

Effect of Turmeric, Turmerin and Curcumin on Ca²⁺, Na/K⁺ ATPases in Concanavalin A-Stimulated Human Blood Mononuclear Cells

Hari H. P. Cohly¹, Maheshwara-Rajeswara Rao², Vijaya K. Kanji², Babu Patlolla³, Anelle Taylor¹, Melanie T. Wilson¹, Michael F. Angel¹, and Suman K. Das¹

¹Departments of Surgery and ²Medicine, University of Mississippi Medical Center, Jackson, MS, 39217-4505, E-mail: hcohly@surgery.umsmed.edu

³Department of Biology, Alcorn State University, Lorman, MS, 39096

Received: 7 June 2002 / Accepted: 30 October 2002 / Published: 31 January 2003

Abstract: Ion transport enzymes may play an important role in T cell activation. This study investigates the role of turmeric and its individual components, turmerin-and curcumin-on Ca²⁺ and Na/K⁺ adenosine triphosphatases (ATPase) in the course of T cell activation. Concanavalin A (Con A) stimulated human blood mononuclear T cell proliferation paradigm was investigated for 3, 5 and 7 day periods with different concentrations of turmeric, curcumin and turmerin. Con A-stimulated cells treated with turmeric (250, 50, 5 µg/ml) for 3 and 5 days inhibited ATPase levels when compared to base levels obtained by cells in media alone. At day 7, there was a 3-fold increase for Ca²⁺ATPase levels and a 2-fold increase for Na/K⁺ATPase. Curcumin (250, 50, 5 µg/ml) showed the same pattern for ATPase activity as turmeric at 3 and 5 days with a 2-fold increase at day 7. Turmerin (2500, 1250, 250, 25 ng/ml) for Na/K⁺ ATPase activity showed an increase at day 3, a decrease on day 5, and a 2-fold increase on day 7. Ca²⁺ ATPase activity in the presence of turmerin showed an increase in ATPase levels at day 3 (except at 2500ng/ml where it decreased) and a decrease in day 5 (except at 25 ng/ml where it increased). Turmeric and curcumin generally inhibited Ca²⁺ATPase and Na/K⁺ATPases in early (day 3) and intermediate (day 5) stages of mitogen stimulation. However, the effect after 7 days incubation for turmeric, curcumin and turmerin showed a marked increase up to three fold.

Keywords: Turmeric, turmerin, curcumin, Ca²⁺ATPase, Na/K⁺ATPase.

Introduction

The sodium (Na^+) and potassium (K^+) adenosine triphosphatase (ATPase) is an integral cell membrane protein. Three types of Ca^{2+} ATPases have been described in human tissues, one in the plasma membrane, and two in the sarcoplasmic-reticulum [1, 2]. Na/K^+ ATPase functions as an ion pump and is essential for the maintenance of cellular homeostasis. It is required for the establishment of ionic and electrochemical transmembrane gradient [3] and provides energy for uptake of nutrients [4] and neurotransmitter function [5] through co-transport and counter-transport systems. As a result of such activities, Na/K^+ ATPase is thought to contribute to the overall level of cellular activation. Similarly, Ca^{2+} transporting ATPases (Ca^{2+} pumps) translocate Ca^{2+} ions against a steep concentration gradient through cellular membranes. Ca^{2+} fluctuation between the cytoplasm, intracellular Ca^{2+} -storage organelles and the plasma membrane play a key role in cellular homeostasis and signal transduction. Upon stimulation of a wide variety of receptors, Ca^{2+} enters the cytoplasm from intracellular pools as well as from the extracellular compartment through Ca^{2+} channels [3-5]. In a reversible activation event, Ca^{2+} is re-sequestered in extracellular storage pools and eliminated from the cell through the plasma membrane [6]. Ca^{2+} transport ATPases thus plays an important role in the control of cell activation.

In its native state, one molecule of concanavalin A (Con A) binds two metal atoms: one Ca^{2+} and one ion of a transitional metal, usually Mn^{2+} [7]. Con A is mitogenic to T cells and has been used to activate T lymphocytes [8-12]. Con A interacts with antigen presenting cells and T-helper cells thereby promoting production of interleukin 2 (IL-2) which supports the growth and function of other immune effector cells, including macrophages [13]. Thus, Con A can be used as a model reagent to understand the interaction of turmeric and its components with ion transporters coupled to ATPases in activated immune cells.

Turmeric is a yellow powder obtained from the plant *Curcuma longa*. Turmeric has two active derivatives: a water-soluble moiety termed turmerin and water insoluble moiety termed curcumin. Turmeric and its products have demonstrated antioxidant activity [14-18]. Purification and extraction of plant products may generate products whose biological activity may be altered or diminished.

