ABSTRACT

Classic galactosemia is an inborn error of metabolism associated with mutations that impair the activity and the stability of the dimeric enzyme galactose-1-phosphate uridylyltransferase (GALT), which catalyzes the third step in galactose metabolism. Out of more than 300 known mutations, p.Gln188Arg, a missense mutation located at the active site and in the dimer interface, is the most frequently found for GALT. It causes the almost total inactivation of the enzyme and impairs its stability, resulting in the most severe phenotype of the disease. In the past, and more recently, the structural effects of this mutation were deduced on the static structure of the wild-type human enzyme; however, we feel that a dynamic view of the protein is necessary to deeply understand their behavior and obtain tips for possible therapeutic interventions.

We performed molecular dynamics simulations of both wild type and p.Gln188Arg GALT proteins in the absence or in the presence of the substrates in different conditions of temperature. Our results suggest the importance of the intersubunit interactions for the correct activity of this enzyme and can be used as a starting point for the search of drugs able to rescue the activity of this enzyme in galactosemic patients.

Since no treatments, including the current one (the removal of galactose from the diet), are adequate to solve lifelong physical and cognitive disability, some research groups started searching for pharmaceutical chaperones towards GALT. Pharmaceutical chaperones are small molecules able to bind specific target proteins and to stabilize their native conformation or to correct misfolding in proteins affected by mutations thus rescuing their original function. In particular, it has been found that arginine was able to rescue the activity of several mutant GALT enzymes including p.Gln188Arg in a bacterial model of the disease. However, more recently, this rescue was not confirmed testing Arg directly on four galactosemic patients affected by p.Gln188Arg mutation. Given that no molecular characterization of the possible effects of arginine on GALT has been performed, and given that the number of patients treated with arginine is extremely limited for drawing definitive conclusions at the clinical level, we performed computational simulations to predict the interactions (if any) between this amino acid

and the enzyme. Our results do not support the possibility that arginine could function as a pharmacochaperone for GALT, but information obtained by this study could be useful for identifying, in the future, possible pharmacochaperones for this enzyme.

Simultaneously, we wondered if there might be an allosteric site in the GALT enzyme and if it could be used as a target to develop new pharmacochaperones for this enzyme. Through a computational predictor and considering our previous results, we identified a potential allosteric site corresponding also the to portion of the enzyme to which arginine interacts. This potential allosteric site can be a target for new candidate pharmachochaperones for human GALT.

A possible interaction between putative pharmacochaperones was simulated by molecular docking of both wild type and p.Gln188Arg GALT proteins. Starting from the best conformation of docking, the next step was to proceed with the search for pharmacophores, using the method of receptor-based pharmacomodelling. This led to the identification of five new ligands, which were selected for further docking on the allosteric site. All ligands selected showed promising results. These results were used to set up further molecular dynamics studies that are currently ongoing.

Preliminary tests of these ligands on fibroblasts from galactosemic patients showed their ability to lower galactose-1-phosphate concentration when fibroblasts are stressed by galactose. These preliminary data obviously need to be confirmed, but they are promising for the development of pharmacochaperon therapy for galactosemia.