Inhibition of Steroid Sulphatase Activity *via* the Percutaneous Route: A New Option for Breast Cancer Therapy

A. PUROHIT[1], S.K. CHANDER[1], L.W.L. WOO[2], M.F.C. PARSONS[1], R. JHALLI[1], B.V.L. POTTER[2] and M. J. REED[1]

[1]Endocrinology and Metabolic Medicine and Sterix Ltd, Imperial College London, St. Mary's Hospital, London, W2 1NY;
[2]Medicinal Chemistry, Department of Pharmacy and Pharmacology, and Sterix Ltd, University of Bath, Bath, BA2 7AY, U.K.

Abstract. *Steroid sulphatase (STS) inhibitors have been developed primarily for the treatment of hormone-dependent breast cancer, but may also have utility for the treatment of a number of androgen-dependent skin conditions. STS regulates the hydrolysis of steroid sulphates, such as oestrone sulphate (E1S) and dehydroepiandrosterone sulphate, (DHEAS). Liberated oestrone (E1) can be converted to biologically active oestradiol (E2) while dehydroepiandrosterone (DHEA) can undergo reduction to testosterone or aromatisation to E1. In this study the ability of the STS inhibitor STX64 (BN83495) and its N,N-dimethyl analogue (STX289) to inhibit liver and skin STS when applied orally or topically to nude mice was examined. Oral administration at 1 and 10 mg/kg resulted in almost complete inhibition of skin and liver STS. When applied topically to the dorsal neck region at 1.0 and 10 mg/kg not only skin but, unexpectedly, also liver STS was effectively inhibited. An investigation into the metabolism of these two compounds by HepG2 liver carcinoma cells, with high-performance liquid chromatography (HPLC) analysis, was also undertaken. In the presence of HepG2 cells a similar degree of desulphamoylation of STX64 (68%) or de-N, N-dimethylsulphamoylation of STX289 (66%) occurred over a 3h period. In the absence of cells, however, STX289 was resistant to de-N, N-dimethylsulphamoylation whereas STX64 was completely desulphamoylated, demonstrating the more favourable pharmaceutical profile of STX289 for development for topical application. It is concluded that both STX64 and STX289 are not only effective inhibitors of skin STS, but also liver STS when applied topically. These findings suggest that it may be possible to develop a formulation for the percutaneous administration of STS inhibitors, but also that this class of compound may have therapeutic potential for the treatment of a number of skin disorders.*

Correspondence to: Prof. M.J. Reed, Endocrinology and Metabolic Medicine, Imperial College London, St. Mary's Hospital, London, W2 1NY, U.K. Tel: +44 207 886 1738, Fax: +44 207 886 1790, e-mail: m.reed@imperial.ac.uk

Key Words: Steroid sulphatase, oestrogen, androgen, breast cancer, skin, acne, hirsutism, sulphatase inhibitor.

Oestrogens have a crucial role in supporting the development and growth of breast tumours. Most breast malignancies occur in postmenopausal women at a time when ovarian oestrogen production has ceased, but with low levels of oestrogens continuing to be synthesized in peripheral tissues (1). Such tissues include adipose tissue, but also normal and malignant breast tissues (2, 3). Studies have revealed that the enzymes required for the synthesis of biologically active oestradiol (E2) are present in breast tissues and include: aromatase, which converts androstenedione to oestrone (E1); steroid sulphatase (STS) which regulates the formation of E1 from oestrone sulphate (E1S) and 17β-hydroxysteroid dehydrogenase Type 1 (17β-HSD1), which reduces E1 to E2 to allow binding to the oestrogen receptor with high affinity (4). As an alternative to blocking the interaction of E2 with its receptor by the use of anti-oestrogens (*e.g.* tamoxifen) a number of drugs have been developed to block oestrogen synthesis. These include aromatase inhibitors (*e.g.* letrozole, anastrozole, exemestane), which are now in clinical use, and sulphatase inhibitors (*e.g.* STX64 also known as BN83495) that are at an earlier stage of development (5-7).

