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COMPARISON OF ACTIVITIES OF GLUTATHIONE ENZYMES IN CEACUM AND LIVER OF CATTLE, HORSE, PIG, RABBIT AND SHEEP

O. FEDETS
Stepan Gzhytskyi Lviv National University of Veterinary Medicine and Biotechnologies, Department of Biological and General Chemistry, 79010 Lviv, Ukraine

Abstract

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The level of glutathione and activities of glutathione S-transferase, glutathione peroxidase and glutathione reductase in caecum and liver of food-producing animals have been investigated and compared. The activity of glutathione S-transferase is the highest in the liver, but that of glutathione reductase is the highest in mucosa of caecum. Pig and rabbit have significant differences of glutathione peroxidase activity in caecum and liver. The highest content of glutathione and activity of glutathione S-transferase were in sheep liver and rabbit caecum, the lowest were in cattle. The highest glutathione peroxidase activity was in pig liver and cattle caecum. In horse the activities of glutathione peroxidase and glutathione reductase were the lowest. The highest glutathione reductase activity was in liver of cattle and caecum of cattle and pig.

Key words: glutathione enzymes, caecum, liver, farm animals

Abbreviations: GST – glutathione S-transferase; GPx – glutathione peroxidase; GR – glutathione reductase; GSH – glutathione; GSSG – oxidized glutathione

Introduction

The importance of research on biotransformation processes in food-producing animals grows not only because of permanent exposure to industrial or agricultural contaminants but also because of the frequent use of pharmacologically active substances. However, biotransformation in food-producing species is not only of relevance for veterinary pharmacotherapy and toxicology but also for health of man (Szotakova et al., 2004).

It is difficult to extrapolate information derived from one species of animal to another species. However, it is possible to utilize one species as a model for another, based on interpolations of data for each species (Smith et al., 1984). Comparison of data from numerous laboratories is difficult because many factors influence on biotransformation including age and developmental stage, sex, species, nutritional status and exposure to various chemicals. In addition, the existence of multiple enzymes in each pathway with different, although, overlapping substrate specificity further clouds comparison of the data available in the literature (Watkins and Klaassen, 1986).

GST catalyzes the binding of a large variety of electrophiles to the sulphydryl group of GSH. Since the reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles. GST takes considerable importance as a mechanism for carcinogen detoxication (Hayes et al., 1995). Another parameter of importance in inhibiting carcinogenesis is prevention of oxidative damage by GPx. GPx is enzyme that catalyzes the reduction of organic hydroperoxides and hydrogen peroxide. The final product of this reaction is oxidized GSSG. Within cells GSH is regenerated from GSSG by the reaction catalysed by GR.

Little information is as yet available concerning the GSH levels and GSH-dependent enzyme activities of large intestine tissues of food-producing animals. Much data are about rat (Sharma et al., 2001; Munday et al., 2006), mouse (Guo et al., 2002; Irons et al., 2006) and human (Stoehlmacher et al., 2002; Skrzydlewska et al., 2005). Therefore I determined the glutathione content and related enzyme activities in the

E-mail: olehfedets@gmail.com

animals ceacum mucosa and liver and focused my interest in particular on the comparison of intraspecies differences.

Materials and Methods

Studies were performed on mature males 3 cattle (weight 400-450 kg), 3 horses (weight 500-550 kg), 3 pigs (weight 80-90 kg), 9 rabbits (weight 2-2.5 kg) and 3 sheep (weight 50-60 kg). Animals were slaughtered according to veterinary law.

The ceacum was removed, opened longitudinally and flushed with cold isotonic saline. The mucosa was scraped off with a plexiglass. Liver was removed, perfused with isotonic saline, and cut into small pieces. Samples were homogenized in tris-HCl buffer (5 mM, pH 7.0; EDTA 5 mM; PMSF 1 mM), using a Potter-Elvehjem homogenizer, and centrifuged at 10 000 g for 15 min at 4°C. The supernatant was taken and used for the analysis.

