Pharmacological Reports
https://doi.org/10.1007/s43440-024-00643-z

SHORT COMMUNICATION

A hydrogen sulphide-releasing non-steroidal anti-inflammatory, ATB-346, significantly attenuates human myometrial contractions

Ana Mijušković[1,3] · Susan Wray[1] · Sarah Arrowsmith[1,2]

Received: 26 March 2024 / Revised: 9 August 2024 / Accepted: 21 August 2024

Abstract

Background Spontaneous preterm birth is the leading cause of perinatal morbidity and mortality. Tocolytics are drugs used to inhibit uterine contractions in cases of imminent preterm birth, however, few are effective in stopping labour once initiated and all have side effects. Combination approaches involving drugs that target multiple signalling pathways that regulate contractions may increase efficacy, reduce dosage and improve tolerability. Both non-steroidal anti-inflammatory drugs (NSAIDs) and hydrogen sulphide (H_2S)-releasing compounds can reduce myometrial contractions. In a novel approach we evaluated the tocolytic properties of ATB-346–a H_2S-releasing derivative of the NSAID naproxen, shown clinically to reduce pain and inflammation in arthritis.

Methods Using organ baths, paired strips of human myometrium were exposed to increasing concentrations of ATB-346, or equimolar concentrations (10µM and 30µM) of the parent drug, naproxen, or the H_2S-releasing moiety, 4-hydroxythiobenzamide (TBZ), alone. The ability of ATB-346 versus the individual components of ATB-346 to decrease ex vivo spontaneous contractions was investigated, and the potency was compared to a known H_2S donor, Na_2S.

Results Acute application of Na_2S produced a concentration-dependent decrease in force amplitude and force integral (area under the curve) of contraction. ATB-346 produced a more profound decrease in contraction compared to equimolar concentrations of naproxen or TZB alone and was more potent than the equivalent concentration of Na_2S.

Conclusions ATB-346 exhibits potent tocolytic properties in human myometrium. These exciting results call for further exploration of ATB-346, with a view to repurposing this or similar drugs as novel therapies for delaying preterm labour.

Keywords ATB-346 · Hydrogen sulphide · Myometrium · Contraction · Tocolytics · Preterm birth

Introduction

Hydrogen sulphide (H_2S) is an endogenous gaseous signalling molecule, akin to nitric oxide and carbon monoxide. Since the discovery of endogenous H_2S in the mammalian brain, it has been shown to play a prominent role in many physiological and pathological processes, including blood vessel relaxation, inflammation and cellular protection [1]. In addition to its role in vasodilation, H_2S has also been found to regulate the contraction of other smooth muscles, including the uterus (myometrium) [2].

In rodent and human myometrium, H_2S and H_2S-releasing compounds such the classical H_2S-releasing donors, sodium hydrosulphide (NaHS) and sodium sulphide (Na_2S), and the synthetic H_2S-releasing GYY4137 (morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate), are potent inhibitors of myometrial contractions [3–6]. H_2S modulates the activity of several ion channels; K_{ATP}, L-type Ca channels [6] and chloride channels [5] in myometrium to dampen excitability. These effects maybe via protein-S-sulphydration affecting their permeability, (as shown for K_{ATP} channels in other tissues [7, 8]) as well as downregulating the expression of proteins involved in contraction [9].

In mammalian tissues, H_2S is predominantly produced from L-cysteine via the enzymatic activity of cystathionine

✉ Sarah Arrowsmith
s.arrowsmith@mmu.ac.uk

1 Harris-Wellbeing Research Centre, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, UK

2 Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester M1 5GD, UK

3 Department of Physiology, Institute for Biological Research 'Siniša Stanković', University of Belgrade, Belgrade, Serbia

Published online: 04 September 2024

γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptosulfurtransferase [10]. Both CSE and CBS are expressed in reproductive tissues during pregnancy including the placenta, fetal membranes and myometrium [3, 11]. Application of L-cysteine also reduces myometrial contraction [12], suggesting that uterine cells can endogenously produce H_2S. In non-pregnant mouse myometrium, estrogen levels have been shown to regulate CSE and CBS enzyme expression during the estrous cycle [13]. In humans, the expression of these enzymes in myometrium is downregulated towards term and labour onset, [3, 9] and enzyme expression and H_2S production is also reduced in term and preterm labouring chorionic tissues [14]. Hence, endogenous H_2S may have an important role in maintaining uterine quiescence which is necessary to continue a pregnancy to term. In accordance with this theory, the inhibitory effects of H_2S on contraction are reversed in normal labour [6] whilst NaHS has been shown to delay inflammation-induced preterm birth in mice [15].

