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Vasodilatory effect of nitroglycerin in Japanese subjects with different aldehyde dehydrogenase 2 (*ALDH2*) genotypes

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Abbreviations:
GTN
glyceryl trinitrate
NO
nitric oxide
GDN
1, 2-glyceryl dinitrate
NMD
nitroglycerin-mediated dilation
FMD
flow-mediated dilation
ADH
alcohol dehydrogenases

ABSTRACT

The functional genetic polymorphism of aldehyde dehydrogenase 2 (*ALDH2*) influences the enzymatic activities of its wild type (Glu504 encoded by *ALDH2*1*) and mutant type (Lys504 encoded by *ALDH2*2*) proteins. The enzymatic activities of mutant-type *ALDH2* are limited compared with those of the wild type. ALDH2 has been suggested as a critical factor for nitroglycerin-mediated vasodilation by some human studies and *in vitro* studies. Currently, there is no research on direct observations of the vasodilatory effect of nitroglycerin sublingual tablets, which is the generally used dosage form. In the present study, the contribution of ALDH2 to the vasodilatory effect of nitroglycerin sublingual tablets was investigated among three genotype groups (*ALDH2*1/*1*, *ALDH2*1/*2*, and *ALDH2*2/*2*) in Japanese. The results by direct assessments of *in vivo* nitroglycerin-mediated dilation showed no apparent difference in vasodilation among all genotypes of *ALDH2*. Furthermore, to analyze the effect of other factors (age and flow-mediated dilation), multiple regression analysis and Pearson's correlation coefficient analysis were carried out. These analyses also indicated that the genotypes of *ALDH2* were not related to the degree of vasodilation. These results suggest the existence of other predominant pathway(s) for nitroglycerin biotransformation, at least with regard to clinical nitroglycerin (*e.g.*, a sublingual tablet) in Japanese subjects.

1. Introduction

Nitroglycerin (glyceryl trinitrate, GTN) was originally synthesized by Ascanio Sobreno, and Alfred Nobel later used the compound as an explosive, commonly known as “dynamite”. The antianginal effect of GTN was first observed in Nobel's dynamite factories [1]. Since Murrell [2] administered it to patients with angina pectoris, several delivery forms have been developed, and currently, the drug is commonly used to treat acute angina [3].

GTN is biotransformed to nitric oxide (NO) or related species *in vivo* [4,5]. This activates soluble guanylate cyclase, increases cellular cGMP, and enhances protein kinase G activity, which promotes the relaxation of smooth muscle cells. Vasodilation occurs in both arterial and venous systems [6]. In the arterial systems, GTN reduces afterload, dilates coronary arteries, and prevents vasospasms. Furthermore, the drug reduces preload by vasodilating veins. These complex hemodynamic effects ensure a balance

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between the consumption and supply of oxygen in the heart. Therefore, GTN biotransformation is a critical step for its therapeutic effect.

Feelisch et al. [7] discovered that a heat-sensitive enzymatic pathway is involved in GTN biotransformation. In humans and animals, 1, 2-glyceryl dinitrate (GDN) is a major product of the enzymatic reaction [8–10], suggesting that the enzyme selectively produces 1, 2-GDN, not 1, 3-GDN, and releases NO or related species in the biotransformation reaction. Several enzymes have been proposed as catalysts, particularly glutathione S-transferase [11,12], cytochrome P450 [13,14], cytochrome P450 reductase [15], and xanthine oxidase [16,17]. However, xanthine oxidase appears to be unrelated to GTN biotransformation *in vivo* [18], and the specific role of other enzymes on the vasodilatory effect of GTN is unclear [19]. The involvement of enzymes including aldehyde dehydrogenase 1 (ALDH1), aldehyde dehydrogenase 3A1 (ALDH3A1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), recently identified as GTN biotransformation enzymes, in the vasodilatory effect of GTN remains to be investigated [20–22].

Presently, aldehyde dehydrogenase 2 (ALDH2) is believed to be one of the significant candidates in GTN biotransformation [23]. Since the primary enzymatic product is 1, 2-GDN, it is suggested that the enzyme is a key regulator of GTN biotransformation [24,25]. ALDH2 is a member of the human ALDH family, which comprises $NAD(P)^+$-dependent enzymes [26]. The functional polymorphism of the *ALDH2* gene is at exon 12, wherein glutamate (wild type coded by *ALDH2*1*) is replaced by lysine (mutant type coded by *ALDH2*2*) at position 504 (Glu504Lys). The enzymatic activity of ALDH2 protein in *ALDH2*1*2* and *ALDH2*2*2* are essentially eliminated [27], and therefore the clearance of acetaldehyde, a substrate of ALDH2, is limited in subjects with *ALDH2*1*2* and *ALDH2*2*2*. This is illustrated by symptoms such as facial flushing, resulting from the poor metabolism of alcohol. It was noted that mutant-type *ALDH2* heterozygotes and homozygotes showed limited GTN biotransformation (approximately 10% in contrast to wild-type enzymatic activity) [27]. Under ALDH2 inhibitory conditions, some human studies indirectly evaluated the vasodilatory effect of GTN (increases in blood flow measured by venous occlusion plethysmography, decreases in blood pressure, or subjective assessments of pain relief), and suggested that ALDH2 contributes to this vasodilatory effect [27–30].

