***IN VIVO* INTERNALIZATION DYNAMICS OF SOYBEAN PROTEASE ISONHIBITORS, IBB1 AND IBBD2, OF THE BOWMAN-BIRK FAMILY IN HT29 COLORECTAL CANCER CELLS**

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IBB1 and IBB2D are major soybean protease isoinhibitors of the Bowman-Birk family with an ability to inhibit either trypsin/chymotrypsin or trypsin activities, respectively, that are currently being investigated as chemopreventive agents of colorectal cancer in humans (Clemente *et al*., 2012). In order to gain insight about the action mechanism of Bowman-Birk inhibitors (BBIs) and their potential therapeutic targets, the *in vivo* internalization dynamics of IBB1 and IBBD2 proteins, in their active and chemically inactive forms, was monitored in human colorectal adenocarcinoma HT29 cells by fluorescence microscopy. For this purpose, a new fluorescent labeling protocol recently developed in our laboratory was used (Castro *et al*., 2020). We observed that the internalization process started after a few minutes. At short times of culture (< 3 h), HT29 cells showed a green fluorescent signal coming from CyDye2-labeled IBB1 and IBBD2 proteins, forming patches randomly distributed across the cytoplasm. The fluorescent signal intensity gradually increased throughout the culture, indicating that IBBs cross the plasma membrane of HT29 colon cancer cells and accumulate inside HT29 cells. On the contrary, no fluorescent labeling was observed at t0 min or in the negative controls with unlabeled BBI proteins. The internalization process was independent of the inhibitory activities of BBIs since both active and chemically inactivated proteins were internalized. In multiplex experiments, we found that the red fluorescent signal from the endosome marker FM 4-64 clearly overlapped with labeling from IBB1 and IBBD2 proteins in the cytoplasm, giving a yellowish fluorescence. These results suggest that BBIs proteins are internalized into the cytoplasm of HT29 cells through one of the existing endocytic pathways. Interestingly, at longer times of culture (>12 h), the fluorescent signal of IBB proteins clearly overlapped with the Hoechst 33342-labeled nucleus, thus indicating that BBI proteins are also capable to internalize into the cell nucleus.

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