Turmeric and curcumin have been shown to inhibit Na/K^+ ATPases [19] and curcumin has also been shown to inhibit peripheral blood mononuclear cell activation by a mitogen [20].

We hypothesized that turmeric and its components would alter ion transport enzyme activity in peripheral blood lymphocytes after interaction with Con A. To test this hypothesis different concentrations of turmeric, turmerin and curcumin were used to alter Ca^{2+} ATPase and Na/K^+ ATPases.

Materials and Methods

Chemicals

Turmeric was obtained from Raja Foods (Lincolnwood, IL). Curcumin was obtained from Aldrich Chemical (St Louis, MO). Turmerin was prepared by dissolving 1 gram of turmeric in 100ml of boiling

water for 30 min, centrifuging at 1200 x g for 10 min and passing the supernatant through 0.22 μm filter whose protein concentration corresponded to 80 $\mu\text{g}/\text{ml}$. Chemicals, media and concanavalin A (Con A) were obtained from Sigma (St. Louis, MO) and Gibco (Grand Island, NY).

Cells

Peripheral blood from healthy individuals was separated by ficoll-hypaque centrifugation and mononuclear cells were harvested and washed 2x in Hanks balanced salt solution and finally re-suspended in RPMI 1640 containing 5% fetal bovine serum supplemented with antibiotics (100 units/ml penicillin, 100 mg/ml streptomycin). Cells (1×10^6 cells/ml) were re-suspended and plated into-96 well plates in aliquots of 100 μl . Ten μl of media were added to the first six wells media control and the 10 μl of Con A were added to the remaining 6 wells in the row. The final concentration of Con A used was 10 $\mu\text{g}/\text{ml}$. Different concentrations of turmeric (100 μl), turmerin (100 μl) or curcumin (100 μl) mixed concurrently with 10 μl of Con A were added to the next row in triplicate in 3 plates and incubated for different lengths of time for 3, 5 and 7 days at 37 $^{\circ}\text{C}$ and 5% CO_2 . The cells were then sonicated, re-suspended and placed in a glass-test tube for further analysis.

Determination of Na/K⁺ ATPases Activity

The ATPase activity of the mononuclear cells was measured using the method of Fritz and Hamrick [21] as modified by Desiah and Ho [22] and end point phosphate analysis by the method of Fiske and Subha Rao [23]. A 1-ml reaction mixture was used and contained in final concentrations 5 mM ATP, 100 mM NaCl, 20 mM KCl, 5mM MgCl_2 , 135 mM imidazole/HCL Buffer (pH 7.5), and 30 to 50 μg of enzyme protein ($\sim 40\mu\text{l}$). The reaction rate was proportional to the amount of protein present. The total ATPase activity was measured with Na/K^+ and Mg^{2+} present in the reaction mixtures. The Mg^{2+} ATPase was measured in the presence of 1mM Ouabain, a specific inhibitor of Na/K^+ ATPase. Thus, delineation of the (Na/K^+)-activated component of ATPase was obtained by the difference between total ATPase and Mg^{2+} ATPase. The incubation was carried out at 37 $^{\circ}\text{C}$ for 20 min, then stopped with a 5% trichloroacetic acid. Thirty minutes after addition of 1ml reagent containing 1% (w/v) ammonium heptamolybdate, 40 mg/ml FeSO_4 , and 1.15N H_2SO_4 to each tube, the released phosphate was measured at 690 nm. The enzyme activity was expressed as $\mu\text{MPi}/\text{mg}$ protein/h.

Determination of Ca²⁺ ATPase Activity

The ATPase activity of mononuclear cells was determined by measuring the inorganic phosphate liberated from the hydrolysis of ATP. The reaction medium contained 135 mM imidazole-HCL buffer pH 7.5, 0.5 mM CaCl_2 , 5mM MgCl_2 , 4 mM ATP, 25-40 μg of protein as an enzyme source. The reaction was carried out at 37 $^{\circ}\text{C}$ for 20 min then stopped by addition of 5% trichloroacetic acid. Thirty minutes after addition of 1ml reagent containing 1% (w/v) ammonium heptamolybdate, 40 mg/ml FeSO_4 , and 1.15N H_2SO_4 to each tube the released phosphate was measured using a spectrophotometer equipped with

a 690 nm filter. The total ATPase activity was measured with Ca^{2+} and Mg^{2+} present in the reaction mixtures, while Mg^{2+} -ATPase was measured in the presence of 0.5mM EGTA. Ca^{2+} activity was obtained by subtracting Mg^{2+} activity from total ATPase activity. The enzyme activity was expressed as $\mu\text{MPi}/\text{mg protein}/\text{h}$.