In addition to regulating the hydrolysis of E1S to E1, STS also controls the formation of dehydroepiandrosterone (DHEA) from DHEA sulphate (DHEAS) (7). DHEA can be reduced to androstenediol by 17β-HSD1 and is a steroid with potent oestrogenic properties (8). DHEA can also be converted to androstenedione which can undergo subsequent reduction to testosterone or aromatisation to E1. In the first ever phase I trial of a STS inhibitor, STX64, in postmenopausal women with advanced breast cancer plasma E1 and E2 concentrations were significantly reduced (9). In

addition, levels of androstenedione and testosterone were also reduced. This finding shows that in postmenopausal women the major substrates for the aromatase enzyme, *i.e.* androstenedione and testosterone, are derived from the peripheral conversion of DHEAS and not, as previously thought, by direct secretion from the adrenal cortex.

Although STS inhibitors were developed primarily for the treatment of breast cancer they could also have therapeutic applications for the treatment of other hormone-dependent conditions. Such conditions include endometriosis, polycystic ovarian disease, prostate cancer and a number of androgen-dependent skin conditions, such as acne and hirsutism (10-12). There is good evidence that STS in skin and the dermal hair papilla can regulate the hydrolysis of DHEAS to DHEA which can then subsequently be converted to the biologically active androgens, testosterone and 5α-dihydrotestosterone (13).

While steroid enzyme inhibitors have so far been administered by the oral route, the delivery of oestrogens *via* the transdermal route for hormone replacement therapy (HRT) is well established. Transdermal delivery of oestrogens reduces the production of oestrogen sensitive liver proteins. This can reduce the adverse events associated with HRT when given orally (14).

In this study the possibility of delivering the STS inhibitor STX 64 and a related analogue (STX 289) *via* the percutaneous route was explored using a nude mouse model. STX289 is inactive *in vitro* and has to undergo demethylation to be activated (15).

Materials and Methods

Compound synthesis. STX64 (Figure 1, **1**) was prepared by sulphamoylating the phenolic precursor 667 COUMARIN (Figure 1, **2**) which is the product of a Pechmann synthesis between resorcinol and ethyl-2-oxocycloheptanecarboxylate (16). STX289 (*N,N*-dimethyl 667 COUMATE, Figure 1, **3**) was prepared by heating a solution of 667 COUMARIN in *N, N*-dimethyl-cyclohexylamine with *N,N*-dimethylsulphamoyl chloride. Full details of the synthesis of STX289 will be reported elsewhere. The compounds exhibited spectroscopic and analytical data in accordance with their structure and were pure, as shown by high-performance liquid chromatography, (HPLC).

