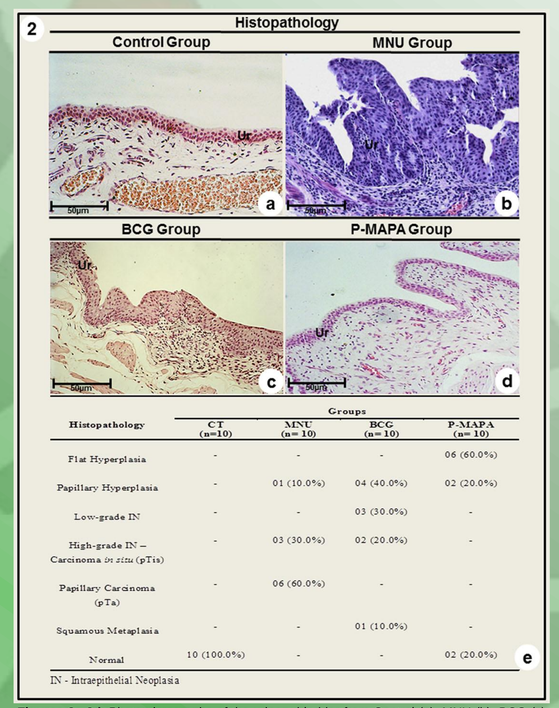
Parameters	Control (n=10)	MNU (n=10)	BCG (n=10)	P-MAPA (n=10)
ALT (U/L)	49.23±1.61	46.03±0.63	46.30±3.60	51.61±7.07
AST (U/L)	189.72±28.74	133.05±2.29	201.61±23.84	208.38±31.34
Alkaline Phosfatase (U/L)	42.97±0.87[a]	128.11±8.21	38.88±3.51[a]	40.56±3.93[a]
Urea (mg/dL)	55.45±3.90[a]	163.09±14.14	60.52±5.90[a]	57.32±7.16[a]
Creatinine (mg/dL)	1.18±0.26[a]	10.33±0.10	1.22±0.25[a]	1.94±1.24[a]

Data expressed as mean ± SEM, $p < 0.05$; Letter *a*: significantly different from MNU group. MNU= *n-methyl-n-nitrosourea*; BCG= Bacillus Calmette-Guerin.



Figures 1a – 1e: Urinary tract of the animals from CT (a), MNU (b, c), BCG (d) and P-MAPA (e) groups. In (a) and (e) the urinary tract showed normal features. (b) Lesion widespread in different points of the urinary tract: hydronephrosis and papillary lesions **(arrows)** in the kidneys; dilation and thickening of the ureters (**open arrowheads**); thickening and papillary lesions (**solid arrowhead**) in the urinary bladder. (c) Intravesical papillary lesions **(arrow)**; dilation and thickening of the ureters. (d) Cystic lesions (**arrow**) in the kidney; dilation and thickening of the ureter (**open arrowhead**); thickening and papillary lesions (**solid arrowhead**) in the urinary bladder. a – e: **LK –** left kidney, **LU –** left ureter, **RK –** right kidney, **RU –** right ureter, **UB –** urinary bladder.
Figure 1f: The animals from the MNU and BCG group showed macroscopic haematuria **(arrow)**. P-MAPA group showed no macroscopic haematuria.





Figures 2a-2d: Photomicrographs of the urinary bladder from Control (a), MNU (b), BCG (c) and P-MAPA (d) groups. (a) Normal urothelium; (b) Papillary carcinoma; (c) Carcinoma *in situ*; (d) Flat hyperplasia. a – d: **Ur –** urothelium.

Figure 2e: Percentage of histopathological changes of the urinary bladder of rats from CT, MNU, BCG and P-MAPA groups.

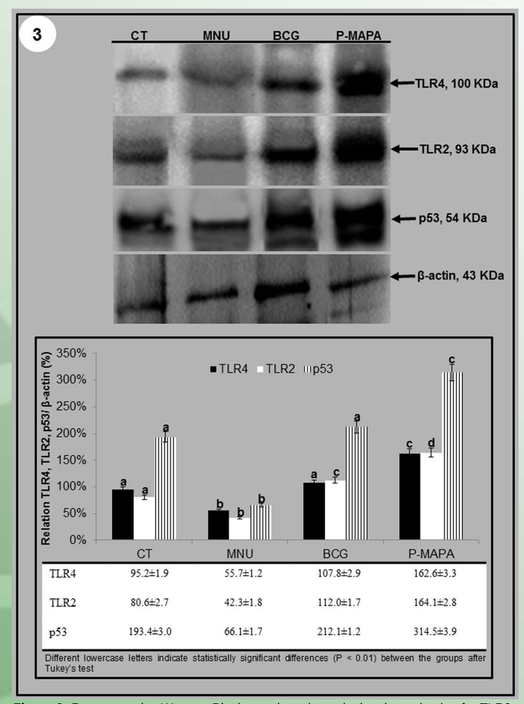


Figure 3: Representative Western Blotting and semiquantitative determination for TLR2, TLR4 and p53 proteins of the urinary bladder extracts in the four experimental groups. The protein levels were identified in the blots. β-Actin was used as the endogenous control. Data were expressed as the mean ± standard deviation (n=5).

CONCLUSIONS

In conclusion, P-MAPA acted as TLR ligand *in vitro* and improved the immunological status *in vivo*, including TLR2 and TLR4 protein levels. P-MAPA immunotherapy was more effective in restoring p53 and TLRs reactivities and showed significant antitumor activity than other immunotherapies. The activation of TLRs and p53 may provide a hypothetical mechanism for the therapeutic effects found in NMIBC. Taking together, the data warrant the further assessment of P-MAPA as a potential candidate for treatment of NMIBC.