Glutathione-S-Transferase Polymorphism and Clinical Features of Acute Drug Poisoning in Children

V. A. Vavilin, O. G. Safronova, N. A. Manankin*, L. F. Kaznacheeva*, and V. V. Lyakhovich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 4, pp. 424-427, April, 2005
Original article submitted December 8, 2004

We studied the association of *GSTM1* and *GSTT1* and *GSTP1* Ile_{105}-Val_{105} polymorphism with the duration of intoxication, polyorgan failure, and severity of drug poisoning in children. The combination of *GSTM1* and *GSTT1* zero genotypes is a favorable sign for the duration of intoxication and severity of the disease.

Key Words: *children; drug poisoning; clinical features; M1, T1, P1 glutathione-S-transferase polymorphism*

Pathogenesis of critical states irrespective of the etiological factor includes a series of universal processes associated with hypoxia, intoxication, immunological disorders, and oxidative stress [1,2]. Glutathione-S-transferases (GST) play an important role in the metabolism of foreign compounds, *e. g.* drugs, and in detoxification of some products formed in the reactions initiated by reactive oxygen forms. Genes of the *GST* superfamily are presented by functionally different polymorphic variants, which can be essential for the individual sensitivity to acute intoxication. We studied the associations between *GSTM1*, *T1*, and *P1* polymorphism and clinical course of acute drug poisoning in children.

MATERIALS AND METHODS

A total of 103 children with acute drug poisoning were examined. Association of GST polymorphism with the duration of intoxication syndrome, polyorgan failure (decompensation of two and more functional systems), and severity of intoxication was studied. The severity of poisoning was evaluated individually with consideration for the toxin, severity of vital function suppression, time course, and changes in the body, including polyorgan failure, and scored: 1 (mild), 2 (medium), and 3 (severe intoxication).

GSTM1 and *GSTT1* zero polymorphism in children was evaluated by PCR ([11] and [7], respectively), which differentiate between homozygotic deletion of these genes (zero genotypes, *GSTM1*0/0*, *GSTT1*0/0*) and plus-genotypes (*GSTM1+*, *GSTT1+*), *i.e.* normal homozygotes and heterozygotes. *GSTP1* Ile_{105}-Val_{105} polymorphism was evaluated by PCR with subsequent restriction analysis [5]. Restriction was carried out using BsoMA I endonuclease (Sibenzim) during 16 h at 55°C with subsequent dilution of fragments in 7.5% PAAG.

The mean values, standard deviations, significance of difference between the two parameters were evaluated using Student's *t* test, 95% confidence interval (CI) was calculated, and analysis of correlations was carried out. Associations of genotypes with clinical features of intoxication were evaluated by the odds ratio (OR), the significance of differences in genotype distribution in compared groups was evaluated using χ^2 test with Yates' correction and, if necessary, using Fisher's bilateral precise test.

RESULTS

The parameters of exposure and individual characteristics of the patient are essential for the formation of

Institute of Molecular Biology and Biophysics, Siberian Division of Russian Academy of Medical Sciences; *First Municipal Pediatric Clinical Hospital, Novosibirsk. ***Address for correspondence:*** drugsmet@soramn.ru. V. A. Vavilin

0007-4888/05/1394-0431

toxic response [3]. In our study severe poisoning (72 children) was caused by neurotropic (54.2%) and cardiovascular drugs (29.1%), while moderate poisoning (31 children) was caused by cardiovascular (58.1%) and neurotropic drugs (25.8%). In order to evaluate the relationship between the exposure (interval between the moment of poisoning and medical care) and the severity of intoxication, we distinguished the following periods: before 1, 2, 6 h and after 6 h. Patients with exposure from 2 and to 6 h predominated (72%) among severe cases. Poisoning with exposure longer than 6 h were rare (12.5%). In the group with moderate exogenous intoxication all periods were represented equally: 25.8, 25.8, 29.0, and 19.4%. The frequency of severe cases directly depended on the duration of exposure for the period from less than 1 h to 6 h (r=0.87; p<0.05), while after 6 h this relationship was not traced. Polyorgan failure, characterizing the somatogenic phase of poisoning, did not directly depend on the exposure, except liver involvement, which directly depended on the duration of exposure (r=0.65; p<0.05). The percent of children with CNS involvement during the somatogenic phase was lower in the group with 1-h exposure (83.3 *vs.* 100% for all other periods).

As the severity of drug poisoning depended on exposure, we compared this characteristic in children with mutant and wild *GST* genotypes. No differences were found (p=0.410-0.978). The distribution of *GST* genotypes did not depend on the drugs causing intoxication (neurotropic or cardiovascular).