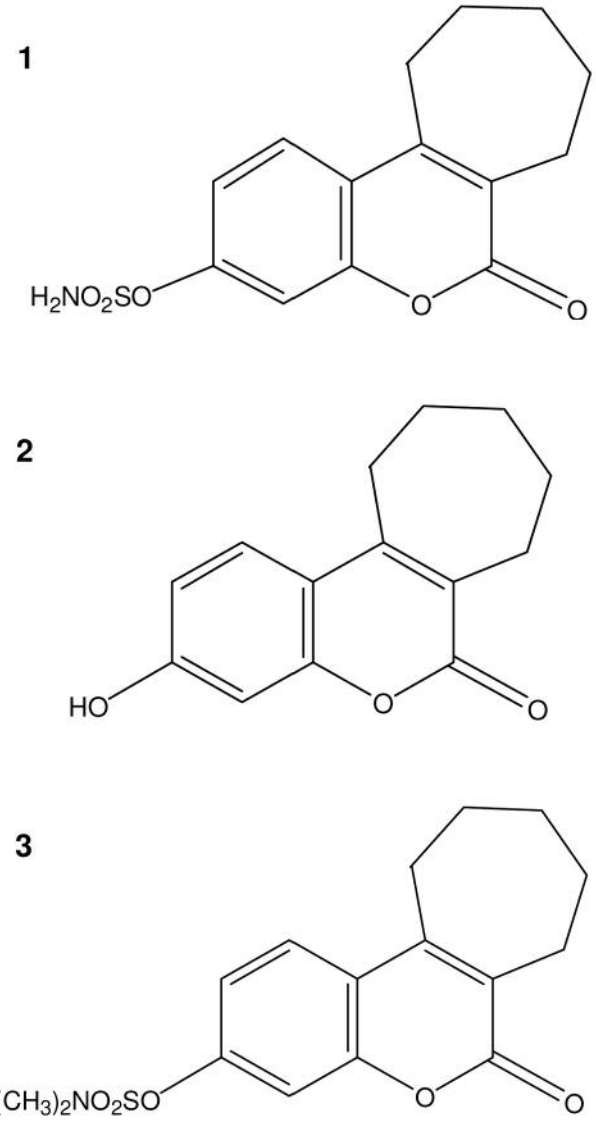

Figure 1. *Compound structures:* ***1****, STX64,* ***2****, 667 COUMARIN,* ***3****, STX289.*

Animal studies. Athymic, female MF-1 nude mice (*nu-/nu-*) were purchased from Harlan (Bicester, Oxon, UK) at 5 weeks of age (approximately 20-25 g in weight). All the experiments were carried out under conditions that complied with institutional requirements. The animals were kept in a 12 h light/dark cycle and given food and water *ad libitum*. Before the experiments the animals were transferred to individual cages to avoid the possibility of contamination by oral ingestion of topically applied compounds. The test compounds were either administered orally (in tetrahydrofuran (THF): propylene glycol, 1:10 v/v) or applied topically (in 10 μl THF) to the skin of the dorsal neck region (0.1, 1.0 and 10.0 mg/kg) with 3 mice per group of treated animals. Mice were killed, using an approved procedure, 24 h after application of the dose. Samples of skin and liver were collected from animals dosed orally. For the animals receiving topical applications of the compounds samples of liver were obtained together with samples of skin from the treated and untreated (ventral region) areas of skin to examine the level of inhibition achieved at a site remote from the point of application. The tissue samples were frozen on solid carbon dioxide and kept at –20˚C until assayed for STS activity.

Steroid sulphatase assay. The skin and liver tissue samples were homogenised in phosphate buffered saline (pH 7.4 containing 250 mM sucrose) (17). Duplicate aliquots of the homogenised tissues were incubated for 4 h with [^{3}H-E1S] (53 Ci/mmol, 2-3 nM, Perkin Elmer, Boston, MA, USA) adjusted to a final concentration of 20 μM with unlabelled substrate (Sigma, Poole, Dorset, UK). [4-^{14}C] oestrone (1×10^4