The content of GSH was determined by the method of Beutler et al. (1963). GST (EC 2.5.1.18) activity was assayed using 1-chloro-2,4-dinitrobenzene as a substrate (Habig et al., 1974). Working solution contained 1 mM GSH and 1 mM CDNB in 100 mM potassium phosphate buffer (pH 6.5). GPx (EC 1.11.1.9) activity was measured by the method of Pirie (1965). The reaction system contained 0.5 mM GSH, 0.2 mM H_2O_2, 1.5 mM NaN_3 and 0.02 mM EDTA in 100 mM potassium phosphate buffer (pH 7.0). GR (EC 1.6.4.2) activity was determined by the method of Carlberg and Mannervik (1975). The reaction system contained 1 mM GSSG, 1 mM NADPH and 0.5 mM EDTA in 100 mM potassium phosphate buffer (pH 7.6). Using bovine serum albumin as standard protein, soluble protein concentration was determined by the method of Lowry et al. (1951).

Results are reported as mean ± SEM. Statistical analysis was carried out using Student's t-test. $P < 0.05$ was considered statistically significant.

Results and Discussion

Results obtained for enzymes activities are summarized in Table 1. The GSH level and GST activity were significantly higher in animals liver than in ceacum. In liver the activity of GST in order from high to low was as follows: sheep > rabbit > horse > pig > cattle. In ceacum GST activity was the highest in rabbit, no significant differences were observed

Table 1
Activities of GST, GPx, GR (nmol min^{-1} mg^{-1}) and content of GSH (nmol mg^{-1}) in caecum and liver

Species	Tissue	GST	GPx	GR	GSH
Cattle	Ceacum	21.99±2.586 P_1<0.001	17.05±1.786 P_2<0.02	69.25±4.738 P_1<0.02 P_2<0.01	ND
	Liver	183.4±15.29	18.76±1.381 P_2<0.01	40.97±4.180	34.20±4.031
Horse	Ceacum	37.90±6.374 P_1<0.01	4.374±0.865	28.55±4.077 P_1<0.02	9.184±0.927 P_1<0.001
	Liver	653.8±119.2	7.655±1.155	11.52±1.776	53.60±2.404 P_2<0.02
Pig	Ceacum	17.25±4.928 P_1<0.01	6.606±1.675 P_1<0.001	69.29±8.040 P_1<0.01 P_2<0.02	9.857±0.812 P_1<0.001
	Liver	480.7±70.78 P_2<0.02	69.10±2.065 P_2<0.001	26.51±4.202	72.52±2.116
Rabbit	Ceacum	127.4±9.597 P_1<0.001 P_2<0.001	8.883±0.945 P_1<0.001	27.45±2.073	23.06±2.053 P_1<0.001 P_2<0.001
	Liver	1110±72.01 P_2<0.01	42.07±3.885	21.80±2.087 P_2<0.01	66.76±8.475
Sheep	Liver	1965±243 P_2<0.01	33.50±2.275 P_2<0.01	33.99±1.394	134.0±12.11 P_2<0.001

P_1 – comparison to liver; P_2 – comparison to another animal's species (from high to low); ND - not determined

between another animals species. In liver the level of GSH was the highest in sheep and it was the lowest in cattle. In ceacum of rabbit the GSH level was more 2 times higher than in horse and pig.

The GPx activity was significantly higher in liver of rabbit and pig than in their ceacum. The lowest data were in both organs of horse. In liver the activity of GPx in order from high to low was as follows: pig > rabbit > sheep > cattle > horse. In ceacum GPx activity was the highest in cattle. The differences were significant by comparison to another animals species.

The GR activity was higher in animals caecum than in liver. The differences were significant in cattle, pig and horse but not in rabbit. The enzyme activity was similar in caecum of cattle and pig. In rabbit and horse it was 2-3 times lower. In liver the data in order from high to low were as follows: cattle > sheep > pig > rabbit > horse. The significant difference was between rabbit and horse.

The mammalian small intestine serves principally as the site for absorption of nutrients, water, and both beneficial and potentially harmful xenobiotics. However, it has become apparent that an array of metabolic machinery is also expressed in this organ (Kaminsky and Zhang, 2003). In humans, approximately 6 to 12 liters of partially digested foodstuffs, water, and secretions are delivered daily to the small intestine. Of this, only 10 to 20% are passed on to the colon, because most nutrients, electrolytes, and water are absorbed as they are transported through the small intestine (Lin et al., 1999). In large intestines the activities of glutathione-dependent enzymes are lower than in small intestines (Peters et al., 1991). A lower detoxication capacity could contribute to an enhanced cancer risk (Berkhout et al., 2006). For example, a low GST enzyme activity is present in the colon as compared with the small intestine, whereas the cancer incidence in the colon is much higher as compared with that in small intestine (Peters et al., 1991). Although several authors have proposed this relationship, very few data exist to support it (Kaminsky and Zhang, 2003).

