

# Synergistic actions of atorvastatin with $\gamma$ -tocotrienol and celecoxib against human colon cancer HT29 and HCT116 cells

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The synergistic actions of atorvastatin (ATST) with  $\gamma$ -tocotrienol ( $\gamma$ -TT) and celecoxib (CXIB) were studied in human colon cancer cell lines HT29 and HCT116. The synergistic inhibition of cell growth by ATST and  $\gamma$ -TT was demonstrated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and isobologram analysis.  $\delta$ -TT exhibited a similar inhibitory action when combined with ATST. Mevalonate and geranylgeranyl pyrophosphate eliminated most of the growth inhibitory effect of ATST, but only marginally decreased that of  $\gamma$ -TT; whereas farnesyl pyrophosphate and squalene exhibited little effect on the inhibitory action of ATST and  $\gamma$ -TT, indicating protein geranylgeranylation, but not farnesylation are involved in the inhibition of colon cancer cell growth. Both mevalonate and squalene restored the cellular cholesterol level that was reduced by ATST treatment, but only mevalonate eliminated the cell growth inhibitory effect, suggesting that the cholesterol level in cells does not play an essential role in inhibiting cancer cell growth. Protein level of HMG-CoA reductase increased after ATST treatment, and the presence of  $\gamma$ -TT attenuated the elevated level of HMG-CoA reductase. ATST also decreased membrane-bound RhoA, possibly due to a reduced level of protein geranylgeranylation; addition of  $\gamma$ -TT enhanced this effect. The mediation of HMG-CoA reductase and RhoA provides a possible mechanism for the synergistic action of ATST and  $\gamma$ -TT. The triple combination of ATST,  $\gamma$ -TT and CXIB showed a synergistic inhibition of cancer cell growth in MTT assays. The synergistic action of these three compounds was also illustrated by their induction of  $G_0/G_1$  phase cell cycle arrest and apoptosis.

Atorvastatin (ATST, commercial name Lipitor or Torvast) belongs to the statin family, a class of compounds used to reduce the levels of cholesterol and low density lipoproteins. The efficacy of statins in lowering the risk of cardiovascular disease and the relatively low toxicity of statins has led to their widespread use. Statins function by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis

(Fig. 1).<sup>1</sup> The product of HMG-CoA reductase, mevalonate, is the precursor for many important intermediates in a pathway commonly known as the "mevalonate cascade." Statins inhibit the formation of many intermediate products such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which isoprenylate various proteins, including small G-proteins such as the Ras/Rho super family proteins. The isoprenylation of these proteins is essential for them to anchor to cell membranes and thus become functional.<sup>2</sup> The cancer prevention activity reported for statins is believed to be due to their inhibition of the isoprenylation of G-proteins and the subsequent alteration of downstream signaling pathways.<sup>3,4</sup> In addition, statins can also affect several HMG-CoA reductase-independent targets. For example, simvastatin inhibits the RANKL (the receptor activator of NF- $\kappa$ B ligand)-induced NF- $\kappa$ B activation pathway. This inhibition leads to the suppression of osteoclastogenesis induced by RANKL and by tumor cells.<sup>5</sup> Lovastatin has been shown to bind directly to an integrin called lymphocyte-function-associated antigen 1 (LFA1) and inhibit the interaction of LFA1 with intracellular-adhesion molecule 1 (ICAM1), thus preventing cell-adhesion, invasion and inflammation.<sup>6</sup> Statins also inhibit the protein degradation machinery, specifically the proteasomes.<sup>7</sup> Inhibition of proteasome activity is likely to be related to the effect of statins on  $G_1$  phase cell cycle arrest. In general, statins exert their cancer prevention effects by regulating several

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**Abbreviations:** ATST: atorvastatin; Cox-2: cyclooxygenase-2; CXIB: celecoxib; Fpp: farnesyl pyrophosphate; GGpp: geranylgeranyl pyrophosphate; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PARP: poly ADP-ribose polymerase; TT: tocotrienol

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