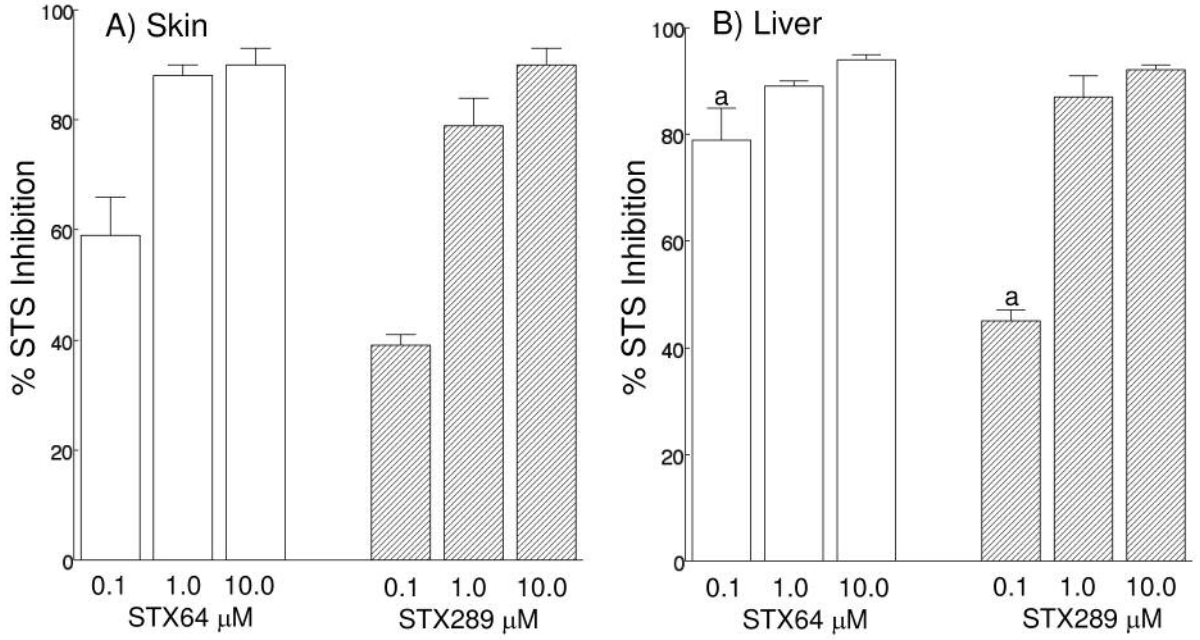

Figure 2. *Inhibition of (A) skin steroid sulphatase (STS) activity after oral administration of STX64 and STX289 (B) liver STS after oral administration of STX64 and STX289. Doses were administered to female nude mice in tetrahydrofuran: propylene glycol (1:10). Twenty-four hours after dosing samples of skin and liver were obtained and assayed for STS activity. Results are expressed as the mean±SEM percentage of inhibition compared to STS activity in mice receiving vehicle only. Inhibition of STS activity in liver after oral application of STX289 at 0.1 mg/kg was significantly lower than that achieved after dosing with STX64: a, p<0.01.*

