PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Differential effects of organic nitrates on arterial diameter among healthy Japanese participants with different mitochondrial aldehyde dehydrogenase 2 genotypes: randomised crossover trial
AUTHORS	Sakata, Satoko; Yoshihara, Tatsuya; Arima, Hisatomi; Shiraishi, Fumie; Oniki, Hideyuki; Takahashi-Yanaga, Fumi; Matsumura, Kiyoshi; Sasaguri, Toshiyuki

VERSION 1 - REVIEW

REVIEWER	***Dr Isla Mackenzie*** Clinical Senior Lecturer and Honorary Consultant Physician, University of Dundee, Dundee, UK. I have no competing interests.
REVIEW RETURNED	04-Apr-2011

THE STUDY	The authors discuss the fact this study was in healthy volunteers rather than angina patients and I think this is reasonable for this type of work and is fully justified.
RESULTS & CONCLUSIONS	I recommend a minor change to the statement on pg 15 line 29-33 to add that ISDN can also cause tolerance. Similarly, I recommend a change to the statement at the end of pg 16 lines 35-44. I think the recommendation to develop new drugs to replace GTN should be toned down as GTN is a very cheap and effective drug.
GENERAL COMMENTS	This is an interesting, well-designed and well-written article that adds significantly to the literature in this area. The authors should add a statement to their discussion regarding whether the sublingual dosing of GTN and ISDN delivered a precise dose of drug or not. The dosing may have been slightly unreliable, although this would have affected all patients similarly so should not have biased the results. I recommend a minor change to the statement on pg 15 line 29-33 to add that ISDN can also cause tolerance. Similarly, I recommend a change to the statement at the end of pg 16 lines 35-44. I think the recommendation to develop new drugs to replace GTN should be toned down as GTN is a very cheap and effective drug. I also recommend that the figures should be improved as follows: Fig 1 - include p values for comparisons made. Figs 2 and 3 include p values for comparisons between the 1/1 and 2/2 genotypes for ISDN to prove there is no difference for each figure.

REVIEWER	***Ulrich Hink, M.D.*** Attending University Medicine Mainz Medical Department, Cardiology Germany

REVIEW RETURNED	13-Apr-2011

RESULTS & CONCLUSIONS	This is an important study highlighting the role of *2/*2 ALDH2 allele carriers who have been associated with a functional loss in GTN bioactivation. However, in this study *2 carriers did not show an attenuated response in maximal vasodilation. 1. In this regard the results of the study do not meet hypothesis. This point is not discussed adaequately in face of earlier studies. The fact that this study, who included more subjects with the *2/*2 genotype than other studies, raises even more the question why there is no reduced GTN effect in maximal dilatation. How does this fit in the context of reduced antianginal GTN or vasodilatory effect of *2 carriers in former studies. Is it a matter of dosage, the studied vascular bed, the method (forearm plethysmography vs. FMD) or is there simply no functional difference compared to non-*2-carriers ? This issue has to be addressed more thoroughly and clearer as the presented findings oppose earlier observations. 2. It is hard to believe that a moderate increase in the 90% time to reach the maximal response may have relevant clinical implications for this short-acting drug GTN. Therefore, this argument should not be over-emphasized, especially, as this parameter was randomly chosen. The time to half maximal effect may reflect a more common and relevant parameter. Nevertheless, this finding reflects the anticipated potential loss in GTN bioactivating function rather then a real clinical problem. 3. If dosage may have been an issue: Have the authors done any studies looking at different GTN or ISDN doses ? Alternatively, are there any reports supporting the choice of the administered doses or showing a dose-relationship ?

VERSION 1 – AUTHOR RESPONSE

Reviewer Dr. Isla Mackenzie

Thank you very much for your encouraging comments.

To the major comment:
We are grateful to you for pointing out what we had forgotten to describe. In this revised version, we stated, “we used sublingual spray formulations instead of tablets for both nitrates to minimise the fluctuation in absorption rate. However, on the other hand, this may have made the dosage slightly unreliable, although this would have affected all the participants similarly and therefore should not have biased the results” in page 13 as the second limitation of the study, with citation of an article concerning the spray formulation of GTN (Ref. 22). We also added this point to “ARTICLE SUMMARY” at page 3.

To the minor comments:
1. As you recommended, we added “although ISDN can also cause tolerance” to the statement at page 15.

2. In line with your suggestion, we toned down the expression at page 16 by changing the statement to “it may be necessary to promote the development of a new medicine that is more beneficial than GTN”.

3. We added P values to Figs. 1, 2, and 3. Accordingly, we deleted “NS, not significant.” from the legends.

Reviewer Dr. Ulrich Hink

Thank you very much for your important comments. We believe that our manuscript has been greatly improved by revisions made in line with your comments.