Evaluation of the duration of intoxication syndrome revealed no differences in the genotype of individual *GST* (Table 1). Analysis of the genotype combinations demonstrated a trend to shortening of those containing double zero genotype *GSTM1*0/0/T1*0/0*, but the level of statistical significance could not be attained because of few number of cases (these combinations are rare in the population). Combinations including plus-genotypes *GSTM1* and *GSTT1* were more favorable for the polyorgan failure syndrome. The differences between the triple combination *GSTM1+/T1+/PIle/Ile* and *GSTM1-/T1-/PIle/Val* were on the border of statistical significance (p=0.1).

Analysis of the relationship between patient genotypes and severity of intoxication revealed an association of the *GSTPVal/Val* genotype with a more benign course (the association bordering with statistically significant, p=0.088) in comparison with the *GSTP1Ile/Ile* genotype (Table 2). Evaluation of the linear trend of the protective effect of accumulation of *GSTP1* Val allele for the severity of disease course showed the following statistics: χ^2=1.93, p=0.165. No relationship of this kind was detected for individual *GSTM1* and *GSTT1* genotypes and their combinations. Summing up the three genotypes, we observe a statistically significant relationship: more benign course of drug poisoning in carriers of the *GSTM1-/T1-/P1Ile/Ile* combination (p=0.022). Evaluation of the relative risk of severe course of intoxication for different *GST* genotypes revealed a protective role of *GSTP1* Val

TABLE 1. Intoxication Syndrome and Polyorgan Failure in Children with Different GST Genotypes

Genotypes and their combinations	Duration of intoxication, h		Number of organs with dysfunction	
	M±*m*	*p*	*M*±*m*	*p*
*GSTM1*0/0* (*n*=39)	21.83±14.88	0.419	3.12±1.10	0.26
GSTM1+ (*n*=61)	24.26±12.97		2.87±0.94	
*GSTT1*0/0* (*n*=19)	21.67±13.30	0.566	3.11±1.18	0.491
GSTT1+ (*n*=81)	23.76±13.86		2.93±0.94	
GSTP1Val/Val (*n*=10)	16.85±7.43		2.57±0.79	
GSTP1Ile/Val (*n*=40)	23.09±14.29	0.27*	3.14±0.97	0.43*
GSTP1Ile/Ile (*n*=50)	24.54±13.88	0.16*	2.89±1.02	0.158*
*M1*0/0/T1*0/0* (*n*=6)	16.50±4.97		3.50±1.22	
*M1*0/0/T1+* (*n*=33)	24.27±12.38	0.138**	2.92±1.17	0.34**
*M1+/T1*0/0* (*n*=13)	22.98±16.08	0.341**	3.04±1.04	0.338**
M1+/T1+ (*n*=48)	24.25±15.5	0.256**	2.86±0.88	0.118**
M1+/T1+/P1Ile/Ile (*n*=25)	22.14±13.27		3.05±0.91	
M1+/T1+/P1Ile/Val (*n*=17)	20.91±10.87	0.08+	2.87±0.64	0.507+
M1-/T1-/P1Ile/Ile (*n*=3)	17.00±7.55	0.17+	3.33±1.53	0.373++
M1-/T1-/P1Ile/Val (*n*=3)	16.0±2.0	0.134+	3.67±1.15	0.1++

Note. *Compared to *GSTP1Val/Val*; **to *M1*0/0/T1*0/0*; +to *M1+/T1+/P1Ile/Ile*; ++to *M1+/T1+/P1Ile/Val*.

TABLE 2. Severity of Drug Poisoning in Children with Different GST Genotypes (*M*±*m*)

Genotypes and their combinations	Severity, points	*p*
*GSTM1*0/0* (*n*=39)	2.69±0.47	0.269
GSTM1+ (*n*=61)	2.79±0.41	
*GSTT1*0/0* (*n*=19)	2.78±0.43	0.52
GSTT1+ (*n*=81)	2.74±0.44	
GSTP1Val/Val (*n*=10)	2.50±0.53	
GSTP1Ile/Val (*n*=40)	2.75±0.43	0.15*
GSTP1Ile/Ile (*n*=50)	2.67±2.90	0.088*
*M1*0/0/T1*0/0* (*n*=6)	2.67±0.52	
*M1*0/0/T1+* (*n*=33)	2.69±0.47	0.915**
*M1+/T1*0/0* (*n*=13)	2.83±0.39	0.45**
M1+/T1+ (*n*=48)	2.78±0.42	0.56**
M1+/T1+/P1Ile/Ile (*n*=25)	2.88±0.34	
M1+/T1+/P1Ile/Val (*n*=17)	2.75±0.45	0.32^{+}
M1-/T1-/P1Ile/Ile (*n*=3)	2.33±0.58	0.022^{+}
M1-/T1-/P1Ile/Val (*n*=3)	3.0±0.0	

Note. *Compared to *GSTP1Val/Val*; **to *M1*0/0/T1*0/0*; $^{+}$to *M1+/T1+/P1Ile/Ile.*

allele for the severity of intoxication course (OR=0.27; 95% CI 0.04-1.77).