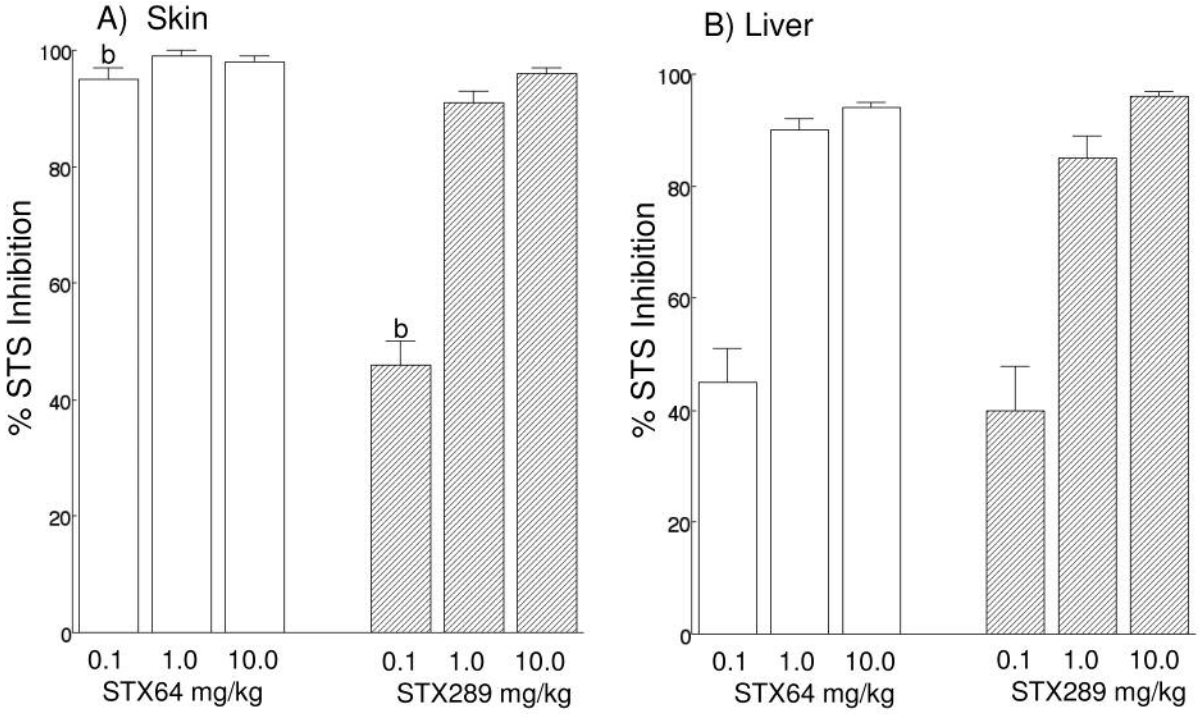

Figure 3. *Inhibition of (A) skin steroid sulphatase (STS) after topical application of STX64 and STX289 (B) liver STS after topical application of STX64 and STX289. Compounds were applied to the dorsal neck region of mice in 10 μl tetrahydrofuran. Twenty-four hours after application of the dose the treated skin area and samples of liver were obtained and assayed for STS activity. Results are expressed as the mean±SEM percentage inhibition compared to STS activity in mice receiving vehicle only. Inhibition of STS activity in skin after topical application of STX289 at 0.1 mg/kg was significantly lower than that achieved after dosing with STX64: b, p<0.001.*

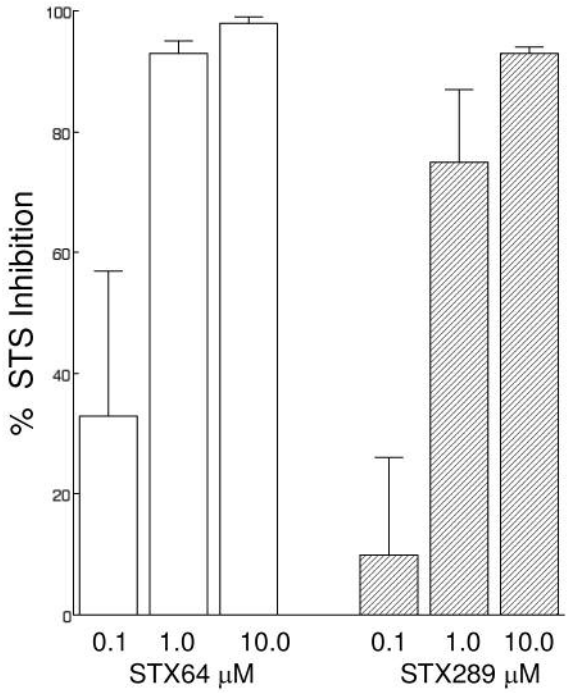

Figure 4. *Inhibition of steroid sulphatase (STS) activity in skin remote from the site of application. STX64 and STX289 were applied to the dorsal neck regions of mice in 10 μl tetrahydrofuran. Twenty four h later skin was obtained from the ventral region of the animal's body, remote from the site of application Results were expressed as the mean±SEM percentage of inhibition compared to STS activity in mice receiving vehicle only.*

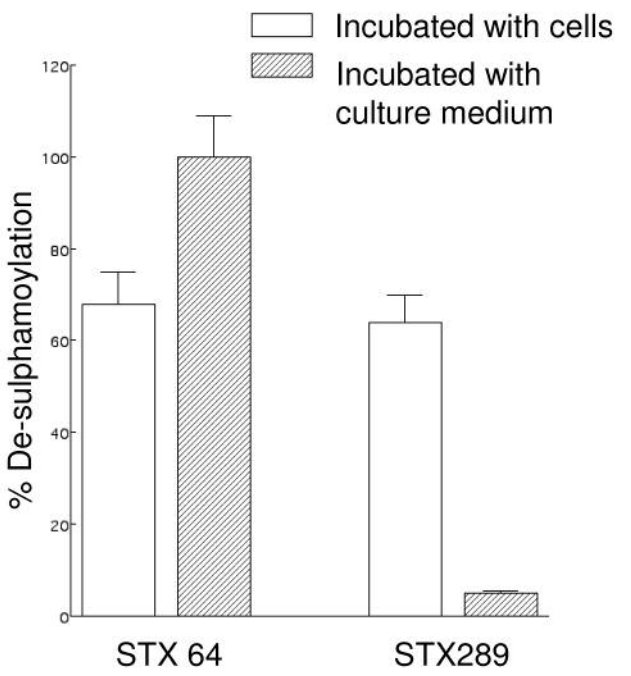

Figure 5. *Metabolism of STX64 and STX289 in the presence or absence of HepG2 cells. STX64 (10 μM) or STX289 (10 μM) were incubated with HepG2 cells for 3 h or in culture medium only. At the end of the incubation period metabolites were extracted with diethyl ether and subjected to analysis and quantification by HPLC analysis. 667 COUMARIN, i.e. desulphamoylated STX64 or de-N, N-dimethyl-sulphamoylated STX289, was the major metabolite detected. Cells were cultured in medium pH 7.4 and both compounds appeared stable during the HPLC analytical procedure used (see methods section). Results show the mean±SEM for the percentage of desulphamoylation of triplicate measurements.*

