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β-glucoronidase in the blood serum and urine of children with urinary tract infection

As protein catalysts stimulating a great number of chemical reactions that take place in every living cell, enzymes play a vital role in the metabolic processes of an organism. Numerous reactions controlled by the enzymes underlie all life symptoms such as growth, respiration or digestion. Hence changes in enzyme activity often signal disruption in the functioning of various biological systems. Identifying the direction of those changes can aid diagnosis and outline the course of therapy.

β-glucoronidase (β-GR) is an enzyme belonging to a group called β-glucosidases. It catalyses β-D-glucoronid breakdown, that is the combination of β-D-glucoronic acid and the compounds containing hydroxylic, carboxylic, amine, imine or thiolic groups. β-glucoronids are the products of detoxicating processes, and the reaction of their hydrolysis (catalyzed by β-glucoronidase) results in the derivation of active forms of aglycones such as sterid hormones or drugs (1,6). β-glucoronidase was found in all mammalian tissues and body fluids as well as in plants, *Invertebrates* and some microorganisms (17).

In peripheral blood cells – neutrophils and lymphocytes – β-glucoronidase is part of the enzymatic composition of lysosomal grain wherefrom, together with other enzymes, it is directly delivered to its destination places without prior synthesis. Like the other lysosomal enzymes of neutrophils β-glucoronidase assists biochemical degradation of fagocytised microorganisms. Therefore in an inflammatory reaction the activity of the enzyme in the nutrophils may increase (2,10). On the other hand, decreased antibacterial immunity (e.g. with the elderly) is accompanied by low levels of glucoronidase (+) pool of neutrophils in the peripheral blood. In lymphocytes, β-glucoronidase probably assists the cleanup of the remnants of organelles after mitosis. Lack or deficiency of the enzyme in patients with significant humoral and cellular immunological response impairment suggests a relationship between β-glucoronidase activity in the lymphocytes and their immunological efficiency (13).

The purpose of the study was to assay the activity of β-glucoronidase in the blood serum and urine of children with urinary tract infections and to find correlations between the activity of the enzyme in the serum and in the urine.

MATERIAL AND METHODS

The studied samples were blood serum and urine of children hospitalized at the Department of Infant Pathology in Children's Clinic in Lublin who were diagnosed as suffering from urinary tract

infection caused mainly by *Escherichia coli, Psudomonas spp., Proteus spp.* Control samples were blood serum and urine of healthy children from the Department of Orthopaedics in the same hospital.

Analysed were 30 samples of the serum and 30 samples of the healthy urine as well as 30 samples of serum and 30 samples of urine obtained from the children with urinary tract infection.

The activity of β-glucoronidase in the studied material was assayed using phenylphtalein β-glucoronide taking advantage of the enzyme's ability to cathalyse the hydrolysis of the compound. The method consists in evaluating the degree of the hydrolysis of the substrate (phenylphtalein β-glucoronide) by the enzyme in acidic conditions. Phenylphtalein – the aglycon freed from the substrate – comes out pink in alkaline conditions. The intensity of the color is measured spectrophotometrically at the wavelength of 540 nm (19).

The results were analysed statictically using t-student test for independent groups, assuming as statistically significant the differences at the significance level of $p < 0,05$.

RESULTS

The activity of β-glucoronidase in the serum of the control was between 550.32 mU/l and that in the urine was 227.32 mU/l. In the infected children its activity in the serum was 625.22 mU/l and in the urine – 54.16 mU/l. Between the activity of the enzyme in the examined group of the sick and that of the control statistically significant differences are only present in the case of the urine. There are none in the serum of either of the groups. No correlations were found between β-glucoronidase activity in the serum and its activity in the urine either in the control or in the infected group of children (in the sick group $r = 0.27$; $p = 0.172$ and in the control $r = -0.25$; $p = 0.247$).

Table 1. The activity of β-glucoronidase in the blood serum and urine of children with urinary tract infections and in healthy children

Material	Patients		Control group		t	p
	median	SD	median	SD		
Blood Serum	625.22	158.31	550.32	187.89	1.64	0.106
Urine	54.16	38.79	227.32	188.45	-4.84	0.0001