Preterm birth (before 37 weeks gestation) is the major cause of neonatal death and morbidity with many surviving babies facing significant neurodevelopment delay and lifelong disability [16]. Survival rates and adverse outcomes are strongly associated with gestational age at delivery. Current management of threatened preterm labour is focussed on supressing uterine contractions using tocolytics to delay delivery to allow administration of fetal neuroprotectants and steroids to mature the fetal lungs before delivery [17]. Most tocolytics however, are not very effective, have adverse effects and difficult routes of administration. The non-steroidal anti-inflammatory drug (NSAID), indomethacin, which inhibits cyclo-oxygenase (COX)-mediated production of prostaglandins, is one class of tocolytic. In pre-clinical studies, it is relatively effective in attenuating uterine contractions [18, 19] and clinically, it has been shown to be more effective delaying delivery than other tocolytic agents [20]. Like most tocolytics, however, it is not utero-specific and has maternal and fetal side effects which limits its use to just a few days [21]. There is therefore a pressing need to develop safer and more potent treatments for preterm labour. Combination tocolytics which target different intracellular signalling pathways, are attractive, as they may produce additive inhibitory effects, but allow for lower therapeutic doses to be used, increasing their efficacy and reduce side effects. To date, most combination approaches have focussed on the administration of two drugs, both of which act to suppress cellular pathways which bring about contraction e.g. calcium channel inactivation and blocking the oxytocin receptor [22]. Few have examined approaches involving co-administration of drugs which act to suppress contraction activating pathways as well as activate endogenous pathways which promote relaxation.

A relatively new class of H_2S donors are the H_2S-releasing non-steroidal anti-inflammatory drugs (H_2S-NSAIDs). They consist of a traditional NSAID to which an H_2S-releasing moiety is covalently attached [23]. ATB-346 (2-(6-methoxynapthalen-2-yl)-propionic acid 4-thiocarbamoyl phenyl ester) is a NSAID derived from naproxen and coupled to 4-hydroxy-thiobenzamide (TBZ), a potent inducer of H_2S release [24]. Hence, ATB-346, like indomethacin, inhibits COX activity but also releases H_2S.

Given the potential for ATB-346 as a 'combined tocolytic,' stemming from having both anti-inflammatory actions and H_2S-releasing properties, we examined the tocolytic properties of ATB-346 on ex vivo human myometrial contractions and compared it to the parent NSAID (naproxen), TBZ alone and the H_2S donor, Na_2S, which has not previously been investigated in human myometrium.

Materials and methods

Reagents

Unless stated otherwise, all reagents were purchased from Merck, UK. ATB-346, and TBZ were kind gifts from Prof John Wallace, University of Calgary, Canada. ATB-346, TBZ and naproxen were prepared in DMSO. Na_2S, was prepared as described below. All working concentrations were prepared freshly on the day and in the case of Na_2S, prepared immediately before application to the tissue baths and stored on ice.

Preparation of Na_2S

Sodium sulphide (Na_2S) stock solution was prepared, and concentration determined by a spectrophotometric assay using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB) which quantifies the concentration of thiol groups in the sample [25]. Briefly, one crystal of Na_2S was washed twice in molecular grade ultrapure water before dissolving. Serial dilutions were prepared, DTNB added to give 100nM and absorbance read at 412nm. The concentration of Na_2S was determined using the extinction coefficient of 14,150 M^{-1} cm^{-1} [26]. Stock concentrations of Na_2S were stored at – 70 °C for up to one week. DTNB was prepared in 0.1M sodium phosphate buffer, 1mM EDTA (pH 8.0) and stored at – 20 °C.

Sample collection and preparation

Myometrial tissue was obtained with written informed consent from 11 women who underwent elective (pre-labour) caesarean section (CS) delivery. The women were between 23 and 43 years of age, with a singleton pregnancy and

delivered between 38 and 41 weeks of gestation. Indications for CS were CS delivery in previous delivery (n = 8), previous traumatic vaginal delivery (n = 2) and breech presentation (n = 1). Women with multiple (e.g. twin) pregnancy, a history of diabetes or hypertension or receiving medications at the time which may affect uterine contractions were excluded from the study. The study received approval from the Local Research Ethics committee (Liverpool East, REC Ref 10/H1002/49 + 5) and institutional review boards of the Research and Development Department, Liverpool Women's Hospital and University of Liverpool.

During surgery, myometrial tissue was excised from the upper lip of the lower uterine incision site following delivery of the baby and placenta and placed into cooled Hank's balanced salt solution. Samples were used immediately or within 16 h of collection with storage at 4 °C. In the laboratory, multiple strips (5 × 2 × 1 mm) were dissected along the direction of longitudinal fibres, as previously described [27]. Aluminium clips were attached to each end and the strips were mounted horizontally within 1-mL organ baths continually superfused with physiological saline solution (PSS; in mM: 154 NaCl, 5.6 KCl, 1.2 $MgSO_4$, 7.8 glucose, 10.9 HEPES, and 2.0 $CaCl_2$) at a rate of 1.0 mL/min, pH 7.4 at 37 °C. One end was attached to a fixed hook, and the other was attached to a FORT 10 g force transducer (World Precision Instruments, Hertfordshire, UK). Each sample was stretched to produce 2 mN force and left to equilibrate in PSS until spontaneous contractions were established, typically within 2 h [28].

