Purification of adenoviral vector serotype 5 for gene therapy against alcoholism using anion exchange chromatography

By: Lucero, AT (Lucero, Alicia T.)[1]; Mercado, SA (Mercado, Sergio A.)[1]; Sanchez, AC (Sanchez, Anamaria C.)[1]; Contador, CA (Contador, Carolina A.)[1]; Andrews, BA (Andrews, Barbara A.)[1]; Asenjo, JA (Asenjo, Juan A.)[1]

Abstract

BACKGROUND: Gene therapy is a potent alternative for long-lasting inhibition of alcohol consumption. This study compares the purification of a recombinant adenoviral vector serotype 5 (rAdV5) for use in gene therapy against alcoholism using two anion-exchange methods.

RESULTS: Two anion-exchange chromatography methods using fast protein liquid chromatography were compared using a packed-bed column (Q-Sepharose (TM) XL) and two monolithic columns (CIM (TM) QA-1 and CIM (TM) DEAE-1). An improved and reproducible separation of recombinant adenovirus type 5 from cell lysate contaminants was achieved using the two strong anion-exchange columns in a two-step gradient chromatography. Higher adenovirus yields were achieved using the CIM QA-1 tube monolithic column at sample volumes of both 1 and 10 mL compared with the Q-Sepharose XL column. At higher flow rates, the CIM QA-1 tube monolithic column achieved better separation of the target fraction. Process recovery was improved from 28% using the Q-Sepharose XL column to 34% with the CIM QA-1 tube monolithic column quantified as vector genome. Analysis by SDS-PAGE demonstrated a purity of 70% for purified adenovirus using the CIM QA-1 tube monolithic column.

CONCLUSION: This study indicated that the use of a CIM QA-1 tube monolithic column is a better alternative than Q-Sepharose XL, and CIM DEAE-1 tube monolithic columns for the primary purification process of rAdV5 carrying the human aldehyde dehydrogenase-2 antisense gene. This purification strategy has been used as a basis to scale-up a GLP process for the production of material at the National Research Council of Canada to be used in preclinical trials of this gene therapy against alcoholism. (C) 2017 Society of Chemical Industry