However, other studies have described the controversial results regarding the predominant role of ALDH2 in GTN pharmacology. Bennett et al. [31] clearly showed the uncoupling of ALDH2 activity and elevation of cellular cGMP in GTN treatment with their unique cell system. Their results showed that ALDH2 may not contribute to the vasodilatory effect of GTN, although the enzyme plays a role in the biotransformation of GTN to 1, 2-GDN. Furthermore, a clinical study of Japanese volunteers on GTN-mediated vasodilation revealed that the maximal vasodilator response to a lingual spray of GTN is independent of any functional loss of ALDH2 [32].

There is currently no report that investigated on whether *ALDH2* genotypes affect the vasodilatory effect of sublingual GTN, a common dosage form of GTN, in humans based on a direct observation of vasodilatory effect of GTN, although the effect of GTN in each genotype had been indirectly assessed through changes in systemic circulation, blood flow or pain relief [27–30]. Considering that the effect of GTN differs according to the formulation, such as the spray or tablet form [33], further study on the direct observation of vasodilation by sublingual GTN focusing on *ALDH2* genotypes is required to elucidate the effect of ALDH2 on GTN-induced vasodilation.

In the present study, we designed a clinical study comprising three groups of Japanese subjects with different genotypes (homozygous of each *ALDH2*1* and *ALDH2*2*, and heterozygous). After the sublingual administration of a GTN tablet (not spray), vessel diameter (nitroglycerin-mediated dilation; NMD) was continuously and directly assessed by using ultrasonography. Considering that several enzymes may be related to GTN biotransformation *in vivo*, low doses of sublingual GTN (0.1 mg) that were sufficient to induce adequate vasodilation were administered, which allows the simple detection of the pathway with a high catalytic efficiency. This is, to our knowledge, the first report regarding the direct comparison of the vasodilatory effect (not indirect effects: *e.g.*, blood flow, blood pressure, or subjective assessments of pain relief) of sublingual GTN among different genotype groups of ALDH2.

2. Materials and methods

2.1. Genotyping

Genotyping was carried out by using a direct sequencing method. Whole blood was collected from each subject by using a blood collection tube containing dried EDTA. To avoid cross-contamination in experimental operations, DNA fragments of the *ALDH2* gene were amplified by blood-direct PCR by using a Phusion Blood Direct PCR kit (Finnzymes Inc, Woburn, MA, USA) without the extraction of genomic DNA (forward primer, 5′-tgg gca aca gag aaa gat tct atc-3′; reverse primer, 5′-cca cca gca gac cct caa g-3′). The amplifications were performed as follows: preheating at 98 °C for 5 min, denaturing at 95 °C for 1 s, annealing at 62 °C for 5 s, extension at 72 °C for 15 s (35 cycles), and then an additional extension at 72 °C for 1 min.

DNA obtained in the supernatant was washed with Wizard SV Gel and a PCR Clean-Up System (Promega, Madison, WI, USA), and the nucleotide sequence was confirmed by using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Japan, Ltd., Tokyo, Japan) (sequencing primer, 5′-taa ccc ata acc ccc aag a-3′).

2.2. Subjects

All subjects were of Japanese ethnicity. The study was approved by the Ethics Committee of National Hospital Organization Hakodate Hospital (No. 18) and the Ethics Committee of Osaka Ohtani University (No. BE-004-11), and was performed according to Declaration of Helsinki principles. All subjects gave written informed consent before inclusion. Twenty subjects were enrolled in the study and screened for their *ALDH2* Glu504Lys genotypes: six wild-type homozygotes (*ALDH2*1/*1*), seven heterozygotes (*ALDH2*1/*2*), and seven mutant-type homozygotes (*ALDH2*2/*2*) subjects. Subjects were healthy as determined by medical history checks and a physical examination before participating in the study, and did not have cardiovascular diseases and diabetes. They were administered no pharmacotherapy that affects systemic circulation. Furthermore, no nitrates were administered to the subjects to ensure that they do not develop any nitrate tolerance. Among the groups of each genotype, age (shown as mean ± SD) and sex ratios (male%) were similar (Table 1).

Table 1
Subjects background.

	*ALDH2*1/*1*	*ALDH2*1/*2*	*ALDH2*2/*2*
Number	6	7	7
Age	44.2 ± 13.7	43.3 ± 16.0	42.3 ± 14.3
Male (%)	42.9	57.1	57.1
Alcohol torelance[a]	3.17 ± 0.408	1.71 ± 0.951*	1.00*

[a] A self-assessment survey on alcohol tolerance was performed by using a 4-point Likert scale (1 = low alcohol tolerance, 2 = somewhat low alcohol tolerance, 3 = somewhat high alcohol tolerance, 4 = high alcohol tolerance), as shown in Table 1.*P < 0.05, against *ALDH2*1/*1* (Dunnett's test).

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