disintegrations per minute, Perkin Elmer) was included in the reaction mixture to monitor procedural losses. At the end of the incubation period product oestrone was isolated from the reaction mixture by toluene partition. An aliquot of the toluene was removed and the ^{3}H and ^{14}C radioactivity measured by liquid scintillation spectrometry. The mass of E1S hydrolysed was calculated from the ^{3}H counts detected, corrected for procedural losses, as fmol product formed/4h/mg protein. The protein concentrations were measured using the Bradford procedure. By comparing the level of STS activity in samples from treated animals with those from controls, the percentage of STS inhibition was calculated. Results are expressed as means±SEM.

In vitro metabolism of STX64 and STX289 by HepG2 cells. Experiments were also carried out to investigate the *in vitro* metabolism of STX64 and STX289 using HepG2 liver carcinoma cells. The cells were obtained from ATCC (LGC Promochem, Teddington, Middlesex, UK) and maintained in minimum essential medium Eagle (MEME) with Earle's salts, 10% foetal bovine serum and supplements. STX64 (10 μM) and STX289 (10 μM) were incubated in serum-free medium with the HepG2 cells for 3 h in triplicate after which the culture medium was removed and extracted with diethyl ether. Sample analysis was performed using a reverse-phase HPLC method. The residues were reconstituted in the mobile phase eluent just prior to injection (100 μL of each sample) onto an Agilent 1100 ChemStation HPLC system (Wokingham, Berks, UK). The mobile phase consisted of 60% methanol in 0.02 M ammonium sulphate, pH 6.4. STX64 and STX289 were separated from endogenous cellular components using a Gemini 5 μ C18 column (250×3.0 mm; Phenomenex, Macclesfield, Cheshire, UK) and a flow rate of 0.34 mL/minute with each elution profile taking 15 min to develop. The column temperature was set at 25˚C and the samples were analysed using a photo-diode array detector set to 246 nm. The areas under peaks detected by the HPLC analysis were quantified to determine the percentage of compounds metabolised. Parallel experiments were carried out in which the compounds were incubated for 3 h in the absence of cells. The results are expressed as means±SEM.

Statisical analysis. The Student's *t*-test was used to assess the significance of differences in STS inhibition resulting from oral or topical application of STX64 or STX289.

Results

Oral application. An initial study was carried out to examine the ability of STX64 and STX289 to inhibit mouse liver and skin STS activity after oral application. As shown in Figure

2A and B, almost complete inhibition of liver STS activity was achieved 24 h after administration at the 1 and 10 mg/kg doses with both STX64 and STX289. At 0.1 mg/kg inhibition of liver STS by STX289 (45±4%) was lower ($p<0.01$) than that resulting from the oral application of STX64 at this dose (79±10%). The STS activity in the skin samples obtained after oral application was almost completely inhibited by both compounds at the 1 and 10 mg/kg doses with a lower level of STS inhibition (39-59%) at the 0.1 mg/kg doses tested.

Topical application. Topical application of both STX64 and STX289 at 1 and 10 mg/kg gave good inhibition of skin STS (99±1% and 98±1% respectively, Figure 3A and B). At 0.1 mg/kg skin STS activity was almost completely inhibited by STX64 (95±3%), whereas a lower level of inhibition was detected in the liver (45±10%). For STX289 at 0.1 mg/kg inhibition of skin and liver STS was <50% and significantly lower ($p<0.001$) than that achieved after topical application of STX64 at this dose. As shown in Figure 4 STS activity in the untreated skin regions was effectively inhibited at the 1 and 10 mg/kg doses by both compounds but with a much lower, but similar, level of inhibition (10-33%) being achieved with the 0.1 mg/kg doses.