Human myometrial contractility assay

After the onset of spontaneous contractions, strips were left to contract in PSS until a minimum of 4 consecutive contractions of approximately equal amplitude and regular frequency were achieved. The PSS superfusing the baths was then exchanged for PSS containing the relevant compound. Firstly, the effect of applying increasing concentrations of Na_2S along a log-scale concentration range from 0.3 to 100 µM was investigated (n = 5). Each concentration of Na_2S was applied for 25 min under continual flow without washout between applications.

In a second experiment involving multiple strips from the same biopsy (paired experiments), the effect of ATB-346 (10 µM and 30 µM, which was guided by the effective concentrations observed for Na_2S) was compared to the effect of the equivalent concentration of unconjugated naproxen or TBZ alone (n = 5–6). Application of each concentration was for 25 min under continual flow and without washout between applications. The concentration of vehicle (DMSO) in the tissue bath did not exceed 1/1000 dilution and is known not to cause significant changes to contraction [19].

Data collection and analysis

Contractions were recorded at a sampling rate of 10Hz via a data acquisition system running the associated software (Labscribe 3; World Precision Instruments, UK). Measurement of contractile activity was performed by calculation of the integral area under the tension curve (AUC, arbitrary units) and mean maximum amplitude of contraction (expressed in mN) using Origin Pro 19 (OriginLab Corporation, MA, USA). Control contractile activity was measured in the 25 min preceding the addition of the first drug. The effects of each compound at each concentration were similarly calculated and expressed as a percentage of the integral and amplitude during the control period (i.e., control activity is equal to 100%).

Statistical analysis

Unless stated otherwise, all values represent the mean ± standard error of the mean (SEM), where "n" is the number of samples, and each represents a different woman. Data were found to be normally distributed by Kolmogorov–Smirnov test, and groups were compared by one-way ANOVA followed by Tukey's multiple comparison post hoc tests using GraphPad Prism 5.0. A probability value of < 0.05 was taken as level of significance. F values for ANOVA indicate the ratio of the between group variance to within group variance. Values in subscript indicate degrees of freedom for explained variance (number of groups minus 1) and unexplained variance (number of observations minus number of groups/residual variance) respectively.

Results

Na_2S is a potent relaxant of human myometrial contractions.

Acute application of the H_2S donor, Na_2S, to contracting strips of human myometrium produced a concentration-dependent decrease in contractile activity which was measured based on a decrease in force amplitude and integral of force (area under the curve, AUC) compared to pretreatment control. A representative recording of the effects of Na_2S is shown in (Fig. 1A). The decrease in force amplitude was determined to be significant at 30µM Na_2S, causing contraction amplitude to decrease to 67.9% (± 5.87) of control activity with further reduction to 31.8% (± 9.02) of control following application of 100 µM Na_2S ($F_{6, 28} = 23.0$, $p < 0.0001$, Fig. 1B). For integral of force, significance was achieved at 100 µM Na_2S in which the mean area under the curve was reduced to 51.5% (± 9.62) of control ($F_{6, 28} = 7.62$, $p < 0.0001$, Fig. 1C).