The nature of diet is a major factor regulating the enteric biotransformation pattern (Virkel et al., 2009). The intestinal and colonic epithelium is repeatedly exposed to metabolites and xenobiotics derived from dietary constituents and bacterial metabolism. Products of digestion or drugs taken orally can be removed from the lumen by the intestinal villus cells and both villus and crypt cells are potential sites for absorption of metabolites present in arterial blood (Pinkus et al., 1977). Also extracellular mechanism for detoxication is very effective to protect epithelial cells (Samiec et al., 2000).

Very few studies, however, have been devoted to assessing the ability of domestic animals to handle xenobiotics. Such information will make possible the appreciation of potential species differences in bioactivation, and would facilitate the extrapolation of metabolic and toxicological data from one species to another, and thus allow the rationale extension of medicines originally licensed in a major species, for use in minor or exotic food-producing species. Furthermore, this information would facilitate the risk assessment of drug and other chemical residues in edible tissues and milk that reach the consumer (Sivapathasundaram et al., 2003). The relative lack of drugs specifically registered for animal species commonly results in the extra-label use of medicinal products already authorised for other major species (Gusson et al., 2006).

GSH, GST and GPx were higher in food-producing animals liver than in ceacum. The cytosolic GST activity in intestine of rat was also significantly lower than in liver (Tahir et al., 1985). The response of GST to substrates is different in the liver and intestinal mucosa. This discrepancy can be explained since the liver and intestine are not exposed to the same metabolites of substrates. Indeed, the liver receives both native and metabolised substrates via the blood stream while the intestinal cells are directly in contact with the conjugates and bacterial metabolites on one side and with the metabolites in the blood (Lhoste et al., 2003).

The low activity of GST in cattle is in agreement with another report. Gusson et al. (2006) demonstrated that the activity of cytosolic GST was significantly higher in liver of rabbits, horses and pigs than in rat, broiler chicks and cattle. Most notably, cattle preparations were characterized by very low activities ranging from about one third to one fifteenth of those recorded in the other food-producing species. These results confirm and extend the adage that sheep are not little cows (Watkins et al., 1987). But according to Watkins and Klaassen (1986) hepatic GST activity in cattle and sheep was similar and had about 50% as pig.

GST activities were no significant difference between pig on the one hand and ruminants (goat, sheep, cattle) on the other. On the contrary, the zoologically closest species – sheep and goat – were mostly distant species from the point of view of in vitro activity of biotransformation enzyme (Szotakova et al., 2004). But GPx activity was the highest in ceacum of cattle.

During GPx-mediated detoxication of peroxides GSSG is formed. The cellular GSH pool can be regenerated from GSSG via the NADPH-dependent enzyme GR (Pompella et al., 2003). The oxidation and reduction of GSH through GPx and GR is important for determining the redox state of GSH and of NADP, and also for controlling the degradation of hydroperoxides. It has been demonstrated direct correlation between increased activities of rat GR and weaning on to high-carbohydrate diets. Significant increases in glycolytic activ-

ity in intestinal mucosa may be related to the pronounced increase in activities of GR (States and Segal, 1973). This can cause high GR activity in caecum.

The activity of GST was significantly lower in cattle and deer than in rat. Moreover, the levels of glutathione were markedly lower in the domestic animals compared with the rat. Similarly, GR, the enzyme that maintains glutathione in the reduced state, was markedly lower in the bovine and cervine livers compared with the rat. These observations would suggest that cattle and deer, similar to the human, but contrary to the rat, favour detoxication of epoxides through hydrolysis rather than through glutathione conjugation. In this way, their limited amounts of glutathione are protected. The metabolic and toxicological data may be extrapolated from cattle to deer and vice versa, but not from the rat to the two ruminants (Sivapathasundaram et al., 2003).

In conclusion, major differences were observed between the tested animals. Being aware of the fact that a number of factors including age, sex, diet, the exposures to drugs and environmental pollutants, as well as the occurrence of genetic polymorphisms, may modulate activity of enzymes (Gusson et al., 2006), the investigations should be carried out on all animal species. Extrapolation of data obtained in one species to another (even related one) species could be misleading (Szotakova et al., 2004).

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