To the comments:

1. Although we initially thought that the maximal response to GTN would be reduced in *2 allele carriers based on the earlier studies, results obtained were different from expectation. However, our results do not mean that ALDH2*2 have no influence on the bioactivation of GTN. Although no difference was observed in the maximal response, the time required to attain the maximal response was clearly different. This is reasonably explained if it is hypothesized that other enzymes such as cytochrome P450 are also involved in GTN bioactivation, as we stated at page 16. We think this consideration is natural, because ALDH2 is involved in the pharmacokinetics of GTN but it does not alter the sensitivity to NO (the active metabolite of GTN). However, I am sorry that we cannot specify the reason for the difference in the results between earlier studies and ours. Probably, multiple factors may be involved: such as study design (there had been only one experimental study prior to ours), the number of participants, the method to evaluate the response, studied vascular beds, or GTN dosage. To make the difference in the results between previous studies and ours clearer, we added the statement “Based on the earlier studies, we initially hypothesized that the maximal response to GTN would be reduced in *2 allele carriers, but the results obtained were different from our expectation. Although the time required to obtain the response was different among the genotype groups, there was no difference in the size of response (The reason for this is discussed later). However, we cannot specify the reason for the difference in the results between earlier studies and ours. Probably multiple factors may be involved: such as study design, sample size, GTN dosage, studied vascular beds, measurement method, or outcome measures.” to “Comparison with other studies” at page 15.

2. We believe that a nearly 1-minute delay in reaching maximal effect is significant for patients with angina who desire to be relieved of chest pain as soon as possible, although, as you pointed out, what we observed was only brachial artery dilation but not disappearance of the symptom. According to your comment, we toned down the expression at page 15 by changing the statement to “Given that the purpose of GTN is to provide rapid relief for chest pain, we believe that a 1-min delay cannot be ignored, although we observed only brachial artery dilation but not disappearance of the symptom. To practice personalised medicine for patients with angina, it may be useful to know individual ALDH2 genotypes before treatment”. And moreover, as you suggested, we additionally analysed the time required for 50% maximal dilation. The result obtained was similar to that of the time required for 90% maximal dilation. We showed this data in Supplement Figure and added a statement “A similar result was obtained when the time required for 50% maximal dilation was analysed (Supplement Figure)” to “RESULTS” section at page 12.

3. I am sorry to say that we have not done studies on the dose-response relationship ourselves. According to the package insert of the GTN spray (Myocor Spray), the standard and maximal doses approved in Japan are 0.3 mg (1 squirt) and 0.6 mg (2 squirts), respectively, corresponding to 0.4 mg and 0.8 mg approved in European countries (Nitrolingual Spray). The difference in approved dosage between Europe and Japan probably reflects the difference in the average body size. Similarly, the standard and maximal doses of the ISDN spray (Nitorol Spray) are 1.25 mg (1 squirt) and 2.5 mg (2 squirts), respectively, corresponding to 1.25 (1 squirt) and 3.75 mg (3 squirts) approved in European

countries (Isoket Spray). According to previous studies examining the dose-response relation, in general, 0.3-0.4 mg GTN causes submaximal effect and 0.6-0.8 mg GTN causes near maximal effect (1-3). Similarly, 1.25 mg ISDN and 2.5 mg ISDN causes submaximal and near maximal effects, respectively (4-6). For the present study, we adopted submaximal doses 0.3 mg for GTN and 1.25 mg for ISDN, because we thought that that would make it easier to observe changes in the response to nitrates and moreover that high concentrations of GTN had been suggested to be activated not only by ALDH2 but also by other enzymes such as cytochrome P450.

1) Parker JO et al. Am J Cardiol 1986; 57: 1-5.
2) Miida T et al. Junkankika 1990; 28: 382-391 (written in Japanese).
3) Ducharme A et al. Am J Cardiol 1999; 84: 952-954.
4) Klein R and Sharir T. Am J Cardiol 1990; 65: 39J-42J.
5) Kadota Y et al. Masui 1991; 40: 636-43 (written in Japanese).
6) Maeda T et al. Kokyu To Junkan 1992; 40: 83-7 (written in Japanese).

VERSION 2 - REVIEW

REVIEWER	***Dr Isla S Mackenzie***
REVIEW RETURNED	04-May-2011

THE STUDY	The authors justify the fact they did not use IHD patients adequately.

REVIEWER	***Ulrich Hink***
REVIEW RETURNED	15-May-2011

GENERAL COMMENTS	Very interesting paper that adds new aspects to the individual clinical effects of the well-known and still often used class of nitrates. Your response clarified all open questions more than adequately. minor comment: page 15, line 17: instead of "size", you may use "magnitude or extent"