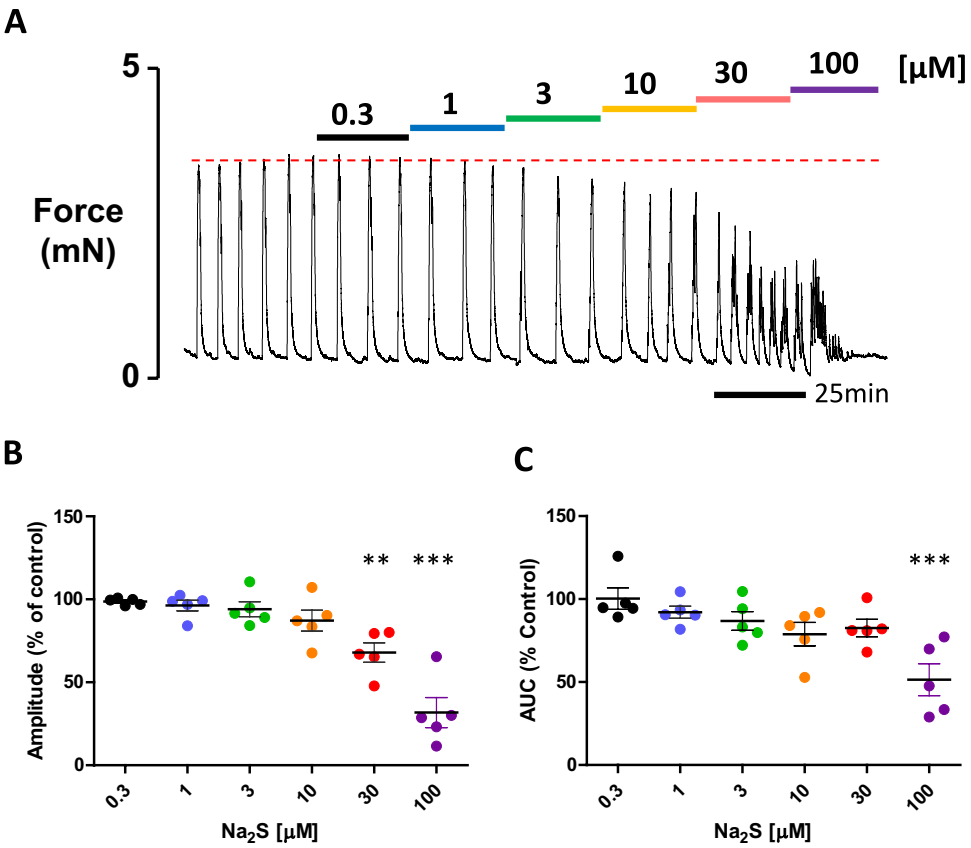

Fig. 1 The effect of Na_2S on human myometrial contractions. **A** Representative recording of spontaneous contractions of human myometrium and the effect of the application of increasing concentrations of Na_2S (μM). Dotted red line indicates control, pre-treatment activity. **B** and **C** Individual data points, each representing a different woman, showing the effect of Na_2S on force amplitude and mean integral force (area under the curve, AUC.) respectively. Black bar indicates mean percentage of control activity (±SEM). Data were compared by one-way ANOVA and Tukey's multiple comparison post-hoc test. P value of <0.05 was taken as significant, $^{**}p<0.01$, $^{***}p<0.001$, $n=5$ women

ATB-346 significantly attenuates human myometrial contraction

Application of naproxen or TBZ alone to spontaneously contracting strips of human myometrium (Fig. 2A) resulted in small and non-significant effects on contraction amplitude and AUC. This was true for both concentrations tested: Expressed as percentage of control amplitude and AUC respectively; 10μM naproxen: 95.6% (±4.8) and 101.4% (±5.6); 30μM naproxen: 93.6% (±4.4) and 96.3% (±7.8); 10μM TBZ: 90.0% (±3.6) and 83.7% (±8.5); 30μM TBZ: 88.6% (±5.2) and 83.0% (±11.7) (For 10μM amplitude, $F_{2,\ 12}=8.25$, $p>0.05$ and AUC, $F_{2,12}=8.57$, $p>0.05$; For 30 μM amplitude $F_{2,\ 14}=5.77$, $p>0.05$ and AUC, $F_{2,\ 14}=9.88$, $p>0.05$, Fig. 2B and C).

In contrast however, application of ATB-346 to multiple strips from the same woman produced a significant decrease in activity which was observed at 10μM: 62.4% (±8.9) of control force amplitude ($F_{2,\ 12}=8.25$, $p=0.0038$) and 62.3% (±5.6) of control AUC ($F_{2,\ 12}=8.57$, $p=0.0033$, Fig. 2A, B*i* and *ii*). This effect was further potentiated at 30μM; 44.9% (±17.8) of control amplitude ($F_{2,\ 14}=5.77$, $p=0.0176$) and 34.1% (±11.4) of control AUC ($F_{2,\ 14}=9.88$, $p=0.0029$, Fig. 2Ci and ii). The effect appeared reversible upon washout with contractions typically being restored to their pre-treatment amplitude and force integral within 30 min of washout (observation only).

Discussion

We present data on human myometrium which suggests that a combination of activating a smooth muscle relaxatory pathway, H_2S, and inhibiting a contraction promoting pathway (COX), produces significantly more attenuation of uterine contractions, than either action alone. Furthermore, these potent effects were produced using a single drug, ATB-346, not previously tested on uterine smooth muscle. The advantages of this novel approach for the treatment of threatened preterm delivery are discussed.

We found sodium sulphide (Na_2S) exerts a concentration-dependent tocolytic effect in pregnant human myometrium. This adds to earlier observations on the effect of Na_2S in non-pregnant rat myometrium [5]. These data also build on findings obtained from other H_2S donors in rodent and human myometrium, that H_2S has a relaxant effect on ex vivo myometrial contractions [3–6, 29].