Protein Assay

Protein was assayed using the Bradford assay (Biorad, Richmond CA) with immunoglobulin gamma (IgG) as the standard.

Statistical Analysis

The data was expressed as means \pm SD of 3 samples assayed in duplicate. Statistical significance was calculated using student's t-test and values of $p \leq 0.05$ were considered statistically significant in comparing first wells values to control values (Con A-treated samples).

Results

Ca^{2+} ATPases

Figure 1A shows that an overall decrease in the ATPase levels in the presence of turmeric when compared with base levels (Con A treated samples). At day 7, there is more than a 3-fold increase in ATPase levels ($p \leq 0.01$).

Figure 2A shows ATPase activity observed in the presence of curcumin. ATPase levels were overall decreased at days 3 and 5. A 2-fold increase in ATPase activity is observed on day 7 ($p \leq 0.05$).

Figure 3A shows there is an increase in ATPase levels on day 3 in the presence of tumerin except at 2500 ng/ml where it decreased, and little changes at day 5 (except at 25ng/ml where a moderate increase is noted). There is approximately a 2-fold increase in ATPase activity noted on day 7 (except at 25 ng/ml) ($p \leq 0.05$).

Na/K^{+} ATPases

Figure 1B shows that at days 3 and 5 there is a general decrease in the ATPase levels across the different concentrations of turmeric when compared with Con A alone. At day 7 there is a 2-fold increase in ATPases levels ($p \leq 0.05$), except for the highest concentration of tumeric where a decrease in the ATPase levels is noted.

Figure 2B shows only marginal inhibition in ATPase levels at days 3 and 5 compared to controls in the presence of curcumin. There is more than a 2-fold increase in ATPase activity noted on day 7 ($p \leq 0.05$).

Figure 3B shows for turmerin an increase from control levels in ATPase activity on day 3 except at 1250 ng/ml where it decreased. By contrast, a decrease from base levels of ATPase activity is noted

