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Beta-glucuronidase in physiology and disease

ß-glucuronidase (GUS) has a systemic name of glucuronohydrolase of ß-D-glucuronides (EC 3.2.1.31). The enzyme catalyses the hydrolysis of ß-D-glucuronides – the compounds arising as a result of the combination of ß-D-glucuronic acid and a number of both exo- and endogenous compounds containing hydroxylic, carboxylic, amine, imine or thiolic groups (3).

ß-glucuronidase belongs to globulins. It is characterized by a high molecular weight (over 200 000). It is a slightly acidic protein with isoelectric point in pH 5–6. The optimum activity of the ß-glucuronidases in the vertebrates lies in the acidic environment with pH 4.5–5.2 and shows species- and organ-dependent differences. The enzyme activity depends on a number of factors. One of them is the concentration of the substrate, whose excessive values inhibit the activity. The non-specific inhibitors irreversibly blocking the enzyme activity are mucopolysaccharides such as the heparin, chondroitin sulfate and hialuronic acid. Strongly inhibitory factors are: cholesterol, ß-carotene and retinol. ß-glucuronidase contains in its structure SH-thiolic groups, which is testified by the inhibitory action of p-chloromercurobensoesane and other organic compounds reacting with thiolic groups and of the cations of heavy metals such as copper, mercury and silver. The activity of the enzyme is controlled by the hormones, especially by the sterid hormones. ß-glucuronidase activators are the DNA and some diamines (10).

METHODS OF ASSAYING ACTIVITY

ß-glucuronidase catalyses the hydrolysis of ß-D-glucuronides, which arise as a result of the combination of ß-D-glucuronic acid with a number of both exo- and endogenous compounds containing hydroxylic, carboxylic, amine, imine or thiolic groups (3). These compounds comprise, among others: tyroxine, corticosteroids, estrogens, testosterone, aldosterone, catecholamines, bilirubin and many drugs and poisons (2). The origination of glucuronides constitutes one of the detoxicating mechanisms, because physiological compounds or those introduced from the outside lose their biological activity or toxic characteristics after combining with the glucuronic acid. Those compounds are better dissolved in water than glucuronides, which favours getting rid of them from the organism. The enzyme affects exclusively ß-glycoside bonds of glucuronides. It does not decompose either α-glucuronides or ß-glucupyranosides, but it hydrolyses ß-galacturonides (3).

Early methods consisting in assaying the freed glucuronic acid or an indecomposed substrate have been replaced by methods in which the freed aglycon is assayed. The most popular testing method is that using phenolphtalein glucuronide as a biosynthetic substrate. The substrate has a high affinity to the enzyme, is quickly hydrolysed and can be used for tissue examination. The activity of ß-glucuronidase is most often assayed using Fishman et al. method

(cit. from 10). It consists in the colorimetric measurement of the quantity of phenolphtalein freed from the substrate. For assaying this enzyme researchers also use ß-glucuronides containing in their aglycon part the following: p-nitrophenol, 8-hydroxychinolin, ß-naphtol or 4-metylo-7-hydroxycumarin. The freed aglycons are basic for the colorimetric assay of the enzyme activity and the last of the aglycons – for the fluorometric method. The activity of ß-glucuronidase is also assayed using histochemical methods.

ß-GLUCURONIDASE ACTIVITY IN PATHOLOGICAL PROCESSES

Changes in the activity can be observed in different physiological and pathological conditions (11,12). This is why a lot of research has been conducted on the enzyme. The studies on the changes of ß-glucuronidase activity influenced by different factors in physiological as well as pathological conditions have been done using experimental animals as well as humans (2). They have provided a lot of information on changes in the activity of the enzyme in peripheral blood cells and in bone marrow, particularly in neutrophils and lymphocytes, which partly explains different patomechanisms occurring in various abnormalities. In the serum of a healthy human one can determine the activity of ß-glucuronidase. Male serum displays a higher activity of the enzyme than the serum of women. Changes in the activity of ß-glucuronidase are observed in different pathological states of the liver (2). In acute liver diseases the activity of ß-glucuronidase in the serum rises noticeably. The increase is sustained for a longer period of time than the increase of the level of bilirubin and the increase of aminotransferase activity. In severe cases of acute hepatitis the activity of ß-glucuronidase in the serum drops abruptly. An increased level of the enzyme in the serum was also observed in the cases of cirrhosis of the liver of mild or medium course of the disease. In severe cases though the activity of ß-glucuronidase in the serum remains normal or is slightly decreased.

According to Rogala (9) ß-glucuronidase activity assay in the serum is the most sensitive laboratory method allowing the detection of balanced mild cirrhosis of the liver (i.e. one without jaundice, ascites or haemorrhages), when almost all the other enzymatic tests yield negative results.