Special analysis was carried out for clofelin poisoning, which was responsible for 14.6% of cases. In addition to standardization of the toxic factor, it was interesting because this provided new data on the involvement of GST in clofelin metabolism, presumably GSTμ [8] and GSTP1 [6]. No relationship with the intoxication severity was detected for *GSTP1* genotypes (OR=1.0; 95% CI 0.07-17.3). Relative risk of severe course of clofelin poisoning for *GSTM1* and *GSTP1* zero genotypes was 0.29 (95% CI 0.02-4.29) and 0.63 (95% CI 0.01-58.78), respectively, which attests to their protective role. The effect was more pronounced in individuals with two zero genotypes, while severe intoxication was observed only in carriers of two plus-genotypes. Presumably, this can be explained by higher toxicity of the conjugate in comparison with the initial compound.

Hence, GST polymorphism is significant for the formation of clinical manifestations of drug poisoning, the genotype effects manifesting during poisoning with the drugs metabolized with GST (clofelin) and in other cases. A lesser number of organ dysfunctions in children with *GSTM1* and *GSTT1* plus-genotypes agrees with traditional concepts on the role of these enzymes in detoxification. However, the protective effects of *GSTM1* and *GSTT1* zero genotypes (in the absence of activities of these enzymes) towards the severity of intoxication is unexpected. Presumably, the detected associations were due to not only GST role in drug metabolism. Under critical conditions GST compete for glutathione pool with other processes, which are no less important than xenobiotic metabolism (inactivation of products of exposure to activated oxygen forms). Weakening of this competition can be favorable for these processes and can explain the association with *GSTM1* and *GSTT1* zero genotype resistance. GST functions as ligand proteins in the signal transduction routes can also be significant. GSTP1 plays an important role in the regulation of the *c-jun*-regulated route, activated under conditions of stress [9]. Presumably, polymorphic variants of GSTP1 possess different characteristics of binding to these protein, but this remains to be studied. GSTM1 forms a complex with another kinase activated by different types of stress, Ask1 (apoptosis signal-regulating kinase 1) [4]. Presumably, polymorphism of these GST involved in signal transduction, associated with cell resistance to different stress factors, can manifest at the organism level by differences in the sensitivity to chemical factors and in the course of pathological processes. For example, *GSTM1* genotype in coronary patients is associated with lower risk of acute myocardial infarction, particularly in tobacco smokers [10]. Favorable effects of *GSTM1* and *GSTT1* on the duration of intoxication and severity of acute toxic exposure partially explain their high incidence in human populations (30-60 and 10-30%, respectively).

REFERENCES

1. A. L. Levit, A. V. Rudnov, A. A. Belkin, *et al.*, *Vestn. Intens. Ter.*, No. 4, 3-11 (2003).
2. I. N. Pasechnik, *Ibid.*, No. 4, 3-9 (2001).
3. *Casarett and Doulls Toxicology: The Basic Science of Poisons*, Ed. C. D. Klaassen, New York (2001).
4. S. Dorion, H. Lambert, and J. Landry, *J. Biol. Chem.*, **277**, No. 34, 30,792-30,797 (2002).
5. L. W. Harries, M. J. Stubbins, D. Forman, *et al.*, *Carcinogenesis*, **18**, No. 4, 641-644 (1997).
6. S. I. James and J. T. Ahokas, *Xenobiotica*, **22**, No. 12, 1433-1439 (1992).
7. S. Pemble, K. R. Schroeder, S. R. Spencer, *et al.*, *Biochem. J.*, **300**, Pt. 1, 271-276 (1994).
8. K. Sano, Y. Totsuka, Y. Ikegami, and T. Uesugi, *J. Pharm. Pharmacol.*, **54**, No. 2, 231-239 (2002).
9. T. Wang, P. Arifoglu, Z. Ronai, and K. D. Tew, *J. Biol. Chem.*, **276**, No. 24, 20,999-21,003 (2001).
10. M. H. Wilson, P. J. Grant, L. J. Hardie, and C. P. Wild, *FASEB J.*, **14**, No. 5, 791-796 (2000).
11. S. Zhong, A. H. Wyllie, D. Barnes, *et al.*, *Carcinogenesis*, **14**, No. 9, 1821-1824 (1993).