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Title: In-vivo impact of prodrug isosorbide-5-nicotinate-2-aspirinate on lipids and prostaglandin D₂: is this a new immediate-release therapeutic option for niacin?

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Abstract

Objectives: To evaluate the pharmacokinetics and effects of the first immediate-release (IR) niacin-aspirin prodrug (ST0702) on lipid, prostaglandin and thromboxane levels in non-human primates (NHPs).

Methods: We compared 28 mg/kg crystalline IR niacin, equimolar doses of crystalline IR ST0702 and control (daily oral gavage) on low density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB) and triglycerides (Tg) in NHPs (6 per group) over 48 hours. In addition, we compared IR niacin and ST0702 effects on prostaglandin (PG)_{D2}, *ex-vivo* thromboxane B₂ (TXB₂) levels and plasma pharmacokinetics.

Results: ST0702 is metabolised *in-vivo* to aspirin, niacin and salicylic acid with $T_{\max}$ values of 30, 45 and 95 minutes respectively using a non-compartmental model. ST0702 resulted in 38% and 40% reductions in LDL-C and ApoB levels compared to control over the 48 hour period ($p=0.027$ and $p=0.012$ respectively). Corresponding values were 32% and 25% for niacin (both $p=NS$ vs control). ST0702, but not niacin, decreased Tg levels ($p=0.017$ for between group difference). Post prandial glycaemia was attenuated vs baseline in the ST0702 group only. *Ex-vivo* serum TXB₂ generation was suppressed at 15 minutes and complete suppression of TXB₂ was sustained at 24 hours ($p<0.01$ vs niacin). ST0702 suppressed PGD₂ exposure eightfold ($p=0.012$) compared to niacin over the first 24 hours.

Conclusions: This two-dose study in NHPs suggests that ST0702 is more effective than IR niacin on lipid profiles, while suppressing TXB₂ and PGD₂ increases and preventing post-prandial glycaemia. ST0702 shows promise as a new IR therapeutic option for niacin.

**In-vivo impact of prodrug isosorbide-5-nicotinate-2-aspirinate on lipids and prostaglandin D₂:
is this a new immediate-release therapeutic option for niacin?**

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Four Figures, zero tables

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Abbreviations

ApoB	Apolipoprotein B
AUC	Area under the curve
BuChE	Butyrylcholinesterase
COX	Cyclooxygenase
SR	Sustained release
ER	Extended release
GPCR	G-protein coupled receptor
HDL-C	High density lipoprotein cholesterol
IR	Immediate-release
LDL-C	Low density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
NHP	Non-human primate
PGD ₂	Prostaglandin D ₂
Tg	Triglycerides
TXB ₂	Thromboxane B ₂
VLDL-C	Very low density lipoprotein cholesterol

Introduction

Niacin favourably modifies the entire lipid panel by reducing low density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), lipoprotein (a) (Lp(a)), apolipoprotein(a), very low density LDL-C (VLDL-C) and triglycerides (Tg) as well as raising high density lipoprotein cholesterol (HDL-C) and apolipoprotein AI¹. While the impact of Tg, Lp(a) and HDL-C modification on outcome is under investigation, the primary goal of lipid management is LDL-C reduction achieved first line with statins¹. Niacin is thought to lower LDL-C and ApoB secondary to reduced VLDL-C production arising from interaction with a G1-protein-coupled transmembrane receptor (GPR109A) in adipose tissue and inhibition of hepatic diacylglycerolacyltransferase. Experimental data also suggest reduction in ApoB results from inhibition of cholesterol esterase transfer protein². Niacin may have additional effects on atherogenesis through anti-inflammatory and anti-oxidant pathways that are independent of its effects on lipids³. Accordingly, immediate release (IR) niacin has demonstrated cardioprotective effects, lowering the risk of myocardial infarction by 27% and long-term mortality by 11%⁴. In the HATS study, the risk of death, myocardial infarction, stroke or revascularization was reduced by 90% when niacin was added to statin therapy⁵.

However, the use of IR niacin in clinical practice is limited by poor adherence and persistence arising from flushing and gastrointestinal toxicity^{6,7}. Niacin-induced flushing is attributed to its activation of GPR109A on epidermal Langerhans cells, resulting in predominantly cyclooxygenase (COX)-1 mediated processing of arachidonic acid⁷. The resultant prostaglandin (PG) D₂ and E₂ increases activate DP1, EP1 and EP2 receptors in the vasculature causing vasodilation. This phenomenon is independent of niacin activation of GPCR 109A receptors on adipocytes which contributes to its lipid modifying effects.⁸ Moreover, increases in thromboxane B₂ (TXB₂) noted in humans following niacin ingestion may mitigate niacin cardioprotective effects⁹. Furthermore, niacin is hydrophilic at plasma pH which may limit its distribution into adipose tissue for GPCR activation necessitating high doses which in turn are associated with liver toxicity and adverse effects on glucose metabolism¹⁰. These adverse effects may be disadvantageous in populations with metabolic abnormalities and diabetes.

Sustained or extended release (SR/ER) formulations of niacin, cause less flushing than IR niacin, but they may be less effective anti-dyslipidaemics and can aggravate gastrointestinal toxicity, hepatotoxicity and dysglycaemia^{7,11}. A formulation of niacin with laropiprant, a DP1 specific antagonist, is marketed in Europe, but not in the USA. This approach does not entirely resolve the flushing effects of niacin which are also mediated by PGE₁ and PGE₂ acting at EP1 and EP2 receptors. An unlicensed approach to niacin mediated flushing is the co-administration of COX-1 inhibitory drugs, especially aspirin, to attenuate peripheral PG production¹². However, some COX inhibitors have independent cardiovascular risks and may not be ideal for chronic usage. Moreover, while it is known that higher doses of cardioprotective aspirin more effectively reduce flushing, the dose-dependent risks of gastrointestinal adverse effects are increased.

