Conclusion
The goal of the present study was to determine the effects of the high doses of AA on process of angiogenesis. Angiogenesis is the process of new blood vessel formation occurring in both normal and cancerous tissues. To make new blood vessels, endothelial cells must migrate toward the angiogenic stimulus, which was released from tumor cells. Endothelial cells must proliferate to provide the necessary number of cells for making new vessels and to form a three-dimensional tubular structure. In addition, circulating endothelial progenitor cells are involved in the development of vasculature, and many tumors are associated with bone marrow-derived endothelial cell infiltration.

According to our study, each of these processes is influenced by high concentration of ascorbic acid: (1) High concentrations of AA alter the metabolic activity of endothelial cells by decreasing the ATP levels by 20% at 300 mg/dl concentration. This prevents significant cell proliferation without changing cell viability. (2) Cell migration: as measured by wound healing assay is decreased by high concentrations of AA. Cell migration was decreased 1.4 times for 200 mg/dl; and 2.4 times for 300 mg/dl. (3) New blood vessel formation: this was measured by in vitro endothelial tube formation assay on Matrigel. The effect of AA on angiogenesis estimated by tube formation assay demonstrated inhibitions of vessel structure after 3 h–24 h of exposure of the cells to ascorbic acid. This appeared secondary to AA inhibition of NO in endothelial cells. NO is known as a major stimulus of new blood vessel formation. Our study measured the level of nitric oxide in response to high concentrations of AA. High concentrations of AA inhibited the production of NO, and as NO pathways are important promoters of tumor angiogenesis, high concentrations of AA have been demonstrated to limit angiogenesis.

The decreasing the availability of NO at high concentrations of AA may be explained by the following mechanisms. As endothelial NO formation depends on the presence of intracellular cofactors such as: NADPH, FAD, FMN and tetrahydrobiopterin (BH4), we can suggest that overloading of AA and DHA in cells can change the oxidative-reduction status inside the cells. This could decrease the availability of nitric oxide, through the formation of peroxynitrite. NO can move very rapidly through membranes, thereby the reactions of inactivation may also occur in the extracellular space between cells. Low concentrations of ascorbic acid protect NO from inactivation by...