SD – standart deviation

DISCUSSION

Changes in the activity of β-glucoronidase can be seen in various pathological as well as physiological conditions. Numerous studies have been conducted concerning the behaviour of that enzyme in illness and in a healthy organism. For example, shifts in the level of β-glucoronidase were noticed in women's menstruation as well as during pregnancy. This phenomenon is a consequence of an increase in the level of estrogens (in the first 15–20 days of menstruation, and, in pregnancy – until the day of delivery), which corresponds with increased values of the enzyme in the peripheral blood (20). During the study we also noticed a meaningful increase in the level of β-GR activity in the amniotic fluid and the blood serum of women with complications of pregnancy in the last trimester as compared with the results of the study on a group of normal pregnancies (15). The bottom line is that changes in the enzyme activity within the amniotic fluid are a sensitive albeit unspecific sign of an overall condition of the fetus and can be a useful additional criterion while evaluating the hazards of pregnancy. At a high level of β-GR activity in the amniotic fluid and in the serum a poor overall condition of a newborn baby is usually observed after

delivery. The activity of β-glucoronidase also increases considerably in gestosis. Its extremely high values were noted in pre-eclampsia (with an accompanying low level of estrogens), which is used in diagnostics as an early symptom of the condition (20).

Studies of an influence of age and sex on plasmatic β-GR activity have also determined an interdependence between them: in female babies increase in the activity of the enzyme lasted longer than in male babies (14).

Changes in β-glucoronidase activity are seen among other things in different liver functioning disorders (11), in infants, in diabetes and in various kidney diseases. Serious liver dysfunction is characterised by an increase in β-GR activity in the serum that lasts longer than that of bilirubin and aminotransferases, while in extremely severe cases β-GR activity drops significantly. A slight increase of the enzyme levels is observed in mild cirrhosis. Assessment of β-glucoronidase activity in the serum is said to be the most reliable laboratory test for diagnosing mild cirrhosis when most enzymatic tests give negative results. An increased activity of β-glucoronidase in the serum characterises 60% of cholestatic jaundice cases. Certain characteristic changes in β-GR activity in the blood serum reflect the functioning status of hepatic cells and the extent of their disorganisation (6).

β-glucoronidase is also one of the biochemical instruments for measuring the advance of neoplastic lesion within the bladder. Increase of the enzyme activity in the urine of patients suffering from bladder cancer, determined in the 1950s, was confirmed in later studies: it was found that the increase was present in 72–25% of the patients (18). Lack of an increase in the remaining group was probably due to the presence of β-glucoronidase inhibitor in their urine (6).

Significant changes in the activity of β-glucoronidase are seen in the blood and tissues of people affected by other neoplasms. Deficiency of the enzyme in the lymphocytes and neutrophils may lead to anti-cancer immunodeficiency of the organism by crippling the cytotoxic potential of those cells. There are data confirming the decrease of β-GR activity in neutrophiles that accompanies chronic lymphatic leukemia, cancer of larynx or acute capillary-cell leukemia (8, 9, 12).

High activity of β-glucoronidase was found in cancer of the breast, cervix, penis, colon, lungs, skin, brain and stomach. Patients with malignancies of the central nervous system exhibit an increased activity of the enzyme in their cerebrospinal fluid while those with vaginitis – in their vaginal discharge and those with stomach cancer – in their gastric juice (10).

Kidney disorders trigger off changes in the activity pattern of the urinary enzymes through the process of filtration and resorption operating within the nephrone (5). The most significant differences affect the renal enzymes like β-glucoronidase present in large quantities in the kidneys. Nephropathies are characterised by intensified eradication of enzymes both of renal and extrarenal origin (3). After examining changes in the activity pattern of three chosen enzymes in children with nephrotic syndrome we found a statistically significant increase as compared with healthy children. The examined enzymes included alanyloaminopeptidase, β-glucoronidase and N-acetylo-beta-D-glucosoaminidase. We assumed that it was due to a damage of the renal tubules accompanying the nephrotic syndrome that there was an increased release of intracellular renal enzymes into the urine. Concomitant lack of significant changes between β-glucoronidase activity in healthy children and those with remission (where alanyloaminopeptidase and N-acetylo-beta-D-glucosoaminidase activity remains high) clearly indicates compensatory ability of β-GR during remission as compared with the remaining two enzymes. Also, there is a correlation between the activity of β-GR and N-acetylo-beta-D-glucosoaminidase. Activity assessment of all three of the above enzymes in children with nephrotic syndrome can serve as a useful tool identifying the locus and degree of nephron damage. It can also facilitate observation of the dynamics of the changes during morbidity process, treatment and remission (4).

Numerous studies have been conducted of the behaviour of β-glucoronidase in different urinary tract disorders. Apart from above mentioned bladder neoplasms we studied the level of β-GR in people with different renal disorders such as pyelonephritis, kidney graft rejection or severe renal failure. We compared the levels of β-glucoronidase and alkaline phosphatase in the urine and the blood serum of patients with the illness mentioned above as opposed to healthy

patients. β-GR levels proved higher in the case of urine samples belonging to each of the examined groups as compared with the control while the enzyme level in the plasma did not change (it was not so with alkaline phosphatase). Therefore, studying the level of the two enzymes both in the blood and in the urine can be of a diagnostic value in various renal dysfunctions (16).

The observations presented above show that the enzyme in question changes its blood and urine activity level in different pathological conditions. It can therefore be a diagnostic tool in certain morbidity states. Encouraged by the results we decided to study the activity of the enzyme in the serum and urine of children with urinary tract infections. Concomitant with the study, we marked the activity of β-glucoronidase in the serum and urine of healthy children, who made up a control group.

The results we arrived at indicate a statistically significant lower activity of the enzyme in the urine of the sick children as compared with the control. Statistically significant differences were found between the activity of the enzyme in the urine of the sick and the healthy children. No correlation was found between the activity of β-glucoronidase in the serum of the blood or the urine of the selected patients (it is true of the control too). The significant changes in the activity of β-GR in urinary tract infections as shown in the present paper confirm the earlier theory on urinary tract infections affecting the enzyme. The direction of the changes, however, stays in opposition to what the other authors found regarding increased activity of β-glucoronidase in the urine for different urinary tract disorders.

CONCLUSIONS

1. The activity of β-glucoronidase in the urine of sick children is lower than in the control group.

2. No correlation was found between the activity of β-glucoronidase in the blood serum or the urine either in children with urinary tract infections or in the control.

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SUMMARY

The purpose of the paper was to study changes in the activity of β-glucoronidase in the blood serum and in the urine of children with urinary tract infections and to find a correlation between the activity of the enzyme in the blood serum and that in the urine of children with urinary tract infections. Finding significant changes in the activity of β-glucoronidase in children with the illness would mean finding a valuable diagnostic tool for those kinds of infections. The analysis was conducted on two groups of children. The first group were the patients of Infant Pathology Department in Prof. Antoni Gębala Children's Clinic in Lublin, diagnosed as having urinary tract infections caused mainly by *Escherichia coli, Pseudomonas spp.* and *Proteus spp.* The other group was made up of healthy children. The material studied were blood serum and urine samples for both groups. The activity of β-glucoronidase in the studied material was assayed using Fishman's method. Based on the results it has been determined that there are noticeable changes in the activity of the enzyme correlated with renal and urinary tract morbidity. The evidence we found confirms the influence of urinary tract infections on the activity of β-GR. Contradictory evidence as compared with the earlier theories on the direction of the changes (a decrease not increase of the β-GR activity in the urine) can suggest a coexisting, modifying influence of some other factors, which requires further study.

Aktywność β-glukuronidazy w surowicy krwi i w moczu u dzieci z zakażeniami dróg moczowych

Celem pracy było zbadanie zmian aktywności β-glukuronidazy w surowicy krwi i w moczu dzieci z zakażeniami dróg moczowych oraz sprawdzenie, czy istnieje korelacja między aktywnością tego enzymu w surowicy krwi a aktywnością w moczu u dzieci z zakażeniami układu moczowego. Stwierdzenie istotnych zmian aktywności β-glukuronidazy u dzieci objętych tą jednostką chorobową miałoby znaczenie jako dodatkowy wskaźnik ułatwiający rozpoznawanie tego rodzaju zakażeń. Analiza została przeprowadzona w dwóch grupach dzieci. Pierwszą stanowili pacjenci Oddziału Patologii Niemowląt w DSK im. prof. A. Gębali w Lublinie, u których rozpoznano zakażenie układu moczowego, spowodowane głównie przez *Escherichia coli*, *Pseudomonas spp.* i *Proteus spp.* Druga grupa to dzieci zdrowe. Materiałami poddawanymi badaniu w obu grupach były surowica krwi oraz mocz. Aktywność β-glukuronidazy w materiale badanym w tej pracy

została oznaczona metodą Fishmana. Na podstawie uzyskanych wyników stwierdzono wyraźne zmiany aktywności enzymu, pozostające w związku z obejmującym nerki lub drogi moczowe procesem chorobowym. Otrzymane w niniejszej pracy wyniki potwierdzają wpływ zakażeń układu moczowego na aktywność β-GR. Inny kierunek zmian w porównaniu z wcześniejszymi doniesieniami (spadek, a nie wzrost aktywności β-GR w moczu) może natomiast sugerować jednoczesny modyfikujący wpływ wielu innych czynników, co sygnalizowałoby konieczność przeprowadzenia dalszych badań.