Glutathione *S*-transferase μ polymorphism does not explain variation in nitroglycerin responsiveness

Objective: To determine whether the considerable interindividual variability in nitroglycerin-induced venodilation in humans is related to the polymorphic expression of the μ class of glutathione *S*-transferase (GSTμ). Recently vascular glutathione *S*-transferase (EC 2.5.1.18) of the μ-class (GSTμ), a polymorphic group of enzymes present in only about 60% of the population, have been identified and shown in vitro to possess high metabolic activity toward nitroglycerin. Their clinical relevance is unknown.

Design: Dose-response relationships to nitroglycerin were constructed in vivo measuring changes in compliance of dorsal hand veins in 26 healthy volunteers during local infusion of small amounts of nitroglycerin. Polymerase chain reaction was applied to detect the deoxyribonucleic acid sequence that codes GSTμ in whole blood samples.

Results: The GSTμ isozyme was present in 15 subjects (58%) and deficient in 11 subjects. Values for mean maximum venodilation (E_{max}) and dose rates producing 50% of E_{max} (ED_{50}) were not significantly different between the groups with or without GSTμ. The respective values were 98% and 103% dilation for E_{max} and 9 and 16 ng/min for ED_{50}. There was no gender difference in the venodilatory response to nitroglycerin.

Conclusions: Subjects lacking GSTμ can clearly respond normally to nitroglycerin, and the large interindividual variability in nitroglycerin potency is not related to the expression of this polymorphic enzyme. Intersubject variability is therefore more likely to be the result of differences in the presence or activity of other vascular enzymes or in steps further distal in the venodilatory cascade. (CLIN PHARMACOL THER 1993;53:463-8.)

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Large interindividual variability in the sensitivity of human blood vessels to the vasodilatory action of nitroglycerin has been reported after systemic administration,[1] and substantial variability has also been found during local venous infusions of systemically ineffective doses.[2-4] The vasodilatory effects of nitroglycerin are believed to be mediated through a nitric oxide–like compound liberated during enzymatic denitration in the vascular wall.[5,6] In rabbits, nitroglycerin metabolism to its dinitrate metabolites is mandatory before vascular action; it is therefore considered to be a pro-drug.[7,8] In humans, different enzymatic pathways have been reported to metabolize the nitrate,[9-12] but at the site of action, the vascular wall, the only enzymes with high activity toward nitroglycerin identified thus far belong to the glutathione *S*-transferase family (GST; EC 2.5.1.18).[13] It is not known whether nitroglycerin metabolism by human glutathione *S*-transferases may produce active intermediates. Indirect evidence obtained in isolated animal vessels suggests that inhibitors of glutathione *S*-trans-

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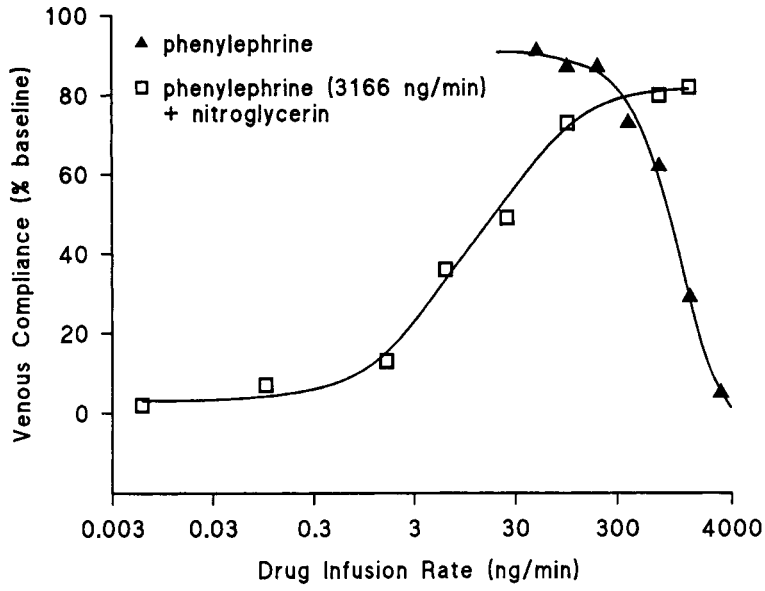

Fig. 1. Vasodilatory action of nitroglycerin in the hand vein of a healthy volunteer expressed as percentage of change from baseline. After constructing a cumulative dose-response curve to phenylephrine *(triangles)*, the highest dose of phenylephrine (3166 ng/min) was infused while increasing concentrations of nitroglycerin were added *(squares)*. Curves are nonlinear least-squares fits to a four-parameter logistic function[24]; estimated dose rates exerting 50% of maximum venodilation for phenylephrine and nitroglycerin were 1474 ng/min and 13 ng/min, respectively.

