Potential synergism of Caffeic acid Phenethyl ester And Dasatinib in C6 Glioma cell Model: Adumbrating the Molecular mechanism

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Gliomas are one of the most invasive, highly recurrent, heterogeneous cancers resistant to most of the current treatment regimes and hence almost incurable. Accounting for 80% of malignant CNS (central nervous system) tumors they are one of the most common primary brain tumors. Gliomagenesis is a multifactorial process involving an intricate interaction between a number of signaling cascades resulting in unrestricted proliferation and invasion. The invasive and proliferative nature of glioblastoma has been one of the impetuses for testing of new therapies to more expeditiously mitigate tumorigenic invasion, proliferation, and survival. Since glioma is a molecularly complex disease thus treatment failure with a single agent that targets one signaling abnormality may result from the uninhibited actions of other molecular abnormalities or from the need to target more than one oncogenic signaling cascade simultaneously. Therefore, treatment with agents that could target several key signaling pathways represents an attractive therapeutic approach. Since there is a substantial need for chemotherapy that is more effective as well as safe, this in vitro study was necessary to establish combining agents to try to more effectively suppress the aberrant signaling pathways by using rationally designed combinations of therapeutics. Despite these limitations, relevant conclusions from this study have helped to figure out how to use a rational combination of drugs that have a strong complementary antitumor effect that is verified in a relevant in vitro model. This study was an effort to put agents together that has a limited overlap of their toxicities and has limited detrimental potential pharmacologic interactions. CAPE and Dasatinib co-treatment inhibited proliferation and invasiveness in C6 cells otherwise resistant to the treatment of the same drugs at the same concentrations singly. Cotreatment with CAPE and Dasatinib not only induces apoptosis in C6 cells but also inhibits cellular clonogenicity as well, indicating CAPE and Dasatinib target different cellular processes in glioma. Our results showed that CAPE and Dasatinib initiate the process of apoptosis in C6 glioma cells. Co-treatment decreases the calcium levels in C6 glioma cells; imperatively it would influence the whole process of gliomagenesis via calcium signaling. Calcium ions are involved in a number of cellular signaling cascades in the brain during tumorigenesis which influences a multitude of cellular reactions due to the diversity of calcium-binding proteins. Calcium ions also play a critical role in glutamate excitotoxicity in the brain. Catalase a primary antioxidant enzyme plays a very important role in the defense against oxidative stress in the brain as such it is constitutively elevated in glioma cells when compared to their normal cell counterpart. Upon cotreatment with CAPE and Dasatinib, the activity of catalase significantly decreased thus countering the stress resistance mechanism in C6 cells. Gliomagenesis is a multifactorial process that involves a complex interplay between the extracellular matrix, adjacent cells, and glial cells which result in active proliferation and invasion of tumor cells into the adjacent boundaries. Cellular proliferation and invasiveness is a highly regulated process and the loss of control on these processes stems from a loss of inhibitory regulation. One of the most understood mechanisms of C6 cell invasion is by metalloproteinase activity. MMPs cause proteolytic processing of extracellular matrix structural proteins, thus regulating cell migration and invasion. Co-treatment with CAPE and Dasatinib resulted in a significant decrease in MMP-2 and MMP-9 activity as well as in the expression of active forms of MMP-2 and MMP-9. Glioma, as indicated by recent studies, seems to results from sequential inactivation of the p14ARF/MDM2/p53, RTK/Pi3K/Akt/mTOR axes, and Ras/MEK/MAPK pathway. 