ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Tereza Staňková

Supervisor: prof. Ing. Vladimír Wsól, Ph.D.

Consultant: Mgr. Lenka Laštovičková, Ph.D.

Title of diploma thesis: Inhibitory effect of zopolrestat, VE-821 and berzosertib on the activity of selected reductases from the AKR and SDR superfamilies in vitro.

Increased activity of carbonyl-reducing enzymes (CRE) of the aldo-keto-reductase (AKR) and short-chain dehydrogenase/reductase (SDR) superfamilies is often associated with the development of some cancers. These enzymes are often overexpressed in these cancer tissues, promote their growth, and are largely involved in the deactivation of various cytostatics, including anthracyclines. One of the most widely used representatives of this group is daunorubicin (DAUN), whose metabolic conversion produces a more toxic alcohol metabolite, daunorubicinol (DAUNol), with reduced antitumor activity.

In the in vitro experiments performed in this thesis, the inhibitory effect of three substances (zopolrestat (ZOP), VE-821 and berzosertib) on the activity of selected CREs (AKR1A1, 1B1, 1B10, 1C3 and CBR1) was investigated. Inhibition of these enzymes leads to a decrease in their activity, and thus to a decrease in the production of the aforementioned metabolite DAUNol. Of the presented compounds, the highest inhibitory effect was observed for ZOP combined with the enzymes AKR1B1 and 1C3. The other two inhibitors did not show a sufficiently large inhibitory potential and therefore they were not included in further experiments. ZOP reduced AKR1B1 activity by 79.9 % at 10μM concentration and by 80.7 % at 50μM concentration. The activity of AKR1C3 was inhibited by 10μM ZOP from 82.7 % and 50μM ZOP from 94.6 %. Other kinetic parameters of ZOP were measured for both enzymes, namely, the IC50 of ZOP in

combination with AKR1B1 was 0.63 ± 0.04 μM and Ki was 0.46 ± 0.16 μM, and in the case of AKR1C3, the IC50 of ZOP was 2.32 ± 0.26 μM and Ki corresponded to 2.32 ± 0.26 μM. ZOP exhibited a mixed type of inhibition to both enzymes with a tendency to be non-competitive, and bound to both enzymes irreversibly. ZOP has been described in the literature, and is currently being investigated, as a potent inhibitor of the AKR1B1 enzyme for the treatment of diabetes-related complications. However, the data found in the present study suggest that ZOP inhibits the enzyme AKR1C3 equally effectively. Thus, given the overexpression of AKR1C3 in hormone-dependent tumor tissues, co-administration of the cytostatic DAUN with ZOP could lead to improved safety and success in the treatment of some cancers.