ferase may decrease nitroglycerin-induced vasodilation[14] and that the extent of inhibition is inversely correlated with the amount of dinitrate metabolites formed,[15] which may indicate that glutathione *S*-transferases are involved not only in catabolism of nitroglycerin but also in formation of active metabolites. However, Chung et al.,[16] using microsomes obtained from bovine coronary artery smooth muscle cells, found that nitric oxide production was not primarily mediated by glutathione *S*-transferase. Schröder,[17] using cultured rat lung fibroblasts, observed that cyclic guanosine monophosphate (cGMP) stimulation was not affected by sulfobromophthalein, an inhibitor of glutathione *S*-transferase. In human aorta, two different classes of glutathione *S*-transferase (GSTμ and GSTα), both of which metabolize nitroglycerin, have been recently isolated; isozymes of the μ class (GSTμ) have higher metabolic activity toward nitroglycerin. Because of a gene deletion the expression of some isoforms of GSTμ in humans is polymorphic.[18] In about 60% of white persons and about the same fraction of most other races, an intact deoxyribonucleic acid (DNA) sequence encoding this protein is present, and these subjects express GSTμ in leukocytes,[19-22] liver samples,[23,24] and other tissues.[25] Because of a homozygous deletion of the gene in the remaining 40% of the population, no detectable amounts of the isozyme are expressed.[18] Smoking-induced lung cancers[24] and chromosomal damage induced by mutagenic drugs[21] are prone to develop in these GSTμ-deficient subjects. The presence or absence of the GSTμ gene can be determined by the polymerase chain reaction with primers designed to detect the intact DNA sequence for human GSTμ.[26] Previous studies have shown that there is complete agreement between the presence or absence of the intact DNA and the metabolic activity of GSTμ as measured by the rate of trans-stilbene-oxide metabolism (Wiencke JK, Kelsey KT. Unpublished data).

This study was designed to determine whether part of the considerable interindividual variability in sensitivity of human veins to nitroglycerin is genetically linked to the presence of the GSTμ isozyme and whether people lacking the enzyme would have an impaired venodilatory response to nitroglycerin.

MATERIAL AND METHODS

Subjects. Twenty-six healthy volunteers (nine women and 17 men) with a mean age of 27 years (age range, 19 to 53 years) and a mean weight of 73 kg (weight range, 51 to 113 kg) entered the study after giving written informed consent. No subjects were

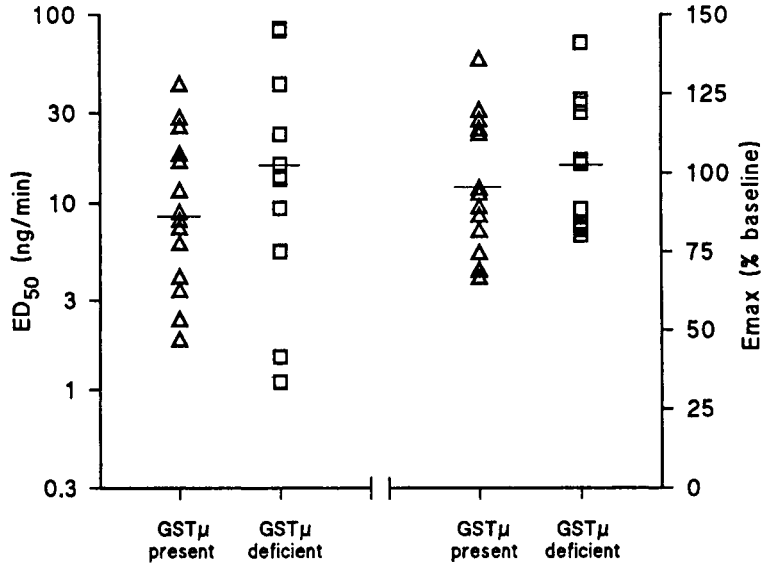

Fig. 2. Dose-response relationship of nitroglycerin in the presence (n = 15; *triangles*) and absence (n = 11; *squares*) of glutathione *S*-transferase (GSTμ). Estimates of maximum venodilation (E_{max}) and dose rates exerting 50% of E_{max} (ED_{50}) were obtained by nonlinear least-squares regression to a four-parameter logistic function.[24] *Horizontal lines* indicate the mean of the respective data set.