Whilst not directly compared, a greater concentration of Na_2S was required to achieve a significant decrease in contraction amplitude and AUC than with the hydrogen sulphide-releasing NSAID, ATB-346; the significant effects of

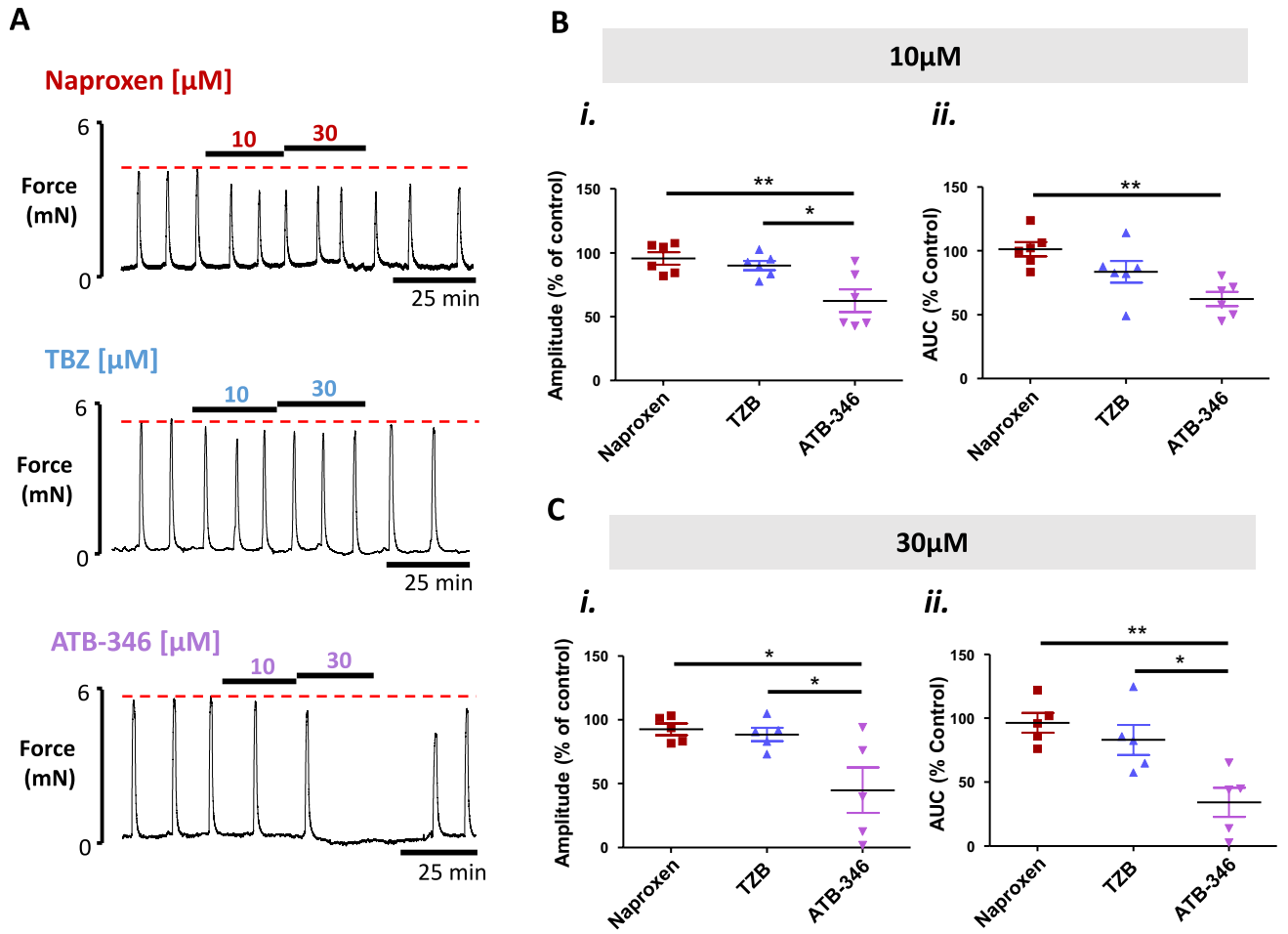

Fig. 2 The effect of naproxen, TBZ and ATB-346 on human myometrial contractions. **A** Representative recordings of spontaneous contractions of human myometrium and the effect of the application of naproxen (top trace), TBZ (middle trace) and ATB-346 (bottom trace) in strips from the same woman. Dotted red line indicates control, pre-treatment activity. **B** and **C** The effect of 10µM and 30µM treatment on (i) force amplitude and (ii) mean integral force (area under the curve, AUC) respectively. Mean percentage of control activity is shown (black bars) ± SEM (coloured bars). Data were compared by one-way ANOVA and Tukey's multiple comparison post-hoc test. P value of <0.05 was taken as significant where $^{*}p<0.05$, $^{**}p<0.01$, n = 5–6 women

ATB-346 were observed at 10µM compared to 30µM (amplitude) and 100 µM (AUC) with Na_2S. ATB-346 was also more potent than the equivalent concentration of the parent NSAID naproxen, or H_2S donor, TBZ when applied in isolation. We suggest that the more profound effect of ATB-346 compared to either naproxen or TBZ alone, or Na_2S, is due to the combination effects resulting from COX inhibition (arising from naproxen moiety) and release of H_2S via the TBZ moiety, which decreases excitability and thus, contraction [6].

