

The effects of taxol (paclitaxel) on chemiluminescence of neutrophils, macrophages and J.774.2 cell line*

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Key words: taxol, chemiluminescence, neutrophils, macrophages

Taxol (paclitaxel) is a chemotherapeutic diterpene with promising anticancer activity that blocks cell division by preventing microtubule depolymerization. Furthermore, recent studies have shown that taxol has other intracellular effects that may contribute to its effect, particularly in macrophages. The signal transduction mechanisms by which taxol stimulates macrophages to anticancer activity are not clear.

The purpose of this study was to determine the effect of taxol on chemiluminescence (an indicator of the production of free radicals) of neutrophils, macrophages and murine macrophage J.774.2 cells. The chemiluminescence was measured in the presence of taxol and/or phorbol myristate acetate (PMA) as a stimulant. Taxol stimulated chemiluminescence (without PMA) of neutrophils and macrophages but not of J.774.2 cells, and modulated chemiluminescence of the cells stimulated with PMA.

Taxol (paclitaxel), originally isolated from the bark of *Taxus brevifolia* is an antineoplastic agent that stabilizes microtubules by preventing microtubule depolymerization [1]. Recent studies have shown that also other intracellular effects of taxol may contribute to its efficacy. Studies with human macrophages have suggested that taxol does not induce but only enhances IL-1 β and TNF- α production in

conjunction with another stimulus [2, 3]. Moreover, taxol stimulates protein-tyrosine phosphorylation and the corresponding gene expression [4, 5]. This stimulation of phosphorylation may suggest the ability of taxol to activate the "respiratory burst" in neutrophils and macrophages.

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*Presented as a poster at the 6th International Symposium on Molecular Aspects of Chemotherapy, July, 1997, Gdańsk, Poland.

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Abbreviations: PMA, phorbol myristate acetate; CL, chemiluminescence; FCS, fetal calf serum; DMSO, dimethylsulphoxide; IL-1 β , interleukin 1 β ; TNF- α , tumor necrosis factor α ; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline solution, pH 7.4.

of human neutrophils, peritoneal murine macrophages and murine macrophages J.774.2.

MATERIALS AND METHODS

Chemicals. Hanks' balanced salt solution (HBSS) without phenol red, phosphate-buffered saline solution, pH 7.4 (PBS), RPMI 1640 medium without phenol red, heat inactivated fetal calf serum (FCS) low in endotoxin, and penicillin – streptomycin (10000 IU/ml–10000 µg/ml) were purchased from GIBCO BRL Life Technologies Ltd. (Paisley, U.K.). Phorbol myristate acetate (PMA), dimethylsulphoxide (DMSO) and Histopaque (density 1.119 and 1.077g/ml) were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was purchased from LKB (Turku, Finland). The tested taxol was purchased from Calbiochem (La Jolla, CA, U.S.A.).

Collection of murine peritoneal macrophages. Macrophages were obtained from BALB/c mice given an intraperitoneal injection of 1 ml sterile thioglycolate broth 4 days prior to harvest. Mice were killed by cervical dislocation, and cells were collected by washing the peritoneum with PBS (5 ml). The populations of macrophages were enriched by adherence to polystyrene tubes 11 mm × 55 mm (Nunc, Roskilde, Denmark) calculated to be 10^6 macrophages per tube. Non-adherent cells were removed after 2 h of incubation in RPMI 1640 without phenol red, supplemented with 10% FCS, penicillin (100 U/ml) and streptomycin (100 µg/ml) (culture medium) at 37°C in a humidified atmosphere of 5% CO₂ in air. Viability of the cells was determined by trypan blue exclusion test.

Neutrophils. Samples of blood were obtained from a healthy volunteer.

Neutrophils were harvested by centrifugation on Histopaque gradient, washed and re-suspended in HBSS.

J.774.2 cells. Murine J.774.2 macrophages were a generous gift from Professor Sir John Vane (William Harvey Research Institute, London) via Professor Janusz Marcinkiewicz (Department of Immunology, Jagiellonian University Medical College, Cracow). J.774.2 cells were grown in RPMI 1640 medium supplemented with 10% FCS, penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C and under 5% CO₂ atmosphere.