Table I. Dose-response relationship of nitroglycerin in healthy volunteers with and without GSTμ

	ED_{50} (ng/min)	*log ED_{50} (ng/min)*	*E_{max} (% baseline)*
GST present (n = 15)	8.5	0.93 ± 0.10	95 ± 5
GST deficient (n = 11)	16.0	1.20 ± 0.16	103 ± 6
Males (n = 17)	11.6	1.06 ± 0.13	98 ± 5
Females (n = 9)	7.9	0.90 ± 0.15	88 ± 12

ED_{50} values are expressed as geometric means of individual dose-response curves. Log transformed data of ED_{50} (log ED_{50}) and E_{max} data are expressed as arithmetic means ± SEM of individual dose-response curves.
GST, Glutathione *S*-transferase; GSTμ, μ class of GST; E_{max}, maximum venodilation; ED_{50}, dose rate exerting 50% of E_{max}.

taking any medications other than oral contraceptives (three subjects), and none had a history of significant medical disease. All had normal physical and electrocardiographic examinations and normal laboratory values (SMA-20, complete blood cell count and urinalysis). The protocol was reviewed and approved by the Medical Committee for the Protection of Human Subjects in Research at Stanford University (Stanford, Calif.).

Volunteers were admitted to the Aging Studies Unit at the Geriatric Research, Education, and Clinical Center at the Veterans Affairs Medical Center, Palo Alto, Calif. They were allowed to eat a light breakfast and were asked to refrain from methylxanthines and alcohol for at least 12 hours before the study. They remained semirecumbent in a quiet room with a constant temperature of 21° ± 1° C throughout the study.

Hand vein compliance technique. Hand vein experiments were performed by use of a general approach and instrumentation similar to that reported by Aellig,[27] with modifications as described previously.[3,4] In brief, through a 23-gauge needle a continuous infusion of physiologic saline solution was delivered into a vein on the dorsal surface of the hand (Harvard Apparatus infusion pump, Harvard Apparatus Inc., South Natick, Mass.). The arm was placed on a padded support above the level of the heart and sloping upward at an angle of 30 degrees from the horizontal to allow for complete emptying of the vein. Changes in the diameter of the vein were recorded with the use of a linear variable differential transformer (Schaevitz type 100 MHR, Schaevitz, Inc., Pennsauken, N.J.) with a freely movable core resting over the summit of the vein under investigation.

Transformer signals were amplified by a Schaevitz signal conditioner (Schaevitz ATA 101) and recorded on a strip-chart recorder (LKB 2210 recorder, LKB Produkter AB, Bromma, Sweden). The difference between the position of the core before and after inflation of a sphygmomanometer cuff on the same arm to 40 mm Hg gives a measure of the diameter changes under a given congestion pressure. For each measurement the blood pressure cuff was inflated and pressure was maintained at 40 mm Hg for approximately 3 minutes, until a stable deflection was observed. The cuff was then deflated to allow continued perfusion of the vein under investigation. After establishing a stable initial baseline with normal saline solution, which was defined as 100% dilation, the vein was constricted with the α_1-adrenergic agonist phenylephrine to about 20% of its initial diameter; this position of the core was considered to represent 0% dilation. Increasing concentrations of nitroglycerin were then infused locally into the vein together with a constant dose of phenylephrine. Each concentration was administered for at least 6 minutes to permit enough time to reach a stable response.[28] Dose rates administered (0.006 ng/min to 1.5 μg/min) were not sufficiently high to result in any systemic effect as evidenced by the lack of changes in blood pressure and heart rate during frequent measurements with an automated blood pressure monitor (Dinamap, Critikon Inc., Tampa, Fla.) placed on the opposite arm. At the completion of each study a heparinized whole blood sample was frozen for analysis of the GSTμ genotype. No untoward effects were observed in any of the subjects.

Polymerase chain reaction for GST μ allele. The GSTμ genotype was assessed in white blood cells of the volunteers with polymerase chain reaction as described earlier.[26] Primers for actin were used as a control. The determination was done after completion of all hand vein experiments.

Materials. Nitroglycerin (Tridil) was purchased from Du Pont Critical Care (Wilmington, Del.) and phenylephrine (Neo-Synephrine) from Winthrop Pharmaceuticals (New York, N.Y.). To avoid an interaction between the nitrates and the plastic infusion set, all studies were done with use of low absorption non-PVC tubing (Accuset, Imed Corporation, San Diego, Calif.) and syringes (Monoject, Sherwood Medical, St. Louis, Mo.). All intravenous solutions were freshly prepared and used within 3 hours.

Curve fitting and statistical analysis. Data are expressed as mean values ± SE unless otherwise indicated. Individual dose-response curves, expressed as percentage change from baseline, were fitted to a four-parameter logistic function, providing estimates of the maximum venodilation (E_{max}) and dose rates exerting 50% of E_{max} (ED_{50}) (Allfit,[29] National Institutes of Health, Bethesda, Md.).