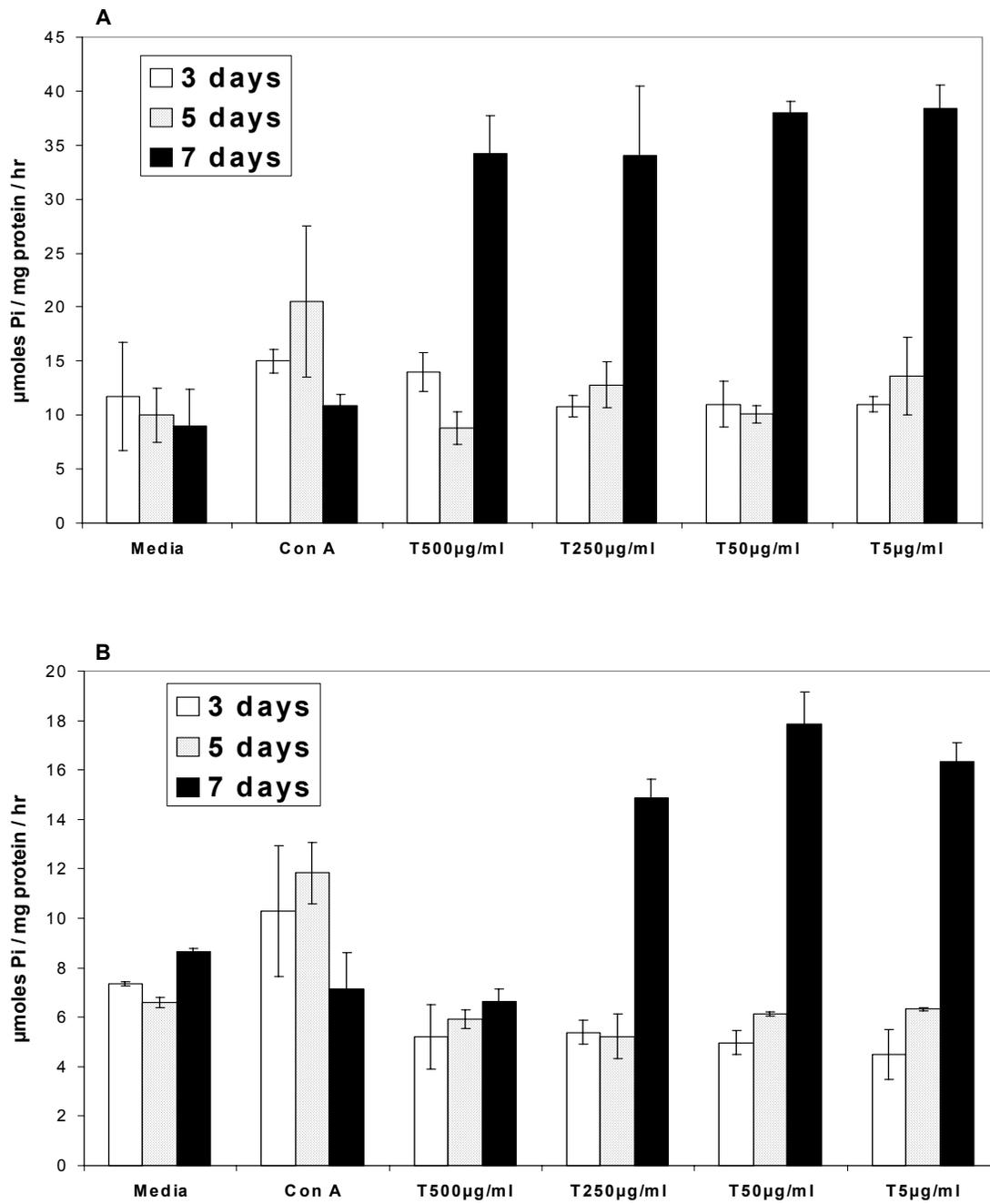


Figure 1. ATPases activity of human mononuclear cells stimulated with Con A after 3, 5 and 7 days of incubation with turmeric (T). The enzyme activity of cells in media alone is compared with the activity of cells in Con A alone and in Con A + T (500, 250, 50 and 5 µg/ml). (A) represents the Ca²⁺ATPase activity. (B) represents the Na/K⁺ATPase activity.

