Abstract

Significant problem and important cause of failure of the cancer chemotherapy is that even originally very sensitive tumors become resistant to effects of cytostatic drugs. Loss of sensitivity to specific chemotherapeutics agent may not directly cause the loss of sensitivity to other chemotherapeutics. However, it have been described that tumors resistant to one type of chemotherapeutics were found to be resistant to several other anticancer drugs that are different in both structure and mode of action. This phenomenon has been described as multidrug resistance (MDR). MDR can develop in several ways, with the predominant mechanism being the overexpression of ATP-binding cassette (ABC) transporters, such as P-glycoprotein. These transporters acts as energy driven pumps and which, maintain intracellular drug concentration below toxic levels.

The aim of this study was to examine the potential of novel polymeric therapeutics based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers bearing either anticancer drug, inhibitor of ABC transporters or both, for overcoming MDR mediated by P-glycoprotein in doxorubicin (Dox) resistant murine monocytic leukemia cell line P388/MDR. Series of low-molecular weight inhibitors reversin 121 (R121), reversin 205 (R205) and ritonavir (RIT) and their derivatives were tested for their ability to block the P-glycoprotein efflux pump. 5-methyl-4-oxohexanoyl R121 (MeOHe-R121) and 5-methyl-4-oxohexanoyl RIT (MeOHe-RIT) showed highest inhibitory activity out of all inhibitors derivatives tested. These derivatives were consequently conjugated to the HPMA copolymer carrier. Conjugate P-Ahx-NH-N=MeOHe-R121 showed superior ability to sensitize P388/MDR cells in dose-dependent manner and achieved almost 50-fold increase of cytostatic activity of Dox at 24 μM (highest concentration tested). Conjugate P-Ahx-NH-N=MeOHe-RIT at the highest tested concentration 12 μM was able to increase cytostatic activity of Dox more than 50 times. Finally, the cytostatic activity of three HPMA copolymer conjugates P-Ahx-NH-N=MeOHe-R121(Dox) bearing both Dox and P-gp inhibitor MeOHe-R121 both bound via pH-sensitive hydrazone bond with three different molar ratios of Dox: P-gp inhibitor (2:1, 1:1, 1:2) were tested and the best outcome was seen in conjugate with the higher content of P-gp inhibitor. The cytostatic activity was almost 45 times higher than that of the conjugate bearing only Dox in P388/MDR cells. Slightly less promising results were observed for P-Ahx-NH-N=MeOHe-RIT(Dox) conjugate with equimolar ratio of Dox and P-gp inhibitor, which exhibited about 10 times higher cytostatic activity than that of the conjugate with Dox only toward the P388/MDR cells.

Keywords: multidrug resistance, ATP-binding cassette transporters P-glycoprotein, ritonavir, reversin 121, reversin 205, inhibitors, *N*-(2-hydroxypropyl)methacrylamide