Mean values of the effect parameters for the two groups were compared by use of the unpaired Student *t* test. The relationship between the genetic trait and variability in E_{max} and log ED_{50} of the two groups was further investigated by linear regression analysis. Confidence intervals of r^2 values were calculated using the Bootstrap method.[30] A p value of less than 0.05 was considered to indicate statistical significance.

RESULTS

The GSTμ enzyme was present in 15 subjects (58%) and deficient in 11 subjects, an enzyme distribution similar to distributions reported earlier.[20-25,31] As an example, the dose-response relationships of phenylephrine and nitroglycerin in a vein preconstricted with phenylephrine in one volunteer are shown in Fig. 1. E_{max} and log ED_{50} values of nitroglycerin of the two groups are depicted in Fig. 2. As we had seen previously,[3,4] nitroglycerin potency, expressed as ED_{50} values, varied substantially over a range of almost two orders of magnitude (1.1 to 85 ng/min). There were no significant differences in either pharmacodynamic parameter between the two groups (Table I). Evaluation of the findings by linear regression revealed no correlation between the sensitivity of the subjects to nitroglycerin (ED_{50}) and the GSTμ genotype ($r^2 = 0.034$; $p = 0.37$; 95% confidence interval, 0 to 0.26). In accordance with findings in arterial beds[32] no gender-related differences in the venodilatory response to nitroglycerin were observed (Table I).

DISCUSSION

Venodilation in response to nitroglycerin is the result of a series of local biochemical events in the vascular wall.[8,33] After entering the vascular smooth muscle cell, the drug is substrate of several GST isozymes[13] that metabolize nitroglycerin in the presence of sulfhydryl-containing cofactors[34] such as glutathione[35] and that mainly belong to the μ class.[13] The nitrate groups are believed to be the vasoactive components in the nitrate molecule[8,36] and their enzymatic cleavage from the parent compound is considered a key step in nitroglycerin-induced vasodilation.[8] After liberation, the nitrate groups exert dilation possibly through formation of an active nitrosothiol intermediate,[33] which activates soluble guanylate cyclase and

subsequently increases in intracellular levels of the second messenger cyclic GMP, a second messenger that is thought to cause vascular relaxation by way of activation of cyclic GMP-dependent protein kinase.[6,33] Consequently, intersubject variation in any of numerous biochemical steps may lead to interindividual differences in the potency of nitroglycerin in human veins.

This study was designed to evaluate a possible influence of the hereditary GSTμ polymorphism on the venous response to nitroglycerin and to relate genetic differences at the very beginning of the vasodilatory cascade to drug action. The findings of this study revealed high potencies of the drug in subjects with and without GSTμ deficiency, indicating that the GSTμ polymorphism did not determine the subjects' ability to activate nitroglycerin (i.e., subjects without the isozyme maintained their vascular response to nitroglycerin). Consequently, the large interindividual variability in nitroglycerin potency is not caused by the presence or deficiency of this polymorphic enzyme. Moreover, the normal response to nitroglycerin in subjects who totally lack GSTμ activity indicates that the polymorphic GSTμ plays a minor role in bioactivation of nitroglycerin in human veins and that this polymorphism is not the cornerstone of interindividual variability in nitroglycerin-induced venodilation. The absence of a correlation between GSTμ and drug potency could indicate that GSTμ is not the rate-limiting step or that other enzymes participate in nitroglycerin bioactivation in humans. Indeed, metabolism of nitroglycerin by enzymes other than GSTμ has been reported in human[37,38] and animal tissues[16,17] and at least one additional glutathione *S*-transferase isozyme, classified as α-type, with activity against nitroglycerin has been found in human aorta.[13] Moreover, at least three further pathways metabolizing nitroglycerin have been recently described that involve plasma proteins, hemoglobin, or cytochrome P450 isozymes with nitrate-metabolizing capacity,[9-12,17] but their involvement in vascular action of nitroglycerin has not been shown thus far. In the case of cytochrome P450 enzymes, animal data suggest that they do not participate significantly in the bioactivation of organic nitrates.[33,39]

In summary, this study shows that the polymorphic GSTμ isozyme is not the cornerstone of interindividual variability in nitroglycerin-induced venodilation and that enzymes other than GSTμ must be involved in vascular bioactivation of nitroglycerin in humans. Intersubject variations in nitroglycerin sensitivity are therefore more likely to be the result of differences in the activity or presence of other enzymes, the availability of cofactors such as sulfhydryl groups, or variation in distal steps in the venodilatory cascade.

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