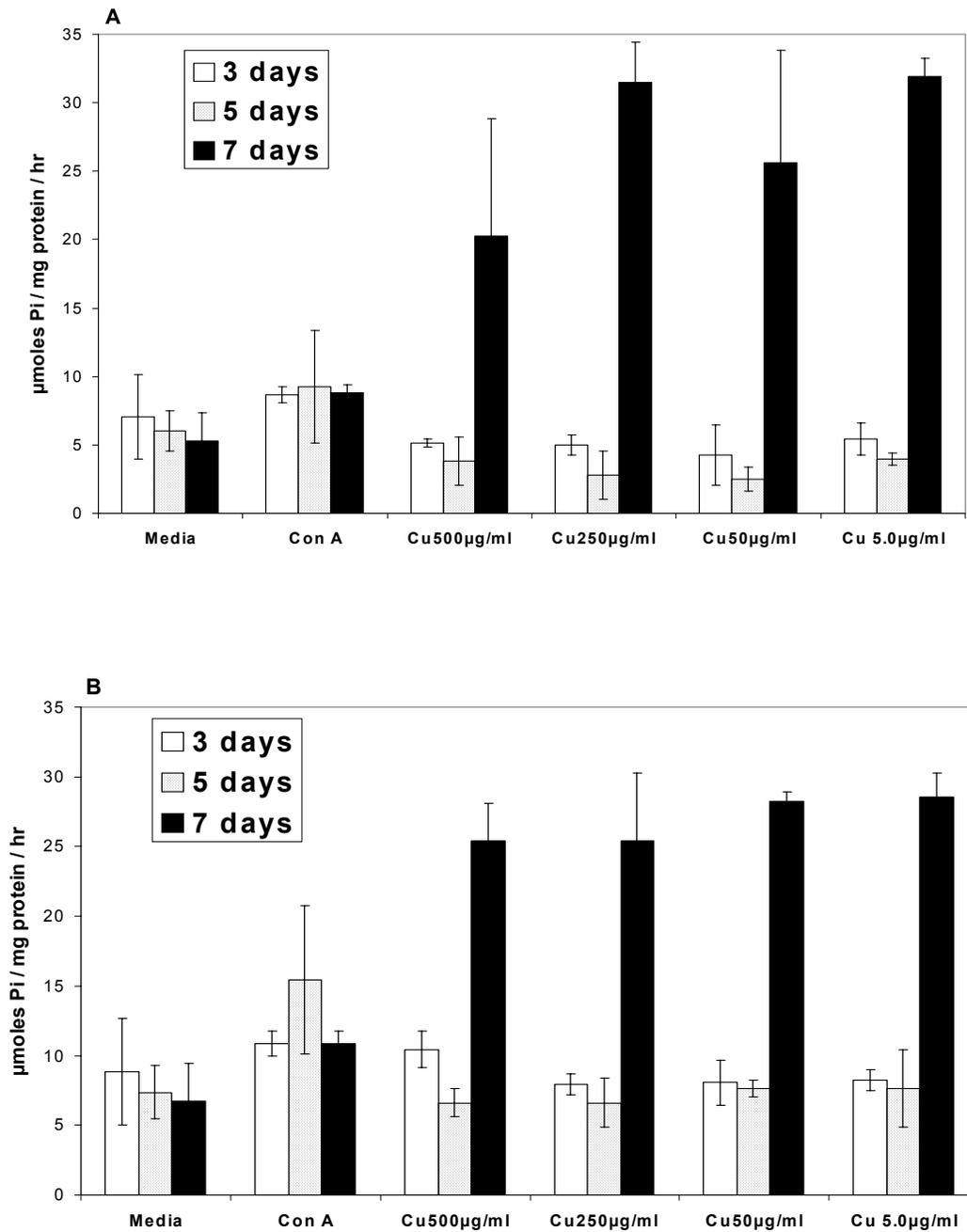


Figure 2. ATPases activity of human mononuclear cells stimulated with Con A after 3, 5 and 7 days of incubation with curcumin (Cu). The enzyme activity of cells in media alone is compared with the activity of cells in Con A alone and in Con A + Cu (500, 250, 50 and 5 µg/ml). (A) represents the Ca^{2+} ATPase activity. (B) represents the Na/K^{+} ATPase activity.

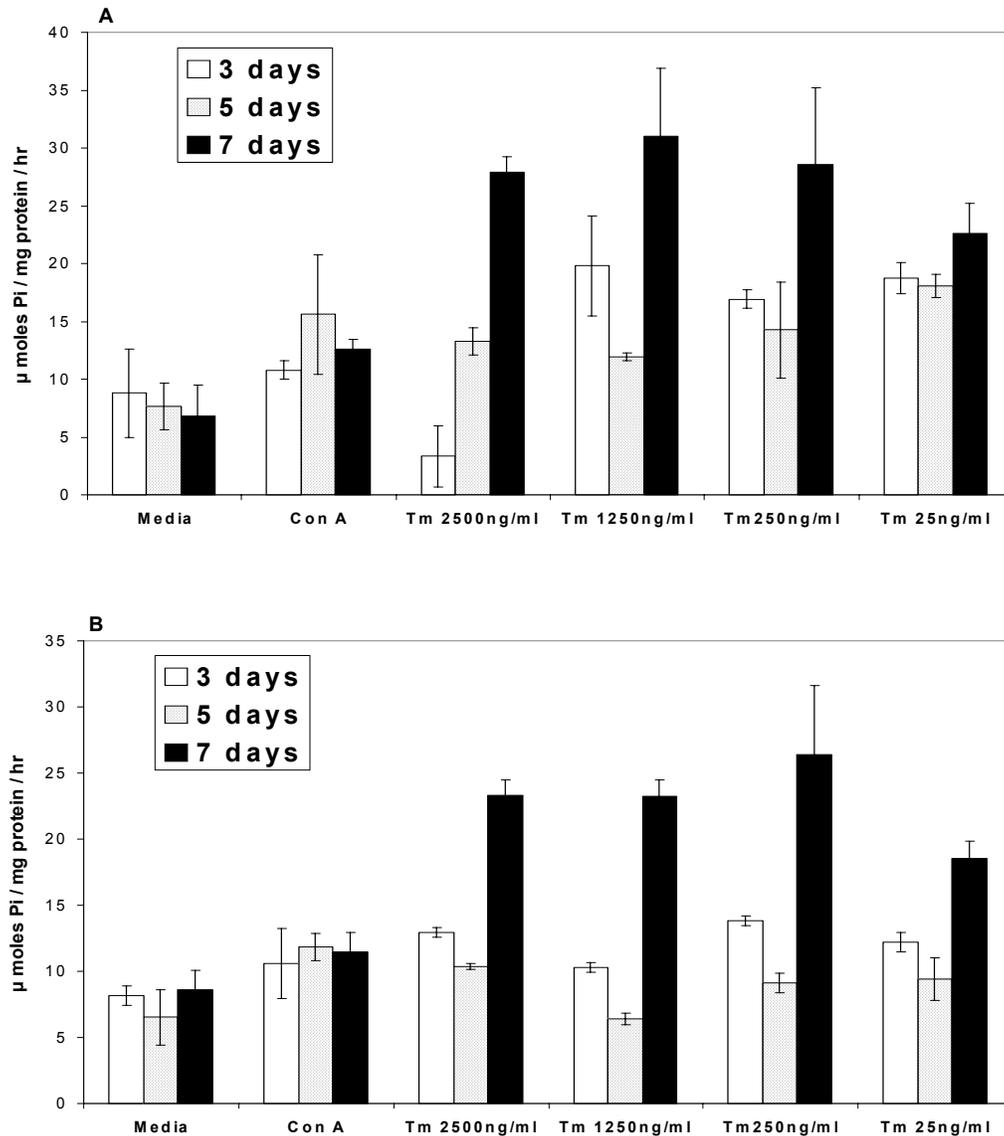


Figure 3. ATPases activity of human mononuclear cells stimulated with Con A after 3, 5 and 7 days of incubation with turmerin (Tm). The enzyme activity of cells in media alone is compared with the activity of cells in con A alone and in Con A + Tm (2500, 1250, 250 and 25 ng/ml). (A) represents the Ca²⁺ ATPase activity. (B) represents the Na/K⁺ ATPase activity.

on day 5. There is more than a 2-fold increase in all the concentrations ($p \leq 0.05$). At the lowest concentration (25 ng/ml), there is only a 1.5 fold increase in ATPase activity at day 7 (not significant).

Discussion

Studies evaluating ATPases have mainly focused on early events. Con A binds to specific glycoproteins on the external surface of intact lymphocytes and causes a marked increase in the number of binding sites for Ouabain in human lymphocytes [24] a specific receptor for Na/K⁺ATPase [25]. It has been reported that mitogenic doses of Con A have no effect on plasma membrane associated calcium ATPases of human lymphocytes [26]. Con A did not alter Na/K⁺ATPase activity in control rats while turmeric and curcumin decreased the Na/K⁺ATPase activity in retinol deficient rats [19]. Curcumin has not only been shown to inhibit Con A mediated proliferation; it also inhibits apoptosis [27]. In another study, curcumin increased the number of T-helper cells in animals that have been fed curcumin over a period of 3-5 weeks [28]. Further, Ca²⁺ ATPase inhibitors induce interleukin-2 synthesis and T cell proliferation. In this study, we wanted to determine the activity of ATPases of Con A-stimulated peripheral blood mononuclear cells after 3, 5 and 7 days of incubation. T cell proliferation by Con A reaches peak stimulation at day 3. Intermediate and later stages of T cell proliferation occur at day 5 and 7, respectively.

Ion transport across the membranes regulates a number of biochemical reactions in the cell [29]. Na/K⁺ ATPase is an integral part of plasma membrane and is responsible for the control of sodium and potassium transport. Inhibition of Ca²⁺ATPase and Na/K⁺ATPases in this study reflects the blockage of ion transport, since ATPases mediate these events in cell organelles. Reduction of Na/K⁺ATPase activity was demonstrated for both turmeric and curcumin. Curcumin alone has been shown to be an inhibitor of Ca²⁺ATPase activity [30]. It has also been shown that curcumin (5-30μM) concentrations inhibit overall ATPase activity and Ca²⁺ transport by interfering with phosphoenzyme formation with ATP or Pi [31, 32]. The lowest concentration of curcumin used in the present study is 5 μg/ml, which corresponds to about 13.5 μM concentration. This study has investigated the relative changes in activity over time. The increase in receptor density for ATPases at day 7 is most likely due to the up-regulation of these receptors.

To our knowledge, this is the first report to show that there is enhancement rather than inhibition in ATPase activity after 7 days of culturing in the presence of turmeric and its products. The late stage of T cell activation lowers the proliferative capacity at day 7, but increases the ATPase activity implying that there is regeneration of the receptors, which are associated with these ATPases. The concept of shedding and resynthesis of immune cell receptor after 3-4 hrs is well documented but the dramatic up-regulation of the receptor at 7 days has not been described previously [33-36].

The differences between the activities of turmeric, curcumin and turmerin on ATPase activity cannot be easily explained although the parent compound and its derivatives generally exert the same effect on ATPase activity. It is possible that the mechanism of action of turmeric and its products may be inhibition of ATPase activity while increasing the intracellular concentration of Ca²⁺ as suggested by other simulation studies [30]. It is also conceivable that turmeric, curcumin and turmerin alter Ca²⁺

mobilization in the early and intermediate phases of T cell proliferation. How Ca^{2+} levels may effect the later stage of proliferation (day 7) remains to be determined. Likewise, the mechanism underlying up-regulation of Na/K^+ ATPases at day 7 is also not known. Why turmeric and curcumin generally inhibit ATPase activity in the early stages of proliferation and why turmeric and its derivatives increase ATPase activity at day 7 is not understood. Turmerin appears to have different properties compared to turmeric and curcumin. The question of why turmerin for the most part increases the ATPase activity at day 3 and decreases the activity at day 5, while turmeric and curcumin decrease activities at days 3 and 5, is interesting and is being further investigated.

In summary the following actions occurred:

- 1) In the presence of turmeric, Ca^{2+} ATPase and Na/K^+ ATPase levels generally decreased above the base level at 3 and 5 days while at 7 days the ATPase activity level exhibited a two-fold increase.
- 2) In the presence of curcumin, Ca^{2+} ATPase and Na/K^+ ATPase levels decreased below the base level at 3 and 5 days while at 7 days ATPase activity exhibited a two-fold increase.
- 3) In the presence of turmerin, Ca^{2+} ATPase and Na/K^+ ATPase levels generally increased on day 3, decreased on day 5 and increased two-fold on day 7.

Acknowledgements

Anelle Taylor and Melanie T. Wilson were supported in part by the Howard Hughes Medical Institute and the Base Pair Program.

References

1. Papp, B.; Enyedi, A.; Paszty, K.; Kovacs, T.; Sarkadi, B.; Gardos, G.; Magnier, C.; Wuytack, F.; Enouf, J. Simultaneous presence of two distinct endoplasmic-reticulum-type calcium-pump isoforms in human cells. *Biochem. J.* **1992**, *288*, 297-302.
2. Schwartz, A.; Lindenmayer, G. E.; Allen, J. C. The sodium-potassium adenosine triphosphatase: Physiological, pharmacological and biochemical aspects. *Pharmacol. Rev.* **1975**, *27*, 3-134.
3. Thomas, R. C. Electrogenic sodium pump in nerve and muscle cells. *Physiol. Rev.* **1972**, *52*, 563-594.
4. Crane, R. K. The gradient hypothesis and other models of carrier mediated active transport. *Rev. Physiol. Biochem. Pharmacol.* **1977**, 69-159.
5. Iversen, L. L., Kelly, J. S. Uptake and metabolism of gamma-aminobutyric acid by neurones and glial cells. *Biochem. Pharmacol.* **1975**, *24*, 933-938.
6. van Breemen, C., Saida, K. Cellular mechanisms regulating $[\text{Ca}^{2+}]_i$ smooth muscle. *Annu. Rev. Physiol.* **1989**, *51*, 315-329.

7. Min, W.; Dunn, A. J.; Jones, D. H. Non-glycosylated recombinant pro-concanavalin A is active without polypeptide cleavage. *EMBO J.* **1992**, *11*, 1303-1307.
8. Ruscetti, F. W., Chervenick, P. A. Regulation of the release of colony-stimulating activity from mitogen-stimulated lymphocytes. *J. Immunol.* **1975**, *114*, 1513-1517.
9. Novogrodsky, A., Katchalski, E. Lymphocyte Transformation Induced by Concanavalin A and its Reversion by Methyl-[[alpha]]-Mannopyranoside. *Biochim. Biophys. Acta.* **1971**, *228*, 579-583.
10. Perlmann, P.; Nilsson, H.; Leon, M. Inhibition of Cytotoxicity of Lymphocytes by Concanavalin A in-vitro. *Science* **1970**, *168*, 1112-1115.
11. Okamoto, T., Kobayashi, T. Effects of Concanavalin A on Cytokine mRNA Expression in Mouse Liver. *J. Pharmacol.* **1997**, *75*, 199-201.
12. Wermerskirchen, A. S.; LaTocha, D. H.; Clarke, B. L. Adrenocorticotrophic hormone controls Concanavalin A activation of rat lymphocytes by modulating IL-2 production. *Life Sci.* **2000**, *67*, 2177-2187.
13. Arai, K. I.; Lee, F.; Miyajima, A.; Miyatake, S.; Arai, N.; Yokota, T. Cytokines: coordinators of immune and inflammatory responses. *Annu. Rev. Biochem.* **1990**, *59*, 783-836.
14. Srinivas, L.; Shalini, V. K.; Shylaja, M. Turmerin: a water soluble antioxidant peptide from turmeric [Curcuma longa]. *Arch. Biochem. Biophys.* **1992**, *292*, 617-623.
15. Selvam, R.; Subramanian, L.; Gayathri, R.; Angayarkanni, N. The anti-oxidant activity of turmeric (Curcuma longa). *J. Ethnopharmacol.* **1995**, *47*, 59-67.
16. Asai, A.; Nakagawa, K.; Miyazawa, T. Antioxidative effects of turmeric, rosemary and capsicum extracts on membrane phospholipid peroxidation and liver lipid metabolism in mice. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 2118-2122.
17. Frautschy, S. A.; Hu, W.; Kim, P.; Miller, S. A.; Chu, T.; Harris-White, M. E.; Cole, G. M. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. *Neurobiol. Aging* **2001**, *22*, 993-1005.
18. Phan, T. T.; See, P.; Lee, S. T.; Chan, S. Y. Protective effects of curcumin against oxidative damage on skin cells in vitro: its implication for wound healing. *J. Trauma* **2001**, *51*, 927-931.
19. Kaul, S., Krishnakanth, T. P. Effect of retinol deficiency and curcumin or turmeric feeding on brain Na(+)-K+ adenosine triphosphatase activity. *Mol. Cell Biochem.* **1994**, *137*, 101-107.
20. Huang, H. C.; Jan, T. R.; Yeh, S. F. Inhibitory effect of curcumin, an anti-inflammatory agent, on vascular smooth muscle cell proliferation. *Eur. J. Pharmacol.* **1992**, *221*, 381-384.
21. Fritz, F. P., Hamrich, M. E. Enzymatic analysis of ATPase. *Enzymol. Acta. Biocat.* **1966**, *30*, 57-64.
22. Desaiyah, D., Ho, I. K. Effects of acute and continuous morphine administration of catecholamine sensitive ATPase in mouse brains. *J. Pharmacol. Exp. Ther.* **1979**, *208*, 80-85.
23. Fiske, C. H., Subba Row, Y. The colorimetric determination of phosphorous. *J. Biol. Chem.* **1921**, *66*, 375-400.
24. Wright, P.; Quastel, M. R.; Kaplan, J. G. Differential sensitivity of antigen- and mitogen-stimulated