HepG2 metabolism. In order to obtain information about the relative rates of metabolism of STX64 and STX289 the compounds were incubated in the presence or absence of HepG2 cells for 3 h after which the major metabolites were isolated and identified by HPLC analysis. To examine the stability of STX64 and STX289 under these experimental conditions, replicate samples were prepared in mobile phase and subjected to HPLC analysis. The coefficients of variation for these replicate analyses were <1% indicating that both compounds were stable during the HPLC analytical procedure. The main product detected was the desulphamoylated derivative of STX64 and the de-*N, N*-dimethylsulphamoylated derivative of STX289 *i.e.* 667 COUMARIN in both cases. When cultured in the presence of the cells compounds underwent desulphamoylation (STX64, 68%) or de-*N, N*-dimethylsulphamoylation (STX289, 64%) to the same extent over the time period investigated (Figure 5). However, when incubated with culture medium (MEME, pH 7.4) in the absence of cells STX64 was completely desulphamoylated whereas only little de-*N, N*-dimethylsulphamoylation (5%) of STX 289 occurred over the 3 h period.

Discussion

The present study showed that STX64 is a very potent oral inhibitor of mouse liver STS activity, confirming previous results obtained in rats (18, 19). The novel *N, N*-dimethyl derivative, STX289, also proved to be a potent inhibitor of liver STS activity when administered orally at the 1.0 and 10 mg/kg dose. At the higher doses tested both compounds also inhibited skin STS after oral application. When applied topically at 1 and 10 mg/kg both compounds effectively inhibited skin STS but, unexpectedly, also inhibited liver STS activity. This finding shows that STX64 and STX289 are able to be absorbed *via* the percutaneous route and can then inhibit STS in the liver and possibly in other tissues throughout the body. This concept is supported by the finding that STS activity in skin from the untreated, ventral, region in mice was also inhibited. This implies that STX289 is not demethylated directly in skin, but after transdermal absorption and subsequent demethylation, most likely in the liver, it then acts on the skin enzyme as STX64.

Previous studies have indicated that at neutral pH, desulphamoylation of STX64 can occur (20). The *N, N*-dimethyl derivative was therefore synthesized and evaluated, as it was thought that this compound would, by blocking the classical elimination mechanism of sulphamate esters, be more robust and act as a pro-drug for the formation of STX64 by demethylation *in vivo* and might, as a more hydrophobic entity, also be suitable for topical application. When incubated with the HepG2 cells, however, HPLC analysis revealed that the compounds underwent desulphamoylation or de-*N, N*-dimethylsulphamoylation to the same extent over the time period studied. In contrast, in the absence of cells at pH 7.4, while STX64 was completely desulphamoylated over a 3 h period, STX289 was resistant to inactivation *via* this route. This finding suggests that STX64 is able to bind to some cellular component that reduces its rate of desulphamoylation. It is known that STX64 binds to carbonic anhydrase II and it is possible that binding to other proteins may also help to protect it from degradation (15, 21). STX289 can only be de-*N, N*-dimethylsulphamoylated in the absence of cells *via* a direct nucleophilic attack at sulphur by water or hydroxide ions. At pH 7.4 neither of these is likely at any appreciable rate, nor is chemical demethylation by any route. Thus, it seems likely that in the HepG2 cells STX289 is enzymatically demethylated to a considerable extent to STX64 that subsequently inhibits the sulphatase.

In skin, STS concentrates in the dermal papilla of the hair follicle and previous studies have demonstrated that the STS inhibitor oestrone-3-*O*-sulphamate, blocked this activity (11). Billich and colleagues evaluated the ability of another sulphamate-based STS inhibitor, 6-[2-(Adamantylidene)-hydroxybenzoxazole]-*O*-sulphamate (AHBS) to block skin activity (12). After oral application at 5 mg/kg >95% inhibition of skin STS was observed 8 h post dosing. Topical application of this inhibitor to the skin of mice resulted in >99% inhibition of STS skin activity. AHBS was also applied topically to the backs of domestic pigs, which are considered to be an appropriate model for human skin with

respect to drug penetration, with complete blockage of STS activity being achieved 6 h post dosing.