An increased activity of ß-glucuronidase in the serum is also observed in 60% cases of cholestatic jaundice. Pineda et al. (cit. from 2) think that the typical changes in the activity of ß-glucuronidase in the serum (an increase and then decrease with accompanying clinical symptoms) are a very sensitive tool indicating the functional state and the stage of disorganisation of a hepatocyte.

β-glucuronidase present in the serum comes mainly from the liver. In hepatocytes it is present not only in lysosomes but also within the endoplasmic reticulum (2). Marogg and Wegmann and Rafałowicz (cit. from 2) observed a higher level of β-glucoronic acid in the blood of diabetic people. According to Rogala (9) the increase in the activity of β-glucuronidase in the serum of diabetic people is to be associated with higher metabolism of the glucuronic acid.

In neutrophils GUS constitutes a part of the enzymatic equipment of lysosomal grains of those cells (8). In old people glucuronidase-positive pool of the neutrophils in peripheral blood decreases considerably, which is connected with a total absence in the circulating blood of cells rich in the enzyme. The phenomenon has been confirmed by reports of decreased antibacterial immunity in those people (7). Glucuronidase thus constitutes a part of the anti-micro-organism enzymatic euqipment of the neutrophils. Its activity in neutrophils increases in inflammatory reactions – a proof of the participation of those cells in phagocytosis and in biochemical degradation of different biological materials especially those of micro-organism origin (4,8).

It should be noted here that GUS has a biological role to play in the lymphocytes. It is not so strictly defined as in the neutrophils. It is estimated that GUS, together with other lysosomal enzymes of the lymphocytes, plays a part in the removal of intracellular remnants of organelles

and other cell components arising during mitotic division. There are data indicating a connection between GUS activity in the lymphocytes and the immunological reactivity of those cells. Lymphocytes showing a lack or intracellular deficiency of the enzyme are less valuable biologically. It has been proved by reports on the presence of low-activity β-glucuronidase in the lymphocytes of people with chronic lymphocytic leukaemia and those with Hodgkin's disease, who present an impaired cellular and humoral immunological reactivity (1,5). The link between GUS activity in the lymphocytes and the immunological competence of these cells is also confirmed by observations of changes in the activity of the enzyme during fetal development. Within 12–14 weeks of fetal life there is almost a complete lack of β-glucuronidase in the lymphocytes. In premature infants the activity of the enzyme in the lymphocytes is lower than in biologically mature ones. Its lowest values appear in premature babies born between 21 and 24 weeks of pregnancy.

Basing on the above data it can be stated that GUS activity is a function of the ontogenetic maturation of the immunological system related to the lymphocytes (6). An increase in β-glucuronidase activity in the lymphocytes was observed in patients with systemic lupus, rheumatoid arthritis, reactive lymphocytoses accompanying Sezary's syndrome, sarcoidosis and tuberculosis. The overall GUS activity in the lymphocytes also increases in people with cat-scratch disease, measles, rubella and cowpox. That activity decreases in Wilson's disease (8).

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SUMMARY

β-glucoronidase (EC 3.2.1.31) is a lysosomal enzyme catylysing the decomposition of β-D-glucoronides – compounds arising as a result of the combination of β-D-glucoronic acid and a number of compounds both exo- and endogenous, containing hydroxylic, carboxylic, amine,

imine or thiol groups. The most common test evaluating the activity of the enzyme is that using phenolphtalein glucoronide as a biosynthetic substrate. The freed aglycons are colorimetrically assayed. The activity of β-glucoronidase increases in many pathological conditions: liver inflammations, cirrhosis of the liver, inflammations of other organs, cholestatic jaundice, tuberculosis, sarcoidosis and also in neoplasms. Many authors point to β-glucoronidase as a sensitive indicator signalling cell damage.

Beta-glukuronidaza w stanie zdrowia i choroby

β-glukuronidaza (E.C. 3.2.1.31) jest enzymem lizosomalnym, katalizującym rozkład β-D-glukuronidów – związków powstałych w wyniku połączenia kwasu β-D-glukuronowego z szeregiem związków, zarówno egzo-, jak i endogennych, zawierających grupy hydroksylowe, karboksylowe, aminowe, iminowe lub tiolowe. Najbardziej rozpowszechnionym badaniem aktywności enzymu jest metoda używająca jako substratu biosyntetycznego glukuronidu fenolftaleiny. Uwolniony aglikon oznacza się kolorymetrycznie. Aktywność β-glukuronidazy wzrasta w wielu stanach patologicznych: chorobach zapalnych wątroby, marskości watroby, stanach zapalnych innych narządów, żółtaczce zastoinowej, gruźlicy, sarkoidozie, a także w chorobach nowotworowych. Wielu autorów wskazuje na β-glukuronidazę jako czuły wskaźnik uszkodzenia komórki.