

PP32**Antipsoriatic activity of GYY4137 (a slow-releasing hydrogen sulphide donor) microemulsion system using a mouse skin model of psoriasis**

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We have previously shown that the systemic administration of fast-releasing hydrogen sulphide (H₂S) donors results in significant inhibition of the acute itching and associated cutaneous inflammation induced by either histamine or the mast cell degranulator compound 48/80, thus supporting the use of H₂S donors as therapeutic alternatives for controlling itch sensation (Nitric Oxide 2013; 31:S54–S55). On the other hand, the systemic administration of H₂S can be potentially life threatening and might have long-term side effects. The aim of this investigation was to assess the antipruritic and anti-inflammatory efficiency of a microemulsion as a drug delivery for the slow-releasing H₂S donor GYY4137 in the therapy of a psoriasis-like skin model. Under isoflurane anaesthesia, the dorsal skin of BALB/c mice was shaved (6 cm²), and psoriasis was induced by the topical application of the TLR-7 receptor agonist imiquimod (IMQ, as a 5% cream; 65 mg/animal; Germed, Brazil), on the dorsal skin and ear surface during 5 consecutive days. Control-treated mice received an equivalent amount of vaseline. Mice with psoriasis were concomitant treated with a microemulsion system containing GYY4137, at 1, 2 or 4 %, or its vehicle (poloxamer + oleic acid and monolein 9:1 w/w; 65 mg/day). The severity of psoriasis-like skin (area and severity index – PASI) and ears was scored daily during six days. The spontaneous itching behaviour was recorded during 30 min at days 1, 4 and 6, when myeloperoxidase activity (MPO, a marker of leucocyte infiltration) was also evaluated. At day 6, the mice were euthanized and skin collected. The single daily topical administration of the GYY4137 microemulsion greatly reduced, in a non-dependent concentration fashion, the increased MPO (12.6 ± 4.2 U/mg for control and 1.1 ± 0.4, 2.8 ± 1.9 and 2.3 ± 2.1 U/mg for GYY4137 at 1, 2 and 4% doses respectively; n = 5), and partially inhibited erythema score (7.5 ± 1.3 control and 4.8 ± 0.3* AUC GYY4137 at 4% dose; n = 5), but only fairly reduced PASI, scaly skin, ear thickness and spontaneous itching, as compared to psoriatic control mice. We conclude that besides the partial beneficial effect that has been demonstrated by the GYY4137 microemulsion, further changes of the current formulation should be performed in order to improve the topic delivery of GYY4137, and thus better suppress PASI and itching in this murine psoriasis-like disease.

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PP33**H₂S-induced increase in hepatic glucose production and the underlying mechanisms**

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Glucose homeostasis is critical for maintaining the physiological function of our body, whereby its sustained disturbance may lead to the metabolic syndrome. The liver is the major glucose-producing organ where gluconeogenesis (the *de novo* synthesis of glucose) occurs via stimulation from the cAMP/protein kinase A (PKA) and glucocorticoid signaling pathways. In the present study, we focused on the crucial role of cystathionine γ -lyase (CSE)-generated hydrogen sulfide (H₂S) in hepatic glucose production under physiological conditions. We found that CSE-knockout (KO) mice had a reduced rate of gluconeogenesis, which was reversed upon administration of NaHS (an H₂S donor) (i.p.). Interestingly, isolated CSE-KO hepatocytes exhibited a reduced glycemic response to chemical-induced activation of the cAMP/PKA and glucocorticoid pathways compared to wild-type (WT) hepatocytes. Treatment with the inhibitors for PKA (KT 5720) or glucocorticoid receptor (RU-486) significantly reduced H₂S-stimulated glucose production from both WT and CSE-KO mouse hepatocytes. NaHS treatment up-regulated the protein levels of key gluconeogenic factors, such as peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), phosphoenolpyruvate carboxykinase (PEPCK), and CCAAT-enhancer-binding proteins- β (C/EBP- β). Additionally, the enzymatic activities of the major gluconeogenic enzymes, fructose-1,6-bisphosphatase (FBPase) and glucose-6-phosphatase (G6Pase), were reduced in CSE-KO hepatocytes compared to WT hepatocytes, which were rescued with exogenous H₂S. Indeed, the sulfhydration levels of PEPCK and FBPase were significantly lower in CSE-KO hepatocytes compared to their WT counterparts, whereby exogenous H₂S significantly enhanced the sulfhydrated forms of both proteins. Finally, knockdown of PGC-1 α , but not C/EBP- β , significantly decreased NaHS-induced glucose production from the primary hepatocytes. This study demonstrates the stimulatory effect of endogenous H₂S on liver glucose production and reveals three underlying mechanisms; i.e. the glucocorticoid receptor pathway-mediated up-regulation of PGC-1 α and PEPCK; the cAMP/PKA-mediated up-regulation of PGC-1 α ; and the increased activities of G6Pase and FBPase. The current study may offer clues for the homeostatic regulation of glucose metabolism under physiological conditions and its dysregulation in the metabolic syndrome.

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PP34**The role of hydrogen sulfide in enhanced sphingomyelinase induced vasorelaxation in db/db mice**

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Sphingomyelinase (SMase) induces changes in vascular tone with an initial contraction and subsequent relaxation in aortic rings of wild type mice and – remarkably – these effects are more pronounced in db/db animals. However, the exact mechanism of these increased effects is not understood; therefore we aimed to investigate the reasons behind the enhanced vasorelaxation. Thoracic aorta segments were isolated from adult db/db and non-diabetic littermate mice. The effect of 0.2 U/ml SMase was investigated after precontraction with 0.1 μ M phenylephrine under isometric

conditions in myographs. Vascular segments were tested in the presence of the following inhibitors: eNOS inhibitor L-NAME (100 μ M), selective thromboxane receptor antagonist SQ 29,548 (1 μ M), ceramidase inhibitor D-erythro-MAPP (50 μ M), PI3-kinase inhibitor Wortmannin (0.1 μ M), Akt-inhibitor MK2206 (1 μ M), PLC inhibitor U73122 (10 μ M) and cystathionine- γ -lyase (CSE) inhibitor propargylglycine (10 mM). We also tested the effects of sodium hydrosulfide (NaHS, 10^{-5} – 10^{-3} M) and sodium nitroprusside (SNP, 10^{-10} – 10^{-5} M) after precontraction induced by 1 μ M phenylephrine in isometric condition in myographs. L-NAME administration completely abolished while SQ 29,548 increased relaxations in both groups, but especially in the db/db group. The use of D-erythro-MAPP, Wortmannin and MK2206 did not alter the vasoactive effects of SMase in control and db/db derived vessels, while U73122 completely inhibited the relaxations in diabetic aortas. The inhibition of H₂S production led to less relaxation in db/db vessels but had no effect on control vessels. Dose–response curves for NaHS were shifted to the left while dose–response curves for SNP were shifted to the right in the db/db group.

Our results indicate that H₂S contributes to the SMase induced and surprisingly enhanced vasorelaxations in db/db animal derived aortas. The altered dose–response curves for NaHS and SNP suggest that phosphodiesterase activity might be enhanced in the aortas of db/db mice. This suggests decreased availability of its endogenous inhibitor H₂S and indicates that sphingomyelinase might lead to enhanced vasorelaxations by massively increased NO production. An interesting aspect of our results is that diabetic vascular tissues have bigger sensitivity to either endogenously or exogenously altered H₂S levels. Taken together our observations strengthen the importance to achieve proper H₂S levels in diabetic vascular tissues.

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PP35

Perivascular adipose tissue derived hydrogen sulphide contributes to hypoxic relaxation of the porcine coronary artery

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Perivascular adipose tissue (PVAT) has been shown to have a paracrine role in the regulation of vascular tone [1] and to enhance vasorelaxation in response to hypoxia in mouse aorta [2]. Hydrogen sulphide (H₂S) is synthesised in PVAT [3] and our studies show a role for H₂S in mediating porcine coronary artery vasodilation induced by hypoxia [4]. The aim of the current study was to investigate the effect of PVAT on the response to hypoxia and determine whether H₂S contributes to its effects.

Segments of porcine coronary arteries, obtained from a local abattoir, were mounted in organ baths, containing Krebs–Henseleit buffer (37 °C) gassed with 95% O₂/5% CO₂, for isometric tension recording. Viability was assessed using 60 mM KCl. Arteries were precontracted with the thromboxane A₂ mimetic, U46619; once a stable tone (~50% of KCl response) was achieved, hypoxia was induced using 95% N₂/5% CO₂ for 30 min. Hypoxic responses were investigated in the absence and presence of inhibitors of cystathionine γ -lyase (CSE), cystathionine β -synthase (CBS) and/or 3-mercaptopyruvate sulphurtransferase (MPST) in segments with and without connected PVAT. Data are presented as mean $\pm$ SEM (n = 8) with contractions and relaxations expressed as a percentage of the U46619-induced tone. Data were analysed using one-way ANOVA with Tukey's post-test, where P < 0.05 was considered significant.

Exposing coronary arteries to hypoxia caused a biphasic response; a transient contraction followed by a prolonged relaxation.

In the presence of PVAT, the contractile phase of the hypoxic response was abolished (control $20 \pm 3\%$; PVAT $-1 \pm 1\%$, P < 0.0001) and the relaxation response was enhanced (control $69 \pm 5\%$; PVAT $104 \pm 9\%$, P < 0.05). In the presence of PVAT, the CSE inhibitor D,L-propargylglycine (10 μ M) and the competitive MPST inhibitor aspartate (1 mM) alone or in combination had no effect on vasodilation in response to hypoxia. By contrast, the CBS inhibitor aminoxyacetate (AOAA, 100 μ M) alone significantly reduced the hypoxic induced vasorelaxation in PVAT intact vessels (PVAT $104 \pm 9\%$; PVAT+AOAA $50 \pm 7\%$, P < 0.01) but had no effect on the relaxation phase in control vessels.

These data suggest that CBS-derived H₂S is released from PVAT, enhancing the cardioprotective vasodilatation elicited in response to an ischemic or hypoxic insult to the coronary vasculature.

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PP36

Hydrogen sulphide rescues preeclampsia-like phenotype aggravated by high sFlt-1 in placenta growth factor (PlGF) deficiency

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Placenta growth factor (PlGF) deficient mice are fertile at a Mendelian ratio. Interestingly, low maternal plasma levels of PlGF are strongly associated with early onset of preeclampsia, a pregnancy hypertensive disorder characterised by high blood pressure, proteinuria and fetal growth restriction. PlGF is increasingly being recognised as an early diagnostic biomarker, but the physiological importance of PlGF in the pathogenesis of preeclampsia is unknown. We investigated whether the decreased levels of PlGF in pregnancy exacerbate the fetal growth restriction associated with preeclampsia in the presence of high sFlt-1 and the potential of hydrogen sulphide to ameliorate these effects. Pregnant PlGF^{-/-} mice were injected with adenovirus encoding sFlt-1 (Ad-sFlt-1) at 1×10^9 pfu/ml at E10.5 and mean arterial blood pressure (MAP), biochemical and histological analysis of maternal kidney, placenta and embryos were assessed at the end of pregnancy. Ad-sFlt-1 significantly increased MAP and induced severe glomerular endotheliosis in PlGF^{-/-} mice compared to wild-type animals. Soluble Flt-1 also significantly elevated albumin–creatinine ratio and increased levels of urinary kidney injury molecule-1, a marker for proximal tubule injury. Furthermore, sFlt-1 overexpression increased fetal resorption rate in the PlGF^{-/-} mice and promoted abnormal placental vascularisation. To determine whether placental PlGF is critical for preventing fetal growth restriction associated with preeclampsia, we generated haploinsufficient PlGF^{+/-} placentas and embryos in dams and exposed to high sFlt-1 environment. These mothers showed reduced fetal resorption, gestational hypertension and proteinuria when compared to pregnant PlGF^{-/-} mice. Furthermore, treatment with hydrogen sulphide-releasing agent, GYY4137,