Unlike our data with Na_2S, the H_2S-releasing TBZ produced only a small relaxant effect. This can be explained by the previously reported small release of H_2S from TBZ alone, compared to when it is covalently bound to another drug such as a NSAID like naproxen [30]. In rat liver homogenates the release of H_2S from ATB-346 was shown to be six times greater than from an equimolar concentration of TBZ [24, 31]. Hence the release of H_2S from TBZ in our system is unlikely to have been as great as from the equivalent concentration of ATB-346.

Like other NSAIDs, naproxen is known to inhibit prostaglandin synthesis via inhibition of COX-2. In myometrium, early work in pregnant rats showed that a 3-day infusion of naproxen delivered by an implanted osmotic pump, significantly reduced release of prostaglandins from uteri, prolonged gestation and reduced in vivo contractions in response to oxytocin [32]. In non-pregnant humans, naproxen therapy has also been shown to reduce menstrual prostaglandin levels and severity of menstrual cramps (dysmenorrhea) compared to placebo [33]. Here we show that acute application of naproxen to ex vivo pregnant human myometrium reduces contractions, but not significantly under the concentration range we tested. This appears to be the first paper examining the effect of acute application

of naproxen ex vivo in human myometrium. Interestingly, the acute application of another NSAID, indomethacin, to ex vivo strips of myometrium causes significant decreases in contractions—~ 60% at 30 µM [19]. Higher concentrations of naproxen may be required to achieve the equivalent tocolytic effects as indomethacin. However, these concentrations are likely to exceed the normal therapeutic range.

Before the onset of term and preterm labour, the expression of pro-inflammatory cytokines (including chemokines) in uterine tissues is increased [34]. These inflammatory mediators stimulate the expression of contraction-associated proteins including oxytocin receptors, connexin 43, and prostaglandin H synthase in myometrium and production of prostaglandins which ultimately leads to labour onset [35, 36]. H_2S donors have also been shown to affect the expression of proinflammatory cytokines and contraction-associated protein expression in human myometrial cells by interfering with NFκB signalling and inhibiting the production of the proinflammatory cytokines IL-1β, IL-6 and TNF-α [9, 37], as well as to delay lipopolysaccharide-induced preterm labour in mice [15].

ATB-346 was originally developed to reduce gastrointestinal ulceration and injury caused by NSAIDs such as naproxen, when taken for chronic pain such as in osteoarthritis. Multiple pre-clinical studies have since demonstrated a superior anti-inflammatory effect of ATB-346 when compared to naproxen whilst also having less damaging effects on the gastrointestinal tract [24, 38, 39]. More recently in a human model of bacterial infection in skin, ATB-346 was shown to reduce neutrophil infiltration [40], and in mice, ATB-346 provides neuroprotection in traumatic brain injury models by reducing secondary inflammation and tissue injury[41]. It would therefore be interesting to examine whether ATB-346 has similar anti-inflammatory activities in the myometrium. If so, the anti-inflammatory properties of H_2S acting in concert with the anti-inflammatory effects associated with inhibition of COX-2 via naproxen, combined with its direct tocolytic actions would present a promising novel therapeutic strategy for reducing rates of preterm birth. For example, in addition to its tocolytic properties in slowing contractions in women in threatened preterm labour, it could potentially be used prophylactically to prevent upregulation of inflammatory signalling pathways and prevent preterm labour in women at high risk of preterm birth.

A limitation to our investigation was that we were unable to study the effects of ATB-346 in preterm myometrium which would better reflect the tissue type in which tocolytics would be used, or in the presence of hormones such as oxytocin and $PGF_{2\alpha}$, which in vitro can reduce potency of NaHS and GYY4137 in myometrium [6]. This action is not particular to H_2S, as it has been found for other tocolytics [19, 22, 42]. Thus, future studies should investigate whether the potency of ATB-346 is reduced in the presence of these hormones and test its potency in tissues from preterm births. The mechanism by which contractions are reduced by ATB-346 also requires investigation. For example, some NSAIDs, including naproxen, are also known to effect cAMP signalling [43], whilst others, e.g. indomethcin, affect calcium influx [18]. Hence, there may be additional actions to those traditional concepts of inhibition of COX-2 and prostaglandin synthesis.

Conclusion

There is an urgent need for better tocolytic (and prophylactic) treatments to prevent or delay preterm birth. Combination approaches targeting different contraction pathways enable greater potency whilst also reducing therapeutic dosages and thereby decreasing maternal/fetal side effects. As with other smooth muscles, H_2S exerts potent relaxatory effects in pregnant human myometrium. The novel and exciting feature about H_2S-releasing NSAIDs in the myometrium is their dual action; supressing contraction (and inflammatory) activation pathways (via COX inhibition) whilst also activating endogenous relaxatory pathways (via H_2S). H_2S-releasing NSAIDs may therefore be a promising alternative therapy for prevention of preterm labour and should be further explored.

Acknowledgment We thank the patients and staff at Liverpool Women's hospital for consenting and collection of myometrial biopsies used in this research. We also thank Prof John Wallace, University of Calgary, Canada for his kind gift of ATB-346 and TBZ.

Author contributions All authors made substantial contributions to the conception and design of the project. AM and SA were responsible for the acquisition, analysis and interpretation of data. All authors contributed equally to the drafting of the manuscript.

Funding Part of this work was funded by a Harris-Wellbeing Preterm Birth Research Centre grant administered by Wellbeing of Women, UK. AM was funded by a Federation of European Biochemical Societies Fellowship.

Data availability The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have nothing to disclose.

References

1. Szabo C. A timeline of hydrogen sulfide (H(2)S) research: from environmental toxin to biological mediator. Biochem Pharmacol. 2018;149:5–19.
2. Dunn WR, Alexander SP, Ralevic V, Roberts RE. Effects of hydrogen sulphide in smooth muscle. Pharmacol Ther. 2016;158:101–13.
3. You XJ, Xu C, Lu JQ, Zhu XY, Gao L, Cui XR, et al. Expression of cystathionine β-synthase and cystathionine γ-lyase in human pregnant myometrium and their roles in the control of uterine contractility. PLoS ONE. 2011;6: e23788.
4. Hu R, Lu J, You X, Zhu X, Hui N, Ni X. Hydrogen sulfide inhibits the spontaneous and oxytocin-induced contractility of human pregnant myometrium. Gynecol Endocrinol. 2011;27:900–4.
5. Mijušković A, Kokić AN, Dušić ZO, Slavić M, Spasić MB, Blagojević D. Chloride channels mediate sodium sulphide-induced relaxation in rat uteri. Br J Pharmacol. 2015;172:3671–86.
6. Robinson H, Wray S. A new slow releasing, H(2)S generating compound, GYY4137 relaxes spontaneous and oxytocin-stimulated contractions of human and rat pregnant myometrium. PLoS ONE. 2012;7: e46278.
7. Jiang B, Tang G, Cao K, Wu L, Wang R. Molecular mechanism for H(2)S-induced activation of K(ATP) channels. Antioxid Redox Signal. 2010;12:1167–78.
8. Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, et al. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circ Res. 2011;109:1259–68.
9. You X, Chen Z, Zhao H, Xu C, Liu W, Sun Q, et al. Endogenous hydrogen sulfide contributes to uterine quiescence during pregnancy. Reproduction. 2017;153:535–43.
10. Li L, Rose P, Moore PK. Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol. 2011;51:169–87.
11. Patel P, Vatish M, Heptinstall J, Wang R, Carson RJ. The endogenous production of hydrogen sulphide in intrauterine tissues. Reprod Biol Endocrinol. 2009;7:10.
12. Sidhu R, Singh M, Samir G, Carson RJ. L-cysteine and sodium hydrosulphide inhibit spontaneous contractility in isolated pregnant rat uterine strips in vitro. Pharmacol Toxicol. 2001;88:198–203.
13. Guerra DD, Bok R, Breen K, Vyas V, Jiang H, MacLean KN, et al. Estrogen regulates local cysteine metabolism in mouse myometrium. Reprod Sci. 2021;28:79–90.
14. Sun Q, Chen Z, He P, Li Y, Ding X, Huang Y, et al. Reduced expression of hydrogen sulfide-generating enzymes down-regulates 15-hydroxyprostaglandin dehydrogenase in chorion during term and preterm labor. Am J Pathol. 2018;188:63–71.
15. Liu W, Xu C, You X, Olson DM, Chemtob S, Gao L, et al. Hydrogen sulfide delays LPS-induced preterm birth in mice via anti-inflammatory pathways. PLoS ONE. 2016;11: e0152838.
16. Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. Lancet. 2023;402:1261–71.
17. Wray S, Arrowsmith S, Sharp A. Pharmacological interventions in labor and delivery. Annu Rev Pharmacol Toxicol. 2023;63:471–89.
18. Sawdy R, Knock GA, Bennett PR, Poston L, Aaronson PI. Effect of nimesulide and indomethacin on contractility and the Ca2+ channel current in myometrial smooth muscle from pregnant women. Br J Pharmacol. 1998;125:1212–7.
19. Arrowsmith S, Neilson J, Bricker L, Wray S. Differing in vitro potencies of tocolytics and progesterone in myometrium from singleton and twin pregnancies. Reprod Sci. 2016;23:98–111.
20. Haas DM, Caldwell DM, Kirkpatrick P, McIntosh JJ, Welton NJ. Tocolytic therapy for preterm delivery: systematic review and network meta-analysis. BMJ. 2012;345: e6226.
21 Reinebrant HE, Pileggi-Castro C, Romero CL, Dos Santos RA, Kumar S, Souza JP, et al. Cyclo-oxygenase (COX) inhibitors for treating preterm labour. Cochrane Database Syst Rev. 2015;2015:Cd001992.
22. Arrowsmith S, Neilson J, Wray S. The combination tocolytic effect of magnesium sulfate and an oxytocin receptor antagonist in myometrium from singleton and twin pregnancies. Am J Obstet Gynecol. 2016;215(789):e1–9.
23. Wallace JL. Hydrogen sulfide-releasing anti-inflammatory drugs. Trends Pharmacol Sci. 2007;28:501–5.
24. Wallace JL, Caliendo G, Santagada V, Cirino G. Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). Br J Pharmacol. 2010;159:1236–46.
25. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–7.
26. Riddles PW, Blakeley RL, Zerner B. Reassessment of Ellman's reagent. Methods Enzymol. 1983;91:49–60.
27. Arrowsmith S, Keov P, Muttenthaler M, Gruber CW. Contractility measurements of human uterine smooth muscle to aid drug development. J Vis Exp. 2018;131: e56639.
28. Arrowsmith S, Quenby S, Weeks A, Burdyga T, Wray S. Poor spontaneous and oxytocin-stimulated contractility in human myometrium from postdates pregnancies. PLoS ONE. 2012;7: e36787.
29. Mitidieri E, Tramontano T, Donnarumma E, Brancaleone V, Cirino G, R DEdVB, et al. l-Cys/CSE/H2S pathway modulates mouse uterus motility and sildenafil effect. Pharmacol Res. 2016;111:283–9.
30. Wallace JL, Blackler RW, Chan MV, Da Silva GJ, Elsheikh W, Flannigan KL, et al. Anti-inflammatory and cytoprotective actions of hydrogen sulfide: translation to therapeutics. Antioxid Redox Signal. 2015;22:398–410.
31. Wallace J, Cirino G, Santagada V, Caliendo G. Hydrogen sulfide derivatives of non-steroidal anti-inflammatory drugs. United States Patent Application No. WO/2008/009127; 2010;
32. Chan WY, Berezin I, Daniel EE. Effects of inhibition of prostaglandin synthesis on uterine oxytocin receptor concentration and myometrial gap junction density in parturient rats. Biol Reprod. 1988;39:1117–28.
33. Chan WY, Fuchs F, Powell AM. Effects of naproxen sodium on menstrual prostaglandins and primary dysmenorrhea. Obstet Gynecol. 1983;61:285–91.
34. Sivarajasingam SP, Imami N, Johnson MR. Myometrial cytokines and their role in the onset of labour. J Endocrinol. 2016;231:R101–19.
35. Cook JL, Zaragoza DB, Sung DH, Olson DM. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. Endocrinology. 2000;141:1718–28.
36. Shynlova O, Lee YH, Srikhajon K, Lye SJ. Physiologic uterine inflammation and labor onset: integration of endocrine and mechanical signals. Reprod Sci. 2013;20:154–67.

