

Abstract

The cytoplasmic assembly complex (AC) in HCMV-infected human foreskin fibroblasts (HFF) is a distinguishable “bulb”-like juxtannuclear structure. The morphology of the AC is dependent on the activity of the viral-encoded serine/threonine kinase, pUL97. The morphology of AC also changes when wt-HCMV infected HFF cells were treated with NGIC-I, kinase C inhibitor. Here we employed a set of serine/threonine kinase inhibitors to test whether the HCMV assembly or the AC structure by itself are affected by serine/threonine activity. Our drug inhibition assays indicated that only staurosporine, a broad range serine/threonine kinase inhibitor affected the subcellular distribution of viral tegument protein and Golgi markers residing the AC. Staurosporine inhibition resulted in a "bulb-like structure of the AC highly punctuated with vacuoles on the rims. Regarding the nature of these vacuoles, we observed a damage of the AC structure and vacuoles with Brefeldin A. Our kinetic experiments using staurosporine inhibitors revealed that the vacuoles were clearly detectable at 60hpi. Staurosporine inhibition also remodelled the nuclear shape into a "boat"-like yet unpublished structure. Interestingly, the effect of staurosporine was reversible in block-release assays indicating that its inhibition activity is restricted to infected cells. Another confirmation of this data came from the moi dependent staurosporine drug inhibition assays. Hereby, the inhibition activity of viral load as measured via real time PCR was highly reduced in higher moi infections and less reduced in lower moi infections. Overall, staurosporine reduced the viral load and the viral titer measured via plaque assay. However, the viral titer was remarkably inhibited, since plaques can only emerge from viable viruses, while DNA from even defected viruses can be measured by viral load assay.

While inhibition with staurosporine reduced the wild type infection, the deletion mutant virus (Δ UL97) was hardly affected. Nor the AC structure in Δ UL97 infected cells, viral titer or viral load were affected during inhibition with staurosporine. However, expression of viral protein pp65 was dramatically affected in Δ UL97 infected cells inhibited with staurosporine similar to wt-HCMV infected cells. Finally, Co-immunoprecipitation assay with pp65 revealed that serine/threonine kinase expression is clearly affected by staurosporine as well as by deletion of the UL97 kinase activity. Taken together, these data provide evidence for an essential role of cellular serine/threonine kinase activity in HCMV assembly. Furthermore, our results propose a possible therapeutic role of kinase inhibitors in designing anti HCMV drugs.