Intracellular trafficking of VB6-845, an immunocytotoxin containing a de-immunized variant of bouganin

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ABSTRACT

Recombinant immunocytotoxins consist of an antibody-binding domain fused with a cytotoxin. The specificity of the antibody fragment permits the targeting of the cytotoxin to tumor cells which, upon internalization, mediates cell killing. Bouganin is a type I ribosomal inactivating protein (RIP) isolated from the plant *Bougainvillea spectabilis* Willd and lacks a cell-binding domain. The bouganin cytotoxin has been further modified by epitope depletion to generate a de-immunized variant (de-bouganin). This de-immunized variant of bouganin has been linked to the Fab fragment *via* a furin-sensitive linker creating VB6-845 immunocytotoxin protein. Cytotoxins traffic intracellularly by one of two means, an endosomal/lysosomal pathway or *via* a Golgi/ER route. Once bound to its cognate antigen, EpCAM, VB6-845 internalizes and effects potent cytotoxic activity (IC = 1 nM). The 50 efficiency of de-bouganin cytotoxicity was correlated with its release from the antibody moiety. To optimize the release of debouganin, we have investigated the intracellular trafficking of De-Bouganin by confocal microscopy and evaluated cell viability in presence of drugs that affect organelle function. For confocal microscopy, EpCAM-positive CAL-27 cells were treated with a VB6-845 variant containing an inactive de-bouganin and then stained with anti-de-bouganin and organelle specific markers. De-Bouganin was shown to co-localize along with the EEA1 endosomal and LAMP-2 lysosomal markers after 15 and 45 minutes in CAL-27 cells, respectively. Only marginal colocalization was detected within the Golgi apparatus after a 3-hour incubation. Treatment with lactacystine, a proteosome inhibitor, or the addition of the KDEL sequence, an ER retrieval sequence, did not improve VB6-845 cytotoxicity. This suggests that de-bouganin does not traffic *via* a Golgi/ER pathway in contrast to other cytotoxic proteins such as *Pseudomonas* exotoxin A and ricin. Treatment with chemical drugs such as chloroquine, ammonium chloride and monensin, which increase the pH of the endosome and lysosome, led to an improvement of the VB6-845 cytotoxicity ranging from 5- to 10-fold. To confirm these results, different constructs containing other proteolytic sites of proteases localized in the endosome and the lysosome compartments were engineered and tested on a variety of tumor cells. VB6-845 cytotoxicity with either a legumain, cathepsin B or D sensitive linker was still in the nanomolar range but less potent than the original molecule. Moreover, minimal additional cytotoxicity was observed when legumain, cathepsin B or D sites were added to the furin linker. Together, these data demonstrate that the De-Bouganin traffics *via* an endosome/lysosome pathway to reach the cytosol and that the furin linker is the optimal configuration of the Fab variant for delivering maximal cytotoxic activity.