- human leukocytes to prolonged inhibition of potassium transport. *Exp. Cell Res.* **1973**, *79*, 87-94.
25. Dahl, J. L., Hokin, L. E. The sodium-potassium adenosinetriphosphatase. *Annu. Rev. Biochem.* **1974**, *43*, 327-356.
 26. Lichtman, A. H.; Segel, G. B.; Lichtman, M. A. Effect of Trifluoperazine and mitogenic lectins on calcium ATPase activity and calcium transport by human lymphocyte plasma membrane vesicles. *J. Cell. Physiol.* **1982**, *111*, 213-217.
 27. Sikora, E.; Bielak-Zmijewska, A.; Piwocka, K.; Skierski, J., Radziszewska E. Inhibition of proliferation and apoptosis of human and rat T lymphocytes by curcumin, a curry pigment. *Biochem. Pharmacol.* **1997**, *54*, 899-907.
 28. Yasni, S.; Yoshiie, K.; Oda, H.; Sugano, M.; Imaizumi, K. Dietary Curcuma xanthorrhiza Roxb. increases mitogenic responses of splenic lymphocytes in rats, and alters populations of the lymphocytes in mice. *J. Nutr. Sci. Vitaminol. (Tokyo)* **1993**, *39*, 345-354.
 29. Rossier, B. C.; Geering, K., Kraenbuhl J. P. Regulation of the sodium pump: how and why? *TIBS* **1987**, *2*, 483-487.
 30. Logan-Smith, M. J.; East, J. M.; Lee, A. G. Evidence for a global inhibitor-induced conformation change on the Ca(2+)-ATPase of sarcoplasmic reticulum from paired inhibitor studies. *Biochemistry* **2002**, *41*, 2869-2875.
 31. Bilmen, J. G.; Khan, S. Z.; Javed, M. H.; Michelangeli, F. Inhibition of the SERCA Ca²⁺ pumps by curcumin. Curcumin putatively stabilizes the interaction between the nucleotide-binding and phosphorylation domains in the absence of ATP. *Eur. J. Biochem.* **2001**, *268*, 6318-6327.
 32. Sumbilla, C.; Lewis, D.; Hammerschmidt, T.; Inesi, G. The slippage of the Ca²⁺ pump and its control by anions and curcumin in skeletal and cardiac sarcoplasmic reticulum. *J. Biol. Chem.* **2002**, *277*, 13900-13906.
 33. Sarmay, G.; Istvan, L.; Gergely, J. Shedding and reappearance of Fc, C3 and SRBC receptors on peripheral lymphocytes from normal donors and chronic lymphatic leukaemia (CLL) patients. *Immunology* **1978**, *34*, 315-321.
 34. Romagnani, S.; Maggi, E.; Lorenzini, M.; Giudizi, G. M.; Biagiotti, R.; Ricci, M. Study of some properties of the receptor for IgM on human lymphocytes. *Clin. Exp. Immunol.* **1979**, *36*, 502-510.
 35. McKimm-Breschkin, J. L., Miller, J. F. Synthesis and turnover of the putative T-lymphocyte antigen receptor. *Scand. J. Immunol.* **1985**, *21*, 539-547.
 36. Dello, S. P., Rovida, E. Transmodulation of cell surface regulatory molecules via ectodomain shedding. *Biol. Chem.* **2002**, *383*, 69-83.