70-80% of gliomas analyzed harbor alterations in all three pathways. p53 is one of the major tumor suppressor proteins in gliomas. However, glioma tumors retain wild-type p53; hence the ability to maintain the tumor phenotype depends on the downregulated expression of p53. Since co-treatment with CAPE and Dasatinib induces apoptosis so we investigated the expression of p53 in C6 cells with and without treatment. Co-treatment with CAPE and Dasatinib induced p53 expression in C6 glioma cells compared with the control, CAPE and Dasatinib only group. Thus CAPE and Dasatinib co-treatment induced reactivation of p53 impeding tumor growth and proliferation of C6 glioma cells. The up-regulation of p53 is believed to be instrumental in cell growth inhibition. However, it has been found that in some cancer cell lines the p53 genomic changes did not overtly correlate to the expression of p53 protein. It has been indicated that the elevation of p53 protein levels in response to DNA damage occurs in the absence of clear changes in mRNA levels. Sometimes moderate increases in mRNA expression are followed by large increases in protein levels [35]. Considering this complexity, after expression analysis of protein we further studied the expression of p53 by qRTPCR. At transcriptional levels, CAPE and Dasatinib co-treatment induced p53 expression in C6 glioma cells compared with the control, CAPE and Dasatinib only group. Thus, a combination of CAPE and Dasatinib induces expression of p53 not only at protein level only but at the transcriptional level also. ERKs are known to play a major role in various cellular responses. ERK pathway is involved in cell growth, proliferation, differentiation, migration in cell systems. It has been observed that an increase in intracellular cAMP strongly inhibits ERK activity in C6 cells and enhances the rate of differentiation into the astrocytic phenotype indicating a possible role for ERK in the negative regulation of GFAP expression and glioma cell differentiation. The oncogenic potential of ERK was demonstrated in part by the finding that ERK-1/2 activity was elevated in untreated C6 cells while the expression of ERK appreciably decreased in the co-treated cells. Downregulation of ERK expression
is an important predictor of antineoplastic activity of combination treatment. AKT is a downstream serine/threonine kinase in the RTK/PTEN/PI3K pathway. The pathway is involved in cell survival, inhibiting apoptosis through phosphorylation and inactivation of various target molecules; in addition, it also plays a critical role in metabolism. Studies have revealed that this pathway is mutated in the majority of gliomas and AKT plays a central role in this signal transduction pathway. Enough studies have shown that AKT can be activated in response to increases in cellular calcium levels, via Calcium/Calmodulin dependent protein kinase. We have earlier showed CAPE and Dasatinib cotreatment modulates calcium levels in C6 cells, thus a highly significant reduction in expression of AKT, as well as modulation in calcium levels, implies that CAPE and Dasatinib target not the only expression of nodal proteins but also the secondary molecules. EGFRs are overexpressed in gliomas and play a significant role in regulating other intracellular signaling pathways. Since EGFR is involved in both mTOR/PI3K/AKT and RAS/MAPK pathway, therefore, we evaluated the effect of CAPE and Dasatinib co-treatment on the transcription of EGFR. From the results, we could clearly understand that reduction in EGFR expression in the co-treated group further implies that this therapy targets multiple pathways. PCNA is well recognized as a proliferative marker in gliomas. Studies have revealed that PCNA plays a coordinating role and has a striking ability to interact with multiple partners who are involved in critical cellular activities including. PCNA transcript levels were assessed after drug treatment since it is a nuclear protein and cofactor for DNA polymerase so it's downregulated expression would suppress cell cycle entry and progression. The mechanism by which such potentiation could be achieved would involve the inhibition of cell proliferation, invasion, and the inhibition of angiogenesis. Since there is a substantial need for chemotherapy that is more effective as well as safe, this in vitro study was necessary to establish combining agents to try to more effectively suppress the aberrant signaling pathways by using rationally designed combinations of therapeutics. Co-treatment inhibits multiple cellular processes involved in tumorigenesis hence decreasing the possibility of drug-induced resistance. These drugs are not only addictive but highly synergistic in activity. CAPE dramatically decreased the dose of Dasatinib needed to achieve inhibition of glioma cell proliferation suggesting that perhaps using two agents together increases the therapeutic potential of drugs thus can be an effective therapy for treating glioma. Clearly, more work is needed to be carried out in vivo glioma models at least to mimic microenvironment in brain tumor patients. Adequately powered, high-quality descriptive studies are needed.