We recently reported the development of the first true aspirin pro-drugs. The panel included a new molecular entity isosorbide-5-nicotinate-2-aspirinate (ST0702) incorporating niacin and aspirin connected to isosorbide, a sugar molecule. ST0702 undergoes metabolism in human blood producing aspirin, salicylic acid and niacin.¹⁴ The characteristics of isosorbide-5-nicotinate-2-aspirinate (ST0702) might be well suited to the management of dyslipidaemia with improved tolerability compared with current SR/ER formulations of niacin: as a prodrug it is intrinsically inactive which should reduce the topical gastrointestinal toxicity associated with conventional aspirin; it is metabolised by butyrylcholinesterase (BuChE) and carboxylesterase in human plasma and liver producing niacin which can improve dyslipidaemia; it releases known metabolites including aspirin and salicylic acid which might improve insulin sensitivity and reduce niacin induced hyperglycaemia; finally it should abolish platelet TXB₂ production and provide antiplatelet efficacy in a patient population for whom this therapy is generally indicated¹⁵.

This study aims to evaluate isosorbide-5-nicotinate-2-aspirinate on lipid markers of niacin efficacy, on COX activity as reflected in *ex-vivo* TXB₂ and peripheral PGD₂, and on glycaemia. The cynomolgus NHP model was chosen because pre-clinical evaluation of pro-drugs is best carried out in primates because of esterase homology with humans. Furthermore, this model is well established for the evaluation of LDL-C and ApoB.¹⁶

Material and methods

In-vivo study design. Studies were carried out by contract research organisation Charles River Laboratories. A total of six purpose bred, NHPs (cynomolgus monkeys, 2.8–4.5 kg) were sourced and allocated in a three-phase crossover design to control, high dose niacin (28 mg/kg) or equimolar doses of ST0702. A washout period of at least two weeks occurred between phases. Animals were dosed daily by oral gavage in aqueous vehicle (below) over a 48 hour period.

The study protocol was approved by PCS-SHG Institutional Animal Care and Use Committee before conduct. During the study, care and use of animals was conducted in accordance with the guidelines of the USA National Research Council and the Canadian Council on Animal Care.

Each animal was identified by a cage label and body tattoo and acclimated to oro-gastric dosing on at least two occasions prior to the initiation of dosing. The test articles were suspended in a vehicle (1% (w/v) tween 80 and 0.5% (w/v), carboxymethylcellulose in deionized water) and administered at time 0 and 24 hours using an orogastric tube inserted through the mouth and advanced into the stomach.

The animals were temporarily restrained (e.g., manually) for dose administration, and were not sedated. Disposable sterile syringes and orogastric tubes were used for each animal/dose. Each dose was followed by a tap water flush of approximately 5 mL. Blood samples were taken at the following time-points: pre-dose (0 hour) and at 0.083, 0.25, 0.50, 1, 2, 4, 12, 24, 48 hour after first administration of test article.

The primary study endpoints were changes in serum ApoB, LDL-C and Tg levels at 48 hours. Secondary endpoints were area under the curve (AUC) values of TXB₂ and PGD₂ as well as plasma pharmacokinetics of aspirin, salicylic acid and niacin over the initial 24 hour period.

Effects of ST0702 and niacin on LDL-C, ApoB, Tg and glucose levels. An aliquot of 300 µL of serum was transferred to a cryovial which was immediately stored at -70°C until analyzed for clinical chemistry (LDL-C, ApoB, Tg, glucose) using a standard Roche Analyser.

Pharmacokinetic profiles of aspirin, niacin and salicylic acid. Relevant pharmacokinetic parameters of aspirin, salicylic acid and niacin in plasma were measured following administration of niacin and ST0702 over the initial 24 hour period. For this, additional 0.4 mL serum aliquots were placed in K₂EDTA tubes and processed to plasma pharmacokinetic analysis using fully validated LCMS/MS methods.

Effects of ST0702 and niacin on TXB₂ and PGD₂ levels. Relative effects of ST0702 and niacin on *ex-vivo* serum TXB₂ and PGD₂ levels were determined based on inhibition of baseline (TXB₂) and total AUC measurements over the initial 24 hour period. For TXB₂, additional 0.4 mL whole blood aliquots were placed in siliconised glass tubes to induce clotting and the supernatants were analysed for TXB₂ using a Luminex ELISA immunoassay. PGD₂ was analysed from serum using a liquid chromatography tandem mass spectrometry (LCMS/MS).

Statistical Methods. Data are presented as mean $\pm$ standard error of the mean (SEM), median, interquartile range (IQR) with 95% confidence intervals for non-normal continuous variables and frequencies and percents for nominal/categorical variables. Interactions between treatment period and animals on drug effects were analysed using analysis of variance. In the absence of animal and period effects, comparisons between ST0702 and niacin groups in the NHP study were made on changes over the study period using paired sample t-tests and paired sample Wilcoxon tests as appropriate. Within group tests, comparing baseline to 48 hour values, were conducted using paired sample t-tests and where appropriate non-parametric equivalents. Analyses were carried out using SPSS V.18 statistical software (Statistical Package for the Social Sciences: SPSS Inc, Chicago, Illinois, 2001).

Experimental results:

Effects of ST0702 and niacin on ApoB, LDL-C, Tg and glucose levels. The effects of ST0702, niacin and vehicle control on LDL-C and ApoB are presented in Figure 1. ST0702 administration was associated with significant 38% and 40% reductions in baseline LDL-C and ApoB respectively ($p=0.027$ for LDL-C and $p=0.012$ versus control for ApoB). Niacin was associated with a 32% and 25% reduction in LDL-C and ApoB respectively ($p=0.084$ for LDL-C and $p=0.187$ versus control for ApoB). There were no animal or period interactions with the drug effects and changes in both biomarkers vs control reached statistical significance only in the ST0702 treated group. The effect sizes of both niacin and ST0702 are consistent with cholesterol reduction observed in cynomolgus monkeys with atorvastatin in the range 1–10 mg/kg/day¹⁷.

Tg levels, which were variable, showed non-significant increases of 23.3%, 11.3% in niacin and control respectively with a 7.9% decrease in the ST0702 group (all $p=NS$ within groups). Tg levels in niacin treated animals were 50 ± 10 mg/dL at baseline and 64 ± 31 mg/dL at follow up whereas ST0702 treated monkeys were 59 ± 29 mg/dL at baseline and 55 ± 24 mg/dL at 48 hours ($p=0.033$ between group change ST0702 vs niacin).

Serum glucose levels over the first 24 hours post dose are presented in Figure 2. A significant decrease in glucose from baseline was observed at 1 hour in both niacin and ST0702 treated groups before feeding. Post prandial glucose levels returned to baseline levels in the niacin treatment group, which coincided with feeding between 1–2 hours. Post-prandial glucose levels remained significantly below baseline in the ST0702 group ($p=0.028$) and also demonstrated a non-significant within-group difference relative to niacin ($p=0.066$). This pattern persisted to 12 hours, but it did not reach statistical significance.

Pharmacokinetic profiles of aspirin, niacin and salicylic acid. The comparative pharmacokinetic profiles of aspirin, niacin and salicylic acid following oral administration of niacin or ST0702 are presented in Figure 3. The $T_{\max}$ values determined for aspirin, niacin and salicylic acid released from ST0702 using a non-compartmental model are 30, 45 and 95 minutes respectively. The $C_{\max}$ values using a non-compartmental model determined for aspirin, niacin and salicylic acid released from ST0702 are 1.1 $\mu\text{g/mL}$ (6.1 μM), 10.7 $\mu\text{g/mL}$ (87 μM) and 42.3 $\mu\text{g/mL}$ (306 μM) respectively. In a separate analysis of $n=3$ monkeys dosed with equimolar amounts of aspirin, the AUC value for the aspirin group was $14.0 \pm 1.02 \mu\text{g/mL.h}$ compared with $3.5 \pm 0.64 \mu\text{g/mL.h}$ for ST0702. This represents a 25% exposure to aspirin relative to the aspirin treated group or equivalent to 9.3 mg/kg. The reduction of LDL-C and ApoB levels over 48 hours noted with this dose of aspirin was $-5.3 \pm 2.9 \text{ mg/dL}$ and $-3.0 \pm 1.52 \text{ mg/dL}$ (both $p=\text{NS}$ vs baseline). The $C_{\max}$ for niacin was higher in the niacin treatment group than in the ST0702 group but the differences were non-significant. There was no significant difference in niacin and ST0702 AUC values ($20.7 \pm 9.77 \mu\text{g/mL.h}$ vs $15.8 \pm 9.1 \mu\text{g/mL.h}$ respectively ($p=0.39$)). Total salicylic acid AUC values were higher in the aspirin treatment group ($515.8 \pm 72.8 \mu\text{g/mL.h}$) than in the ST0702 group ($336.6 \pm 23.7 \mu\text{g/mL.h}$) ($p=0.012$) indicating that 65% of the total salicylic acid component of ST0702 was released.

Effects of ST0702 and niacin on TXB_2 and PGD_2 levels. Serum TXB_2 levels were significantly reduced in the ST0702 group but remained unchanged in the niacin group (AUC $0.41 \pm 0.15 \mu\text{g/mL.h}$ vs $14.2 \pm 2.8 \mu\text{g/mL.h}$, respectively, $p < 0.0001$ for between-group differences) (Figure 4A). In the ST0702 group, *ex-vivo* serum TXB_2 suppression was evident from 15 minutes post dosing and complete suppression ($>95\%$) was sustained at 24 and 48 hours. This hallmark effect of aspirin exposure is predictive of its anti-platelet effects. Similarly, the impact of niacin and equimolar amounts of ST0702 on PGD_2 levels, a biomarker of flushing, is presented in Figure 4B. ST0702 caused an eightfold reduction in PGD_2 exposure compared to niacin treated animals ($16.2 \pm 6.4 \text{ ng/mL.h}$ vs $128.3 \pm 38.2 \text{ ng/mL.hr}$ respectively, $p=0.012$).

Discussion

This study establishes the therapeutic potential of ST0702, the first niacin-aspirin pro-drug, in providing enhanced efficacy and reduced side-effects compared to IR niacin pharmacotherapy of dyslipidaemia. ST0702 is well absorbed orally and rapidly metabolised by primate esterases to aspirin followed by niacin, resulting in a pharmacokinetic profile similar to IR niacin, the form of the drug with established morbidity and mortality benefits. Importantly, in the present study, plasma LDL-C and ApoB levels were reduced in both treatment groups but only reached statistical significance in the ST0702 group. Furthermore, aspirin release from ST0702 caused rapid and profound suppression of platelet competency as reflected in *ex-vivo* TXB₂. This is a favourable biochemical outcome in the target treatment population who are at increased risk of cardiovascular thrombotic events. Systemic platelet COX blockade was consistent with the suppression of peripheral PGD₂, a key mediator of niacin induced flushing. Finally, the significant attenuation of post-prandial glycaemia in the ST0702 group is consistent with salicylate mediated improvements in insulin sensitivity and efficacy¹⁸ and suggests that ST0702 may have important longer term clinical potential in the management of the metabolic syndrome compared to currently marketed niacin formulations.

Reduction of LDL-C and, by inference its ApoB component, is the primary goal of dyslipidaemia management at present¹. Statins are first line therapeutic agents to achieve this goal, but up to 20% of patients discontinue statin treatment due to adverse effects¹⁹. Furthermore, in clinical practice, 40–50% of patients do not achieve their LDL-C goal, with or without statin therapy²⁰. European and US guidelines recommend niacin as second-line treatment in the management of LDL-C, not least because the IR form of the drug was the first LDL-C lowering therapy shown to reduce the risk of myocardial infarction and long-term mortality in high risk patients. Other studies have shown that high-dose IR niacin in combination with statin and other lipid lowering therapy can favourably modify LDL-C, ApoB, atherosclerosis and reduce major adverse cardiovascular events⁵. These clinical benefits are consistent with established data on greater efficacy on Tg, LDL-C and ApoB with high-dose IR niacin compared to lower doses, including marketed doses of SR/ER niacin¹¹. They may also reflect emerging data on new mechanisms of athero-protection achievable with high dose niacin

such as concentration-dependent reduction in vascular inflammation, inflammatory cytokine production and endothelial reactive oxygen species³.

In the present study, ST0702 was, more effective on lipid targets than niacin. Several possible explanations for this observation present themselves: (i) it may be due to effects of salicylate on improved insulin sensitivity via inhibition of serine kinase IKK β and NF κ B²¹ and corresponding reduction on lipids, although no evidence for these effects was obtained in the aspirin data alone; (ii) it may reflect altered niacin bioavailability due to competition from salicylic acid for the glycine conjugation pathway, although no evidence for this was observed in the NHP pharmacokinetic profiles; (iii) finally, potentially improved distribution of ST0702 into lipid tissue before release of nicotinic acid is consistent with the improved efficacy and the pharmacokinetic profile observed. ST0702 has a lipophilic log D_{7.4} of 1.88, unlike hydrophilic niacin, and this is also consistent with more effective platelet penetration of ST0702 compared to aspirin.^{22,23} These combined effects with ST0702 may also result in reduced availability of Tg for nascent ApoB containing particles, promoting their intracellular degradation and reduced production of ApoB containing particles such as LDL and VLDL. Reduced hepatic VLDL-C production with ST0702 versus conventional niacin could open new treatment options, such as Type III Frederickson's disease, where niacin is not currently indicated.²⁴ ST0702 administration was also associated with attenuated post-prandial glycaemia unlike IR niacin treated animals, possibly because of salicylate-mediated improvement of insulin sensitivity. The insulin sensitising effects of ST0702 are in contrast to reduced insulin sensitivity observed with all formulations of conventional niacin. The long held concerns about disturbances in glucose control associated with niacin,^{10,25} cannot be dismissed in the context of the growing patient population with metabolic syndrome, and pre-diabetes who may not be receiving hypoglycaemic agents and it is noteworthy that significantly more patients receiving niacin than control discontinued treatment for dysglycaemia in the AIM HIGH study.²⁶

The severity of the flushing response, as well as niacin availability at GPCR 109A in peripheral adipocytes, are proportional to the rate of absorption and extent of distribution of niacin. In practice, new European Guidelines suggest that only 15% of patients remain on niacin at the end of one year.¹

There are two strategies currently used to attenuate flushing. The first, DP1 receptor blockade with laropiprant, partially blocks the PG mediated flushing response which also results from increased EP1/EP2 receptor activation. The second, albeit unlicensed, approach to preventing flushing is broad prostaglandin suppression via inhibition of constitutive COX-1 enzyme 30 minutes prior to niacin¹². However, this is limited by long term cardiovascular toxicity associated with non-aspirin COX inhibiting drugs and gastro-intestinal adverse effects of aspirin, the COX-1 inhibitor with established cardio-protective benefits. Furthermore, relatively high doses of aspirin are required to reduce flushing symptoms associated with niacin, thus aggravating dose-related gastrointestinal toxicity and niacin-induced gastrointestinal side-effects¹¹. The acid group of aspirin is a key contributor to its gastrointestinal toxicity. ST0702 belongs to a new class of sugar-based compounds that are rapidly activated ($T_{1/2}$ 1 min) on contact with human BuChE present in plasma and liver¹⁴. ST0702 rapidly releases aspirin (T_{max} 30 min), because of a specific interaction with BuChE due to the isosorbide scaffold. This occurs asymmetrically because of a difference in the steric environment of the two drug substituents. In human plasma, this leads to aspirin release before niacin which is important for inhibition of PG release *in-vivo*. In our *in-vivo* primate model, aspirin was released with T_{max} occurring before niacin (30 vs 45 minutes respectively). The aspirin release profile explains the observed PGD₂ reduction and the early and sustained suppression of *ex-vivo* TXB₂. The latter is an accepted marker of systemic COX-1 activity suggesting that ST0702 may have a broader anti-flushing profile than laropiprant.

This is the first study in which an aspirin prodrug administered orally has generated aspirin *in-vivo* in a primate, vindicating the isosorbide-based prodrug design and the plasma metabolism models predicting this behaviour¹⁴. The products of metabolism of ST0702 are either generally recognised as safe (isosorbide) or well characterised pharmacologically (aspirin, niacin, salicylic acid). Furthermore, in this NHP model only one quarter of the molar dose equivalent of aspirin was released *in-vivo* corresponding to an aspirin dose equivalent of < 10 mg/kg. Inhibition of platelet COX-1 activity, demonstrated by complete, sustained TXB₂ suppression, is associated with a reduction in the risk of serious coronary events (myocardial infarction, stroke and vascular death) by 15–18% in patients with

hyperlipidaemia¹⁵. The inherent antiplatelet effect of ST0702 will negate the need for additional therapy with aspirin and improved platelet penetration previously reported^{22,23} may also help with aspirin resistance linked to adherence, dose and glycaemic control in patients with diabetes.²⁷

In addition to its established benefits in patients with elevated LDL-C, high dose, IR niacin is the most effective marketed agent for reducing Lp(a) and raising HDL-C, independent predictors of long-term cardiovascular outcome. While the growing clinical practice of using predominantly “low-flush” ER/SR versions of niacin to lower Lp(a) and elevate HDL-C may be rational, it is not yet supported by a large-scale clinical outcome. There are important retrospective and emerging prospective data to suggest that raising HDL-C is less important when LDL-C levels are very low²⁸, a design feature of AIM HIGH and HPS2-THRIVE studies. Compared to IR niacin, SR/ER niacin formulations cause deteriorations in glucose control, more gastrointestinal disturbances and more liver enzyme abnormalities^{8,10} which have the potential to mask the net clinical benefit of therapy.³⁰ For example, in AIM HIGH, approximately 80% of included patients were diabetic or pre-diabetic and the metabolic disturbance of niacin may have partially offset lipid-modifying benefits of the drug. Furthermore, one quarter of patients did not remain on therapy and the net difference in HDL-C between niacin and the active comparator was only 4 mg/dL. In this context, the physicochemical and pharmacokinetic profile of ST0702 coupled with improved efficacy, better metabolic control and suppression of COX-1 related PGs suggests this is a new therapeutic option for niacin.

Conclusion

ST0702 is metabolized to niacin, salicylic acid and aspirin in a manner which improves the lipid profile more effectively than conventional IR niacin in the cynomolgus NHP model. The mechanism of improved efficacy of ST0702 over IR niacin may be related to pharmacokinetic/pharmacodynamic and distribution properties of the prodrug. There was evidence that ST0702 may also have beneficial effects on glycaemia *in-vivo*. ST0702 uniquely has an aspirin metabolite which suppresses important COX-1 related bio-markers of the flushing adverse effects associated with IR niacin. ST0702 may be a new immediate-release therapeutic option for niacin.

References

- 1 Reiner, Ž., Catapano, A. L., De Backer, G., Graham, I., Taskinen, M.-R., Wiklund, O., Agewall, S., Alegria, E., Chapman, M. J., Durrington, P., Erdine, S., Halcox, J., Hobbs, R., Kjekshus, J., Filardi, P. P., Riccardi, G., Storey, R. F., Wood, D., Bax, J., Vahanian, A., Auricchio, A., Baumgartner, H., Ceconi, C., Dean, V., Deaton, C., Fagard, R., Filippatos, G., Funck-Brentano, C., Hasdai, D., Hoes, A., Kearney, P., Knuuti, J., Kolh, P., McDonagh, T., Moulin, C., Poldermans, D., Popescu, B. A., Sechtem, U., Sirnes, P. A., Tendera, M., Torbicki, A., Vardas, P., Widimsky, P., Windecker, S., Reviewers:, D., Berkenboom, G., De Graaf, J., Descamps, O., Gotcheva, N., Griffith, K., Guida, G. F., Gulec, S., Henkin, Y., Huber, K., Kesaniemi, Y. A., Lekakis, J., Manolis, A. J., Marques-Vidal, P., Masana, L., McMurray, J., Mendes, M., Pagava, Z., Pedersen, T., Prescott, E., Rato, Q., Rosano, G., Sans, S., Stalenhoef, A., Tokgozoglu, L., Viigimaa, M., Wittekoek, M. E. and Zamorano, J. L., ESC/EAS Guidelines for the management of dyslipidaemias, *European Heart Journal*, 2011, 32: 1769-1818.
- 2 Van der Hoorn, J. W. A., de Haan, W., Berbee, J. F. P., Havekes, L. M., Jukema, J. W., Rensen, P. C. N. and Princen, H. M. G., Niacin Increases HDL by Reducing Hepatic Expression and Plasma Levels of Cholesteryl Ester Transfer Protein in APOE*3Leiden.CETP Mice, *Arterioscler Thromb Vasc Biol*, 2008, 28: 2016-2022.
- 3 Ganji, S. H., Qin, S., Zhang, L., Kamanna, V. S. and Kashyap, M. L., Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells, *Atherosclerosis*, 2009, 202: 68-75.
- 4 Canner, P. L., Berge, K. G., Wenger, N. K., Stamler, J., Friedman, L., Prineas, R. J. and Friedewald, W., Fifteen year mortality in Coronary Drug Project patients: Long-term benefit with niacin, *Journal of the American College of Cardiology*, 1986, 8: 1245-1255.
- 5 Brown, B. G., Zhao, X.-Q., Chait, A., Fisher, L. D., Cheung, M. C., Morse, J. S., Dowdy, A. A., Marino, E. K., Bolson, E. L., Alaupovic, P., Frohlich, J., Serafini, L., Huss-Frechette, E., Wang, S., DeAngelis, D., Dodek, A. and Albers, J. J., Simvastatin and Niacin, Antioxidant Vitamins, or the Combination for the Prevention of Coronary Disease, *New England Journal of Medicine*, 2001, 345: 1583-1592.
- 6 McKenney, J., New Perspectives on the Use of Niacin in the Treatment of Lipid Disorders, *Arch Intern Med*, 2004, 164: 697-705.
- 7 Jacobson, T. A., A “Hot” Topic in Dyslipidemia Management—“How to Beat a Flush”: Optimizing Niacin Tolerability to Promote Long-term Treatment Adherence and Coronary Disease Prevention, *Mayo Clinic Proceedings*, 2010, 85: 365-379.

- 8 Benyó, Z., Gille, A., Kero, J., Csiky, M., Suchánková, M. C., Nüsing, R. M., Moers, A., Pfeffer, K. and Offermanns, S., GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing, *The Journal of Clinical Investigation*, 2005, 115: 3634-3640.
- 9 Saareks, V., Ylitalo, P., Mucha, I. and Riutta, A., Opposite Effects of Nicotinic Acid and Pyridoxine on Systemic Prostacyclin, Thromboxane and Leukotriene Production in Man, *Pharmacology & Toxicology*, 2002, 90: 338-342.
- 10 Grundy, S. M., Vega, G. L., McGovern, M. E., Tulloch, B. R., Kendall, D. M., Fitz-Patrick, D., Ganda, O. P., Rosenson, R. S., Buse, J. B., Robertson, D. D., Sheehan, J. P. and for the Diabetes Multicenter Research Group, Efficacy, Safety, and Tolerability of Once-Daily Niacin for the Treatment of Dyslipidemia Associated With Type 2 Diabetes: Results of the Assessment of Diabetes Control and Evaluation of the Efficacy of Niaspan Trial, *Arch Intern Med*, 2002, 162: 1568-1576.
- 11 Knopp, R. H., Ginsberg, J., Albers, J. J., Hoff, C., Ogilvie, J. T., Warnick, G. R., Burrows, E., Retzlaff, B. and Poole, M., Contrasting effects of unmodified and time-release forms of niacin on lipoproteins in hyperlipidemic subjects: Clues to mechanism of action of niacin, *Metabolism*, 1985, 34: 642-650.
- 12 Whelan, A. M., Price, S. O., Fowler, S. F. and Hainer, B. L., The effect of aspirin on niacin-induced cutaneous reactions, *The Journal of Family Practice*, 1992, 34: 165-168.
- 13 Moriarty, L.M., Lally, M.N., Carolan, C.G., Jones, M., Clancy, J.M., Gilmer, J.F., Discovery of a "true" aspirin prodrug, *Journal of Medicinal Chemistry*, 2008, 25: 7991-7999.
- 14 Jones, M., Inkielewicz, I., Medina, C., Santos-Martinez, M. J., Radomski, A., Radomski, M. W., Lally, M. N., Moriarty, L. M., Gaynor, J., Carolan, C. G., Khan, D., O'Byrne, P., Harmon, S., Holland, V., Clancy, J. M. and Gilmer, J. F., Isosorbide-Based Aspirin Prodrugs: Integration of Nitric Oxide Releasing Groups, *Journal of Medicinal Chemistry*, 2009, 52: 6588-6598.
- 15 Antithrombotic Trialists Collaboration, Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials, *Lancet*, 2009, 373: 1849-1860.
- 16 Colvin, P. L., Spray, B. J. and Miller, N. E., Plasma low density lipoprotein cholesterol concentration in cynomolgus monkeys; Differing effects of age and body weight in animals consuming low and high cholesterol diets, *Atherosclerosis*, 1994, 111: 191-197.
- 17 Ito, B. R., Zhang, B. H., Cable, E. E., Song, X., Fujitaki, J. M., MacKenna, D. A., Wilker, C. E., Chi, B., Van Poelje, P. D., Linemeyer, D. L. and Erion, M. D., Thyroid hormone β receptor activation has additive cholesterol lowering activity in combination with atorvastatin in rabbits, dogs and monkeys, *British Journal of Pharmacology*, 2009, 156: 454-465.

- 18 Hundal, R. S., Petersen, K. F., Mayerson, A. B., Randhawa, P. S., Inzucchi, S., Shoelson, S. E. and Shulman, G. I., Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes, *The Journal of Clinical Investigation*, 2002, 109: 1321-26.
- 19 Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy, AIM-HIGH Investigators, *New England J Medicine*. 2011, 365: 2255-67.
- 20 Böhler, S., Scharnagl, H., Freisinger, F., Stojakovic, T., Glaesmer, H., Klotsche, J., Pieper, L., Pittrow, D., Kirch, W., Schneider, H., Stalla, G. K., Lehnert, H., Zeiher, A. M., Silber, S., Koch, U., Ruf, G., März, W. and Wittchen, H. U., Unmet needs in the diagnosis and treatment of dyslipidemia in the primary care setting in Germany, *Atherosclerosis*, 2007, 190: 397-407.
- 21 Goldfine, A. B., Fonseca, V., Jablonski, K. A., Pyle, L., Staten, M. A. and Shoelson, S. E., The Effects of Salsalate on Glycemic Control in Patients With Type 2 Diabetes, *Annals of Internal Medicine*, 2010, 152: 346-357.
- 22 Harmon, S., Inkielewicz-Stepniak, I., Jones, M., Ledwidge, M., Santos-Martinez, M.J., Medina, C., Radomski, M.W., Gilmer, J.F. Mechanisms of aggregation inhibition by aspirin and nitrate-aspirin prodrugs in human platelets, *Journal of Pharmacy and Pharmacol*, 2012, 64: 77-89.
- 23 Medina C, Harmon S, Inkielewicz I, Santos-Martinez MJ, Jones M, Cantwell P, Bazou D, Ledwidge M, Radomski MW, Gilmer JF. Differential inhibition of tumour cell-induced platelet aggregation by nicotinate aspirin prodrug (ST0702) and aspirin, *British Journal of Pharmacology*, 2011 Nov 29. doi: 10.1111/j.1476-5381.2011.01794.
- 24 Physicians' Desk Reference 2011, 65th Edition, Thompson Publications, USA.
- 25 Elam, M. B., Hunninghake, D. B., Davis, K. B., Garg, R., Johnson, C., Egan, D., Kostis, J. B., Sheps, D. S., Brinton, E. A. and Investigators, f. t. A., Effect of Niacin on Lipid and Lipoprotein Levels and Glycemic Control in Patients With Diabetes and Peripheral Arterial Disease, *JAMA*, 2000, 284: 1263-1270.
- 26 Boden, W. E., Probstfield, J.L., Anderson, T., Chaitman, B.R., Desvignes-Nickens, P., Koprowicz, K., McBride. R., Teo, K., Weintraub, W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. AIM-HIGH Investigators, *New England Journal of Medicine*, 2011, 365: 2255-2267.
- 27 Ertugrul, D. T., Tatal, E., Yildiz, M., Akin, O., Yalçın, A.A., Ure, O.S., Yilmaz, H., Yavuz, B., Deveci, O.S., Ata, N., Küçükazman. M. Aspirin resistance is associated with glycemic control, the dose of aspirin, and obesity in type 2 diabetes mellitus. *Journal of Clin Endocrinology and Metabolism*. 2010, 95: 2897-2901.
- 28 Ridker, P. M., Genest, J., Boekholdt, S. M., Libby, P., Gotto, A. M., Nordestgaard, B. G., Mora, S., MacFadyen, J. G., Glynn, R. J. and Kastelein, J. J. P., HDL cholesterol and residual

- risk of first cardiovascular events after treatment with potent statin therapy: an analysis from the JUPITER trial, *The Lancet*, 2010, 376: 333-339.
- 29 AIM-HIGH Investigators, The role of niacin in raising high-density lipoprotein cholesterol to reduce cardiovascular events in patients with atherosclerotic cardiovascular disease and optimally treated low-density lipoprotein cholesterol: Baseline characteristics of study participants. The Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes (AIM-HIGH) trial, *American Heart Journal*, 2011, 161: 538-543.
- 30 McKenney, J. M., Proctor, J. D., Harris, S. and Chinchili, V. M., A Comparison of the Efficacy and Toxic Effects of Sustained- vs Immediate-Release Niacin in Hypercholesterolemic Patients, *JAMA*, 1994, 271: 672-677.

Figure Legends

Figure 1. Impact of test articles on (A) serum LDL-C and (B) ApoB levels in a prospective, randomised, cross-over, NHP study of ST0702 vs equimolar amounts of niacin (n=6 per group). Grey bars are controls, white bars are niacin and black bars are ST0702. Data shown represent the mean and standard error of the mean (SEM) of change values from baseline to 48 hour (both fasting). *P* values shown are vs control.

Figure 2. Impact of test articles on serum glucose levels over 24 hours in a prospective, randomised, cross-over, NHP study of ST0702 (solid circles) vs equimolar doses of niacin (open squares, n=6 per group) vs control (open triangles, n=6 per group). Data shown represent the mean and SEM of values over 24 hours. *P* value shown represents post-prandial change from baseline within group (ST0702 at 4 hours only $p=0.028$, with $p=0.066$ for between group differences with ST0702 (paired sample *t*-tests)). Control group animals did not demonstrate any differences vs ST0702.

Figure 3. Pharmacokinetic profiles of (A) aspirin, (B) niacin and (C) salicylic Acid in a prospective, randomised, cross-over, NHP study of ST0702 (solid circles) vs equimolar amounts of niacin (open squares, n=6 per group) following single daily dose at time 0. Because of the short half-lives of aspirin and niacin, data are shown over the first 12 hours for illustrative purposes. Data represent the mean and SEM of values.

Figure 4. AUC values for (A) *ex vivo* serum TXB₂ profiles and (B) PGD₂ profiles of ST0702 (solid bars) and niacin (open bars) treated animals over 24 hour post dosing in a prospective, randomised, cross-over, NHP study of ST0702 vs equimolar amounts of niacin, (n=6 per group) following single daily dose at time 0. Data represent the mean $\pm$ SEM. The AUC value for TXB₂ in the control group was 8.3 ± 0.9 $\mu\text{g/mL.hr}$ and was non-significantly lower than Niacin (14.2 ± 2.8 $\mu\text{g/mL.hr}$, $p=0.072$ vs control). PGD₂ was not quantified in control animals.

Highlights

- We compared the niacin and niacin-aspirin prodrug ST0702 in the cynomolgus monkey.
- ST0702 was more efficacious on lipid parameters than niacin.
- ST0702 reduced COX-products associated with platelet activation and flushing.
- ST0702 attenuated post-prandial glycaemia, unlike niacin.
- ST0702 may be a new immediate-release therapeutic option for niacin.

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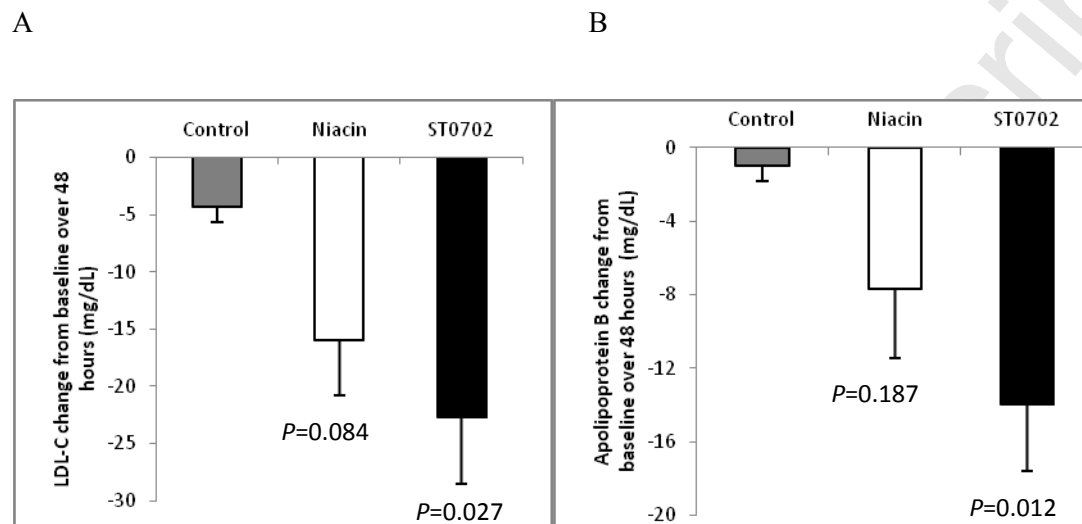


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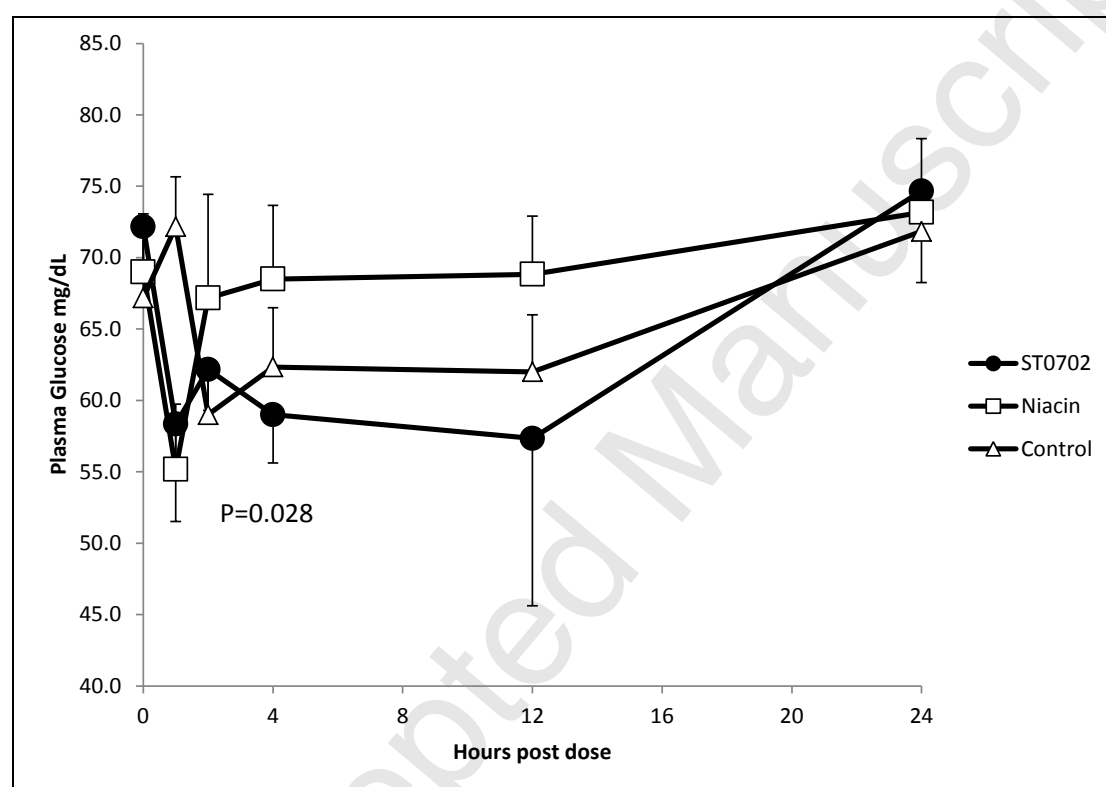
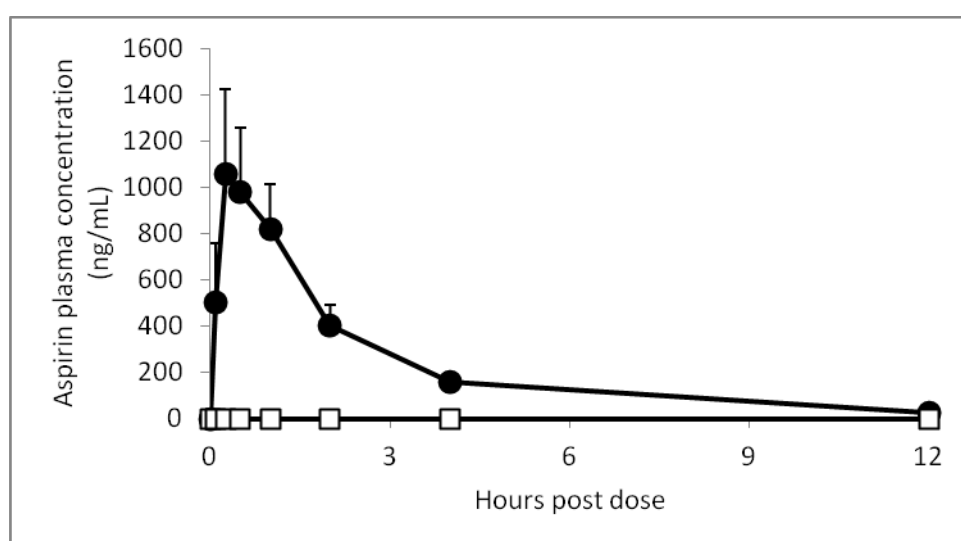
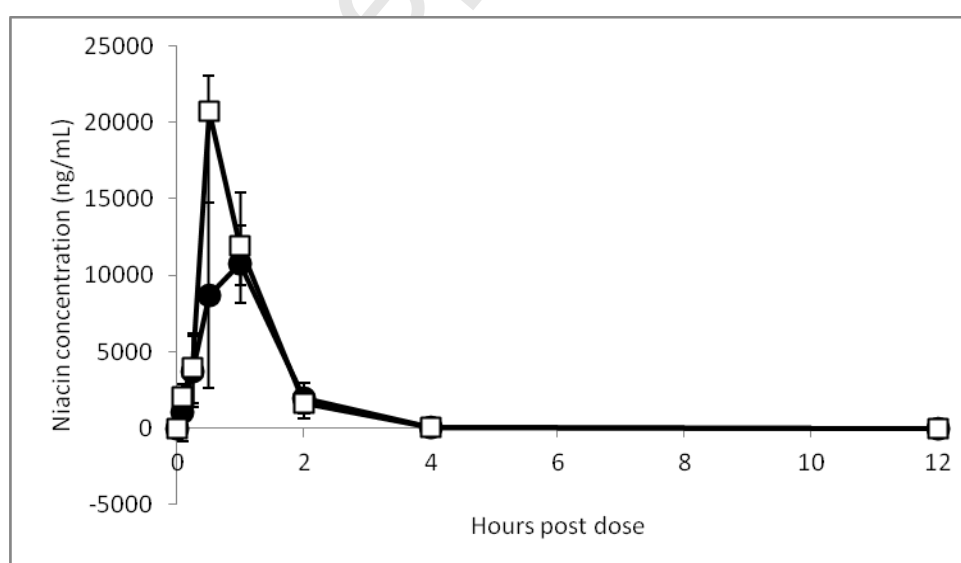


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A



B



C

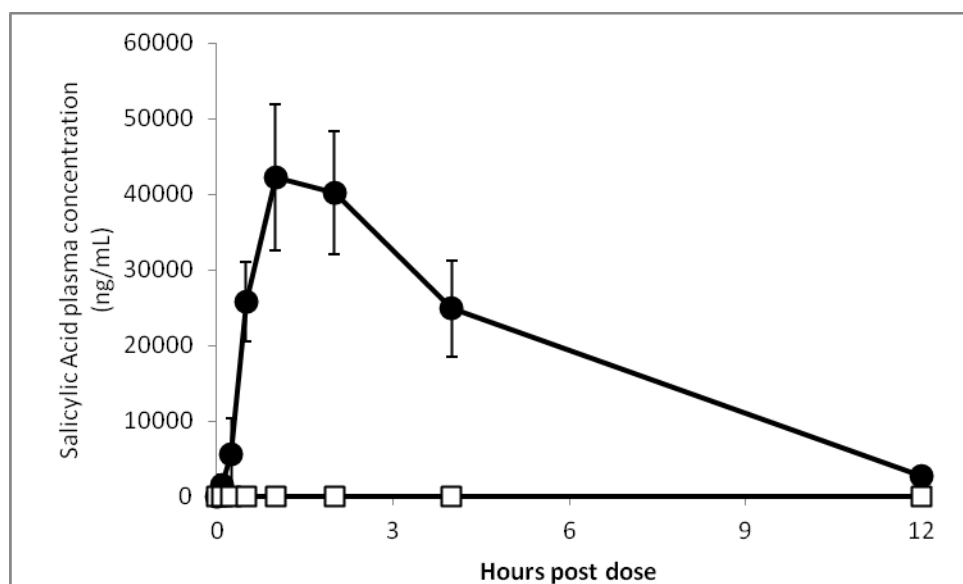


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