Chemiluminescence. Chemiluminescence was measured as described previously [6] using a special low noise-count-rate photomultiplier 9514s (EMI, Middlesex, U.K.). The sample volume was usually 1 ml. The reactions were initiated by dispensing aliquots of solutions of luminol (final concentration – 100 µM for macrophages and J.774.2 cells, 5.6 µM for neutrophils) to cells (10^6) in HBSS in test tubes. After 5 min taxol (final concentration 100 µM or 10 µM) and/or PMA (final concentration – 0.8 µM for macrophages and J.774.2, 0.16 µM for neutrophils) were added. The light emitted was then recorded continuously for 15 min.

RESULTS AND DISCUSSION

Preliminary experiments showed that taxol (0.1 mM, 2 h) had no significant effect on the viability of the cells studied. Neutrophils and murine macrophages in the presence of luminol and taxol (without PMA) generated chemiluminescence (Fig. 1 and 2). This effect was dependent on concentration of taxol and did not appear in J.774.2 cells (not shown). The J.774 cells, originally derived from a BALB/c mouse, have many characteristics of normal macrophages [7] and exhibit a chemiluminescent oxidative burst primed by preincubation with a variety of biological response modifiers in a manner similar to the burst observed in macrophages stimulated *in vivo* [8, 9]. On addition of PMA to the medium containing luminol, the chemiluminescence of J.774.2 cells was generated (302500 ± 4450 counts per 15

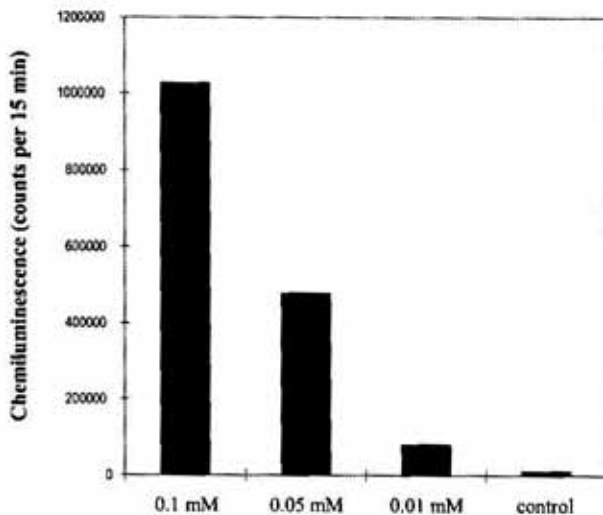


Figure 1. Chemiluminescence of neutrophils (without PMA) stimulated with taxol at different concentrations.

Human neutrophils – 10^6 cells, luminol – final concentration $5.6 \mu\text{M}$, final volume 1 ml. Control presents light emission from the cells without PMA and taxol.

min). Also with neutrophils and macrophages addition of PMA in the presence of luminol generated chemiluminescence (1522800 ± 612000 counts per 15 min and 326000 ± 77700 counts per 15 min, respectively). Chemiluminescence of neutrophils, macro-

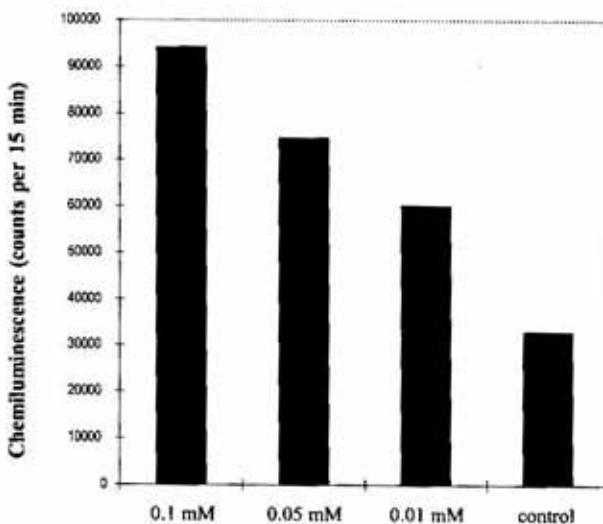


Figure 2. Chemiluminescence of murine peritoneal macrophages (without PMA) stimulated with taxol at different concentration.

Macrophages – 10^6 cells, luminol – final concentration $100 \mu\text{M}$, final volume 1 ml. Control presents light emission from the cells without PMA and taxol.

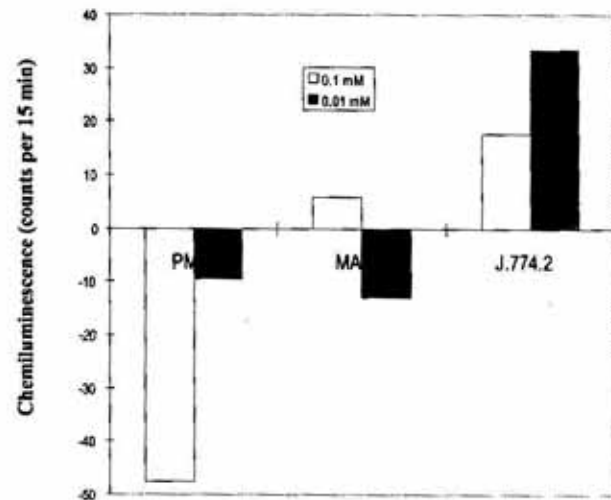


Figure 3. Effect of taxol (0.01 mM or 0.1 mM) on chemiluminescence generated by neutrophils (PMN), peritoneal macrophages (MAC), or J.774.2 cells in the presence of phorbol myristate acetate.

phages and J.774.2 cells (Fig. 3) was affected with taxol at both concentrations (0.1 mM and 0.01 mM) in the presence of PMA. Taxol increased chemiluminescence of neutrophils, modulated CL of macrophages and decreased PMA-dependent CL of J.774.2 cells. These results suggest that the capacity of taxol to increase chemiluminescence of neutrophils and macrophages is expressed *via* the signal transduction pathway of protein kinase C as proposed by Jun *et al.* [10]. In the case of J.774.2 cells this mechanism is not clear and requires further studies.

The authors wish to thank B. Kuryło for valuable technical assistance.

REFERENCES

1. Rowinsky, E.K., Onetto, N., Canetta, R.M. & Arbuck, S.G. (1992) Taxol: The first of the taxanes, an important new class of antitumor agents. *Semin.-Oncol.* **19**, 646–662.
2. Allen, J.N., Moore, S.A. & Wewers, M.D. (1993) Taxol enhances but does not induce interleukin- 1β and tumor necrosis factor- α production. *J. Lab. Clin. Med.* **122**, 374–381.

3. Manthey, C.L., Perera, Pin-Yu, Salkowski, C.A. & Vogel, S.N. (1994) Taxol provides a second signal for murine macrophage tumoricidal activity. *J. Immunol.* **152**, 825-831.
4. Ding, A., Sanchez, E. & Nathan, C.F. (1993) Taxol shares the ability of bacterial lipopolysaccharide to induce tyrosine phosphorylation of microtubule-associated protein kinase. *J. Immunol.* **151**, 5596-5602.
5. Henricson, B.E., Carboni, J.M., Burkhardt, A.L. & Vogel, S.N. (1995) LPS and taxol activate *Lyn* kinase autophosphorylation in *Lpsⁿ*, but not in *Lps^d*, macrophages. *Mol. Med.* **1**, 428-435.
6. Król, W., Czuba, Z., Scheller, S., Gabryś, J., Grabiec, S. & Shani, J. (1990) Antioxidant property of ethanolic extract of propolis (EEP) as evaluated by inhibiting the chemiluminescence oxidation of luminol. *Biochem. Int.* **21**, 593-597.
7. Marcinkiewicz, J. & Grabowska, A. (1996) Influence of macrophage priming on IFN- γ driven production of inflammatory molecules by J.774.2 cells *in vitro*. The memory of macrophages. *Central-Eur. J. Immunol.* **21**, 239-245.
8. Tosk, J., Lau, B.H.S., Lui, P., Myers, R.C. & Torrey, R.R. (1989) Chemiluminescence in a macrophage cell line modulated by biological response modifiers. *J. Leukocyte Biol.* **46**, 103-108.
9. Rittenhouse, J.R., Lui, P.D. & Lau, B.H.S. (1991) Chinese medicinal herbs reverse macrophage suppression induced by urological tumors. *J. Urology* **146**, 486-490.
10. Jun, C.D., Choi, B.M., Kim, H.M. & Chung, H.T. (1995) Involvement of protein kinase C during taxol-induced activation of murine peritoneal macrophages. *J. Immunol.* **154**, 6541-6547.