37. Chen Z, Zhang M, Zhao Y, Xu W, Xiang F, Li X, et al. Hydrogen sulfide contributes to uterine quiescence through inhibition of NLRP3 inflammasome activation by suppressing the TLR4/NF-κB signalling pathway. J Inflamm Res. 2021;14:2753–68.
38. Wallace JL, Nagy P, Feener TD, Allain T, Ditrói T, Vaughan DJ, et al. A proof-of-concept, Phase 2 clinical trial of the gastrointestinal safety of a hydrogen sulfide-releasing anti-inflammatory drug. Br J Pharmacol. 2020;177:769–77.
39. Van Dingenen J, Pieters L, Vral A, Lefebvre RA. The H(2)S-releasing naproxen derivative ATB-346 and the slow-release H(2)S donor GYY4137 reduce intestinal inflammation and restore transit in postoperative ileus. Front Pharmacol. 2019;10:116.
40. Glanville JRW, Jalali P, Flint JD, Patel AA, Maini AA, Wallace JL, et al. Potent anti-inflammatory effects of an H(2) S-releasing naproxen (ATB-346) in a human model of inflammation. FASEB J. 2021;35: e21913.
41. Campolo M, Esposito E, Ahmad A, Di Paola R, Paterniti I, Cordaro M, et al. Hydrogen sulfide-releasing cyclooxygenase inhibitor ATB-346 enhances motor function and reduces cortical lesion volume following traumatic brain injury in mice. J Neuroinflammation. 2014;11:196.
42. Osaghae BE, Arrowsmith S, Wray S. Gestational and hormonal effects on magnesium sulfate's ability to inhibit mouse uterine contractility. Reprod Sci. 2020;27:1570–9.
43. Hidalgo A, Cantabrana B, Pérez-Vallina JR. Contribution of cAMP to the inhibitory effect of non-steroidal anti-inflammatory drugs in rat uterine smooth muscle. J Auton Pharmacol. 1998;18:31–7.