There is, therefore, convincing evidence that sulphamate-based STS inhibitors, when applied topically, can inhibit not only skin STS but also liver STS activity. These findings suggest that, given the potency of STX64 and its related analogue STX289, they could be considered for development for a percutaneous method of delivery for patients with hormone-dependent malignancies. Additonally, in view of the important role that STS has in the skin, topical application of a STS inhibitor could have considerable therapeutic potential for a number of androgen-dependent skin conditions. Increased STS activity has been detected in acne lesions compared with unaffected skin with infiltrating monocytes contributing to the STS activity found in such lesions (22). Monocytes have also been postulated to be involved in the pathogenesis of psoriasis, a chronic proliferative epidermal disease that affects 1-3% of the world's population (23). This condition may result from an inappropriate Th1-type immune response and subsequent cytokine production. As STS has been postulated to regulate the Th1/Th2 cytokine response, by modulating DHEA concentrations, inhibition of STS could also have therapeutic potential for the treatment of psoriasis (24-26).

In summary, results from this investigation have confirmed that STX64 and STX289 are potent inhibitors of skin and liver STS when administered by oral or topical application. As an alternative to oral administration of STS inhibitors it is possible that by using an appropriate formulation these inhibitors could be developed for percutaneous administration for cancer therapy. This route of delivery could be particularly important if STS inhibitors are used in the preventative setting when it may be desirable to avoid any hepatic effects associated with oral administration. However, a more important use of STS inhibitors when applied topically could be for androgen-dependent skin disorders or psoriasis. As topical application of STX64 was also shown to inhibit liver STS its actions could be two fold: firstly direct inhibition of skin STS blocking the hydrolysis of DHEAS delivered to skin from the blood and secondly a systemic effect on the liver, and other tissues, to reduce the levels of weak androgens that are available for uptake by the skin and conversion to more active androgens within the skin. Given the potency of these STS inhibitors it will be important to carry out further clinical trials to assess the feasibility of using the percutaneous route for cancer therapy and their efficacy in treating a range of skin disorders.

Acknowledgements

This research was supported by Sterix, a member of the Ipsen Group.

References

1 Reed MJ, Hutton JD, Baxendale PM, James VHT, Jacobs HS and Fisher RP: The conversion of androstenedione to oestrone and production of oestrone in women with endometrial cancer. J Steroid Biochem *11*: 905-911, 1979.

2 Longcope C, Pratt JH, Schneider SH and Fineberg SE: *In vivo* studies on the metabolism of estrogens by muscle and adipose tissue in normal males. J Clin Endocrinol Metab *45*: 1134-1145, 1977.

3 James VHT, McNeill JM, Lai LC, Newton CJ, Ghilchik MW and Reed MJ: Aromatase activity in normal breast and breast tumor tissue: *in vivo* and *in vitro* studies. Steroids *50*: 269-279, 1987.

4. Peltoketo H, Isoma V, Maentausta O and Vikko R: Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. FEBs Lett *239*: 73-77, 1988.

5 Smith IE and Dowsett M: Aromatase inhibitors in breast cancer. N Engl J Med *348*: 2431-2442, 2003.

6 Coombes RC, Hall E, Gibson LJ *et al*: A randomised trial of exemestane after two to three years of tamoxifen in postmenopausal women with primary breast cancer. N Engl J Med *350*: 1081-1082, 2004.

7 Reed MJ, Purohit A, Woo LWL, Newman SP and Potter BVL: Steroid sulphatase: molecular biology, regulation and inhibition. Endocr Rev *26*: 171-202, 2005.

8 Poulin R and Labrie F: Stimulation of cell proliferation and estrogenic response by adrenal C19-delta 5 steroids in the ZR-75-1 human breast cancer cell line. Cancer Res *46*: 4933-4937, 1986.

9 Stanway SJ, Purohit A, Woo LWL *et al*: Phase I study of STX64 (667 COUMATE) