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The effect of administration of high doses of selected antioxidants upon the activity of beta-glucuronidase (β-GL) in liver, kidney and blood plasma of mice

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The activity of beta-glucuronidase (β-GL) was determined in lysosomal, microsomal and cytosolic cellular subfractions of liver and kidney of mice and in mice blood plasma after intraperitoneal injections of reduced glutathione (GSH) and vitamins A, E and C. The β-GL activities were found to depend on the antioxidant used, organ investigated and cellular subfraction analysed. The least β-GL reactivity was shown in blood plasma. It is concluded that the antioxidants injected can increase the activity of β-GL in cellular subfractions, particularly in microsomal fraction. The increase could be interpreted as physiological response of redox processes. The results presented allow to assume that the activity of β-GL can be used as an indicator of homeostasis disturbance.

KEY WORDS: antioxidants / beta-glucuronidase / cellular subfractions / glutathione / lysosomal enzymes / vitamins

The lysosomal system, which eliminates foregin and endogenous molecules under physiological conditions, is the best developed degradation system of the cell [Kołątaj *et al*. 2001, Lankoff and Kołątaj 2001, Jóźwik *et al*. 2003]. Lysosomes seem especially sensitive to oxidative stress [Hellquist *et al.* 1997, Yuan *et al*. 2000, Śliwa-Jóźwik *et al*. 2004]. Cytotoxic effects of oxidative stress are connected with the destabilizing of lysosomal membranes resulting in the release of lysosomal hydrolases into the cytosol with an induction of apoptosis or necrosis [Roberg 2001, Śliwa-Jóźwik *et al.* 2002ab, Ogawa *et al*. 2004].

Glucuronidation represents a major pathway enhancing the elimination of many lipophilic xenobiotics and endobiotics as well as formation of water-soluble compounds, thus being one of the most important pathways of detoxication in mammals [Dutton 1980, Kasper and Henton 1980]. Some compounds must undergo phase I oxidative, reductive, and hydrolytic modifications before the phase II conjugation reaction takes place [Dwivedi *et al*. 1987]. As glucuronidation plays a role in the clearance of many endogenous compounds, drugs, and toxic intermediates (including carcinogens), important is to examine its contribution to the overall reaction of the organism. Glucuronidation is a metabolic process by which a sugar moiety is enzymatically conjugated to a compound of interest *via* -OH, -NH2, -SH, or -COOH functional groups. The resulting conjugate or glucuronide is more hydrophilic than the original compound, and its elimination through excretion is easier [Mitchell and Whitcombe 2000]. Beta-glucuronidase is one of more important enzymes which can neutralize toxins.

Interactions between antioxidants and activity of lysosomal hydrolases in connection with glucuronidation reactions in cellular subfractions are unknown.

This paper aims at analysing the effect of high doses of exogenous antioxidants (reduced glutathione, L-ascorbic acid, vitamin A, vitamin E) on the reactivity of beta-glucuronidase (β-GL) in lysosomal, microsomal and cytosolic subfractions of the liver and kidney cells, and in blood plasma. Antioxidant doses used (μg/g body weight daily) were derived from their respective recommendations for humans.

Material and methods

Animals

Sixty Swiss male mice aged 8 weeks from the Polish Academy of Sciences Institute of Genetics and Animal Breeding were used, weighing 25±1.2 g. The animals were maintained under standard conditions at 21°C, with light/dark exposure of 12/12 h, fed the standard pelleted diet (16% protein) supplied by the Animal Feed Production Company, Łomna, Poland, and had free access to water.

The mice were divided into six groups, 10 animals per group.

Reduced glutathione – GSH (γ-Glu-Cys-Gly from SIGMA-ALDRICH) – and vitamin C (L-*Acidum ascorbicum* from POLFA, Poland) were administered intraperitoneally in doses of 100 μg/g and of 250 μg/g body weight, respectively, in 0.9% NaCl, in a volume of 12 μL/g body weight, at 8 a.m. and 4 p.m. for seven consecutive days. Ten animals injected with only 0.9% NaCl twice a day were treated as control (Tab. 1).

Vitamin A (*Vitaminum A*, POLFA, Poland) and vitamin E (*Tocopherolum aceticum*, POLFA, Poland) were administered intraperitoneally in a dose of 1.5 μg/g and 10 μg/g body weight, respectively. Solutions of both vitamins in arachidonic oil (POLFA, Poland) in a volume of 6 μL/g body weight were injected once a day at 9 a.m. for seven consecutive days. Ten animals injected daily with only 6 μL/g body weight arachidonic oil were treated as control (Tab. 1).

Table 1. Experimental schema

Group	n	Injection
I control (NaCl)	10	0.9% NaCl
GSH	10	Glutathione (GSH) (L-Glu-Cys-Gly; SIGMA) (100 g/g body weight)
Vitamin C	10	Acidum ascorbicum (POLFA, Cracow) (250 g/g body weight)
II control (oil)	10	oil *per injectionem* (Arachidonic oil, POLFA, Warsaw)
Vitamin E	10	Tocopherolum aceticum (POLFA, Warsaw) (10 g/g body weight)
Vitamin A	10	Vitaminum A (POLFA, Warsaw) (1.5 g/g body weight)

On day 8 of the experiment all animals were killed by decapitation at 8 a.m. and their liver, kidney and blood plasma were immediately prepared for analyses.

The total experimental procedure was approved by the Local Ethics Commission for Animal Research.

Analytical

All the procedures were conducted at 0-4°C. The organs were rinsed with 0.9% NaCl and suspended in a buffer including 0.25 M saccharose solution and 2 mM EDTA in a ratio of 1 g tissue per 7 mL of buffer, and next homogenized in a Potter-Elvehjem-type homogenizer at 200 rpm. The preparation of cellular subfractions was performed according to Marzella and Glaumann [1980].

The activity of beta-D-glucuronidase (β-GL, EC 3.2.1.31) was determined in the liver and kidney lysosomal, microsomal, and cytosolic cellular subfractions and in blood plasma according to Barrett and Heath [1972] with p-nitrophenyl β-D-glucuronide (SIGMA-ALDRICH) as a substrate, measured at 420 nm. The total enzyme activity was measured after incubation at 37°C and expressed in nmol/mg protein/hour. The total protein content in the tissue was determined after Kirschke and Wiederanders [1984] with a bovine serum albumin (SIGMA-ALDRICH) as a standard. The method quoted by Krawczyński and Osiatyński [1967] was used to determine the total plasma protein concentration. All biochemical measurements were performed with the spectrophotometer Lambda Bio 20/1998 (PERKIN-ELMER).

Statistical

The results were expressed as means and their standard deviations (SD). Differences between control and experimental groups in each tissue and fraction were evaluated with three-way analysis of variance, using the Kramer's test with SAS software package [SAS 2001].

Results and discussion

Activities of β-GL were found related to antioxidant, tissue and cellular subfraction investigated.

Figure 1 presents the β-GL activity following injections of GSH and vitamin C. Activities are expressed as per cent of values obtained with the control NaCl injections. In the liver, after GSH injection, observed was a significant decrease in the β-GL activity of lysosomal subfraction, accompanied with its increase in microsomes and cytosol. In kidney an increase in the β-GL activity was oberved in lysosomal and microsomal subfractions. The injection of vitamin C caused an increase of the β-GL activity in all estimated fractions of both organs, with an exception of the liver cytosol.

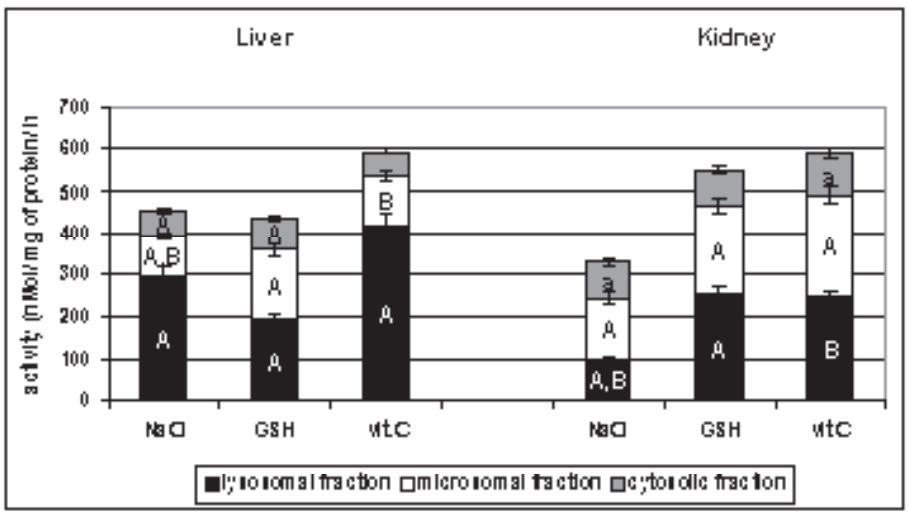

Fig. 1. The influence of GSH and vitamin C on the activity of β-GL in the liver and kidney lysosomal, microsomal and cytosolic fraction. The data are presented as means ±SD in comparison with the control groups. The data were analysed using a three-way analysis of variance (a – P≤0.05; A,B – P≤0.01).

Figure 2 presents the β-GL activity following injections of vitamin A and E. Again, the activities are expressed as per cent of values obtained with the control oil injections. Vitamin A increased the activity of β-GL in all estimated cellular subfractions of both organs. Vitamin E injections led to the increase in activity of β-GL in the lysosomal and microsomal subfractions of the liver and in microsomal and cytosolic subfractions of the kidney.

Figure 3 shows the effect of injection mice with antioxidants on the activity of β-GL in blood plasma. The significant decrease of the activity of β-GL expressed as per cent values of controls was observed only after injection of vitamin E.

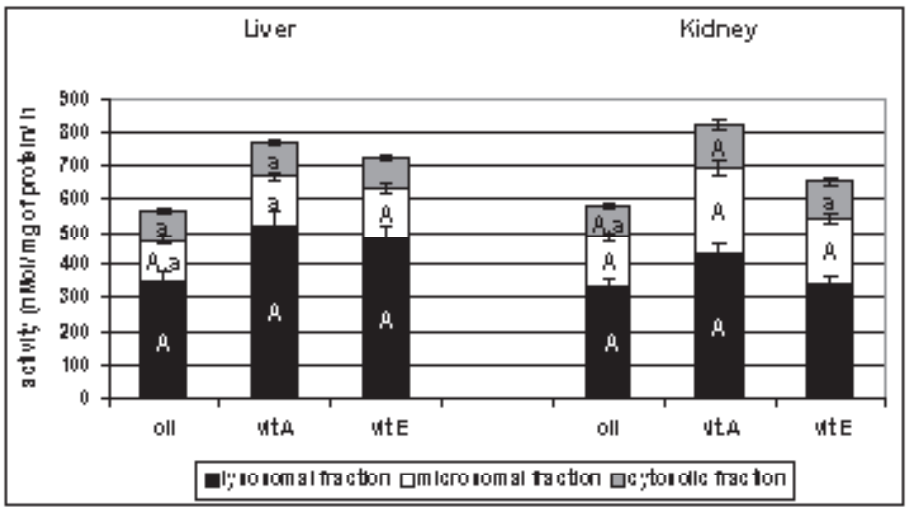

Fig. 2. The influence of vitamin A and vitamin E on the activity of β-GL in the liver and the kidney lysosomal, microsomal and cytosolic fraction. The data are presented as means ±SD in comparison with the control groups. The data were analysed using a three-way analysis of variance (a – P≤0.05; A – P≤0.01).

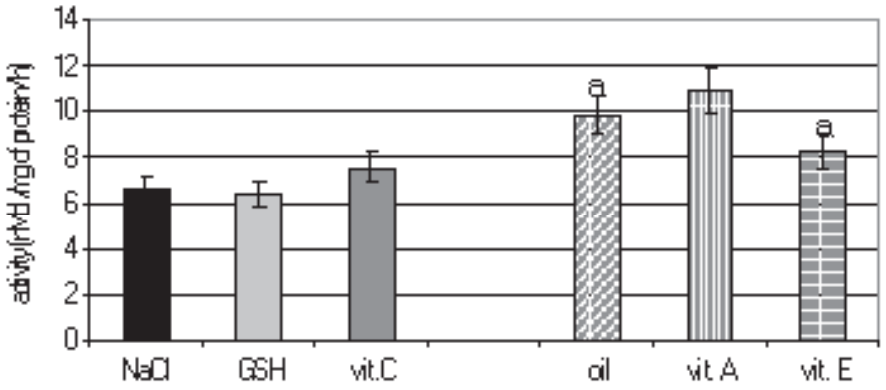

Fig. 3. The influence of antioxidants on the activity of β-GL in blood plasma. The data are presented as means ±SD in comparison with the control groups. Data were analysed using a three-way analysis of variance (a – P≤0.05).

β-GL is an acid glycoprotein in mammalian tissues. In rats and mice β-GL shows a unique subcellular distribution with a significant activity being associated with both microsomal and lysosomal cellular subfractions, at a ratio of 1:3, respectively [Hoja-Łukowicz 1999]. The present study showed high activity of β-GL in the lysosomal subfractions of both liver and kidney, but in the kidney the main source of β-GL activity was the micro-

somal subfraction. In blood plasma the activity of β-GL was the lowest.

Earlier studies by Witek and Kołątaj [1998] and Śliwa-Jóźwik *et al.* [2002b, 2004] showed that injection of reduced glutathione (GSH) led to increased GSH content of blood and some organs in mice.

In this report, GSH caused a significant decrease in the β-GL activity in lysosomal and increase in microsomal and cytosolic subfractions of liver cells. Many endogenous or xenobiotic lipophilic substances are eliminated from the cells by the sequence of oxidation, conjugation to an anionic group (glutathione, glucuronate or sulfate) and transport across the plasma membrane into the extracellular space [Homolya *et al.* 2003]. It was also hypothesized that the reactivity of acyl glucuronides towards smaller nucleophiles such as glutathione may play a role in the toxicity of some acidic drugs as well as enhancing the toxicity of other xenobiotics by glutathione depletion [Shipkova and Wieland 2005]. Lankoff and Kołątaj [2001] showed that toxins like microcystine-YR and nodularin can change the reactivity of β-GL in the mouse liver. Another function of GSH is detoxication of xenobiotics or their metabolites. The conjugates produced are excreted from the cell, and in the case of hepatocytes they reach a bile [Lu 1999]. The present report suggests that under high intake conditions, GSH may cause an efflux of β-GL from lysosomal to other cellular subfractions. An activation of the enzyme by GSH in microsomal and cytosolic subfractions may be important in hepato-bilio-enteric circuit [Mittur *et al.* 1998].

In the kidney, GSH increased the β-GL activity in both lysosomal and microsomal fraction (Fig. 1). It is known that GSH-conjugates of plasma are delivered to kidneys, where they are further metabolized or excreted as mercapturates [Lash *et al.* 1999]. The authors of the present report suggest that the selective cell distribution of β-GL in the kidney and the GSH conjugation pathway both determine the renal selectivity of GSH-derived metabolites.

An increase in the activity of β-GL in microsomes of liver and kidney may be related to its post-translational modifications. The microsomal form of the enzyme is a precursor of the lysosomal enzyme [Hoja-Łukowicz 1999]. Beta-glucuronidase is a thiol hydrolase that requires thiol compounds (e.g. GSH) for its activity. Stimulatory effects of thiols on intralysosomal hydrolysis may be to keep the active-site sulphur of those proteins in the reduced status or inactivate the proteases inhibitors [Śliwa-Jóźwik *et al.* 2004].

Vitamin C caused an increase in the β-GL activity of all cellular subfractions of both organs, with an exception of the liver cytosol (Fig. 1). Roomi *et al*. [1998] suggested that ascorbic acid induces the activity of the phase II enzymes involved in a conjugation and detoxication of xenobiotics. The present report shows that such effect of vitamin C may be related to β-GL activation principally in lysosomal and cytosolic subfractions. It may also be connected with a fact that vitamin C activates the cholesterol 7α-monooxygenase [Benzie 1999]. It may cause an increase in bile acids production and participate in deconjugation reaction related to bilirubine glucuronide hydrolysis. Horio and Horie [1997] suggested that β-GL participates in ascorbic acid biosynthesis stimulated by

xenobiotics. The authors of the present report are of opinion that in this biosynthesis microsomal and lysosomal forms of β-GL play an important role.

Vitamin A led to increased activity of β-GL in all cellular subfractions of both organs (Fig. 2). Early hepatotoxic alterations in different cell organelles induced by hypervitaminosis were reported by Schneider *et al.* [1997]. It is known [Barua *et al.* 1998, Kaul and Olson 1998] that high doses of vitamin A are neutralized in a smooth endoplasmic reticulum through binding by retinoid-binding proteins. This results in all-trans-retinoyl β-glucuronide (RBG) production, which is first identified as a biliary metabolite of vitamin A and next is secreted to urine. The authors of the present report suggest that vitamin A in such large doses increases the detoxitative effect of β-GL activity on retinoyl esters. RBG formation may enhance their rate of biliary and urinary excretion. The data presented show that vitamin A does not affect the activity of β-GL in blood plasma.

After vitamin E injections an increase in the β-GL activity was observed in the lysosomal and microsomal subfractions of the liver (Fig. 2). Lutz *et al.* [1998] showed that an antioxidant vitamin supplementation induces the modifications of the liver microsomal membrane composition in the rat, including an activity of enzymatic system involved in the metabolism of endogenous substances and xenobiotics, e.g. of microsomal glucuronyltransferase (UDP-GT). An increased lysosomal and microsomal β-GL activity in the liver is perhaps related to an intensity of production of glucuronide groups as substrates for UDP-GT. In the kidney an increase was observed in β-GL activity in microsomal and cytosolic fractions. It can be assumed that a necessity of secretion of products of vitamin E metabolism destabilizes the lysosome membranes and leads to enlargement of endoplasmic reticulum and migration of the enzyme into cytosol.

In blood plasma the vitamin E injection caused a decrease in the activity of β-GL (Fig. 3). This may be connected with the metabolic pathway of vitamin E in the tissues.

Results presented here reveal that the injected antioxidants induce the activity of the β-GL in tissue and increase its activity depending on cellular fraction.

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Wpływ wysokich dawek wybranych antyoksydantów na aktywność beta-glukuronidazy (β-GL) w wątrobie, nerkach i osoczu krwi myszy

Streszczenie

Aktywność beta-glukuronidazy (β-GL) oznaczono w podfrakcjach (lizosomowej, mikrosomowej i cytozolowej) mysich komórek wątroby i nerki oraz w osoczu krwi myszy po dootrzewnowym podawaniu przez 7 kolejnych dni zredukowanego glutationu (GSH) i witamin A, E i C. Zaobserwowano zmiany w aktywności β-GL w badanych podfrakcjach komórkowych, zależne od zastosowanego antyoksydantu oraz badanego narządu. Najniższą aktywność badanego enzymu stwierdzono w osoczu krwi. Wnioskuje się, że iniekcje wysokich dawek antyoksydantów mogą zwiększać aktywność β-GL w wybranych rejonach komórki, zwłaszcza w jej frakcji mikrosomowej. Wzrost aktywności tego enzymu może być wynikiem adaptacyjnej odpowiedzi na procesy redoks. Zaprezentowane wyniki potwierdzają możliwość zastosowania β-GL jako markera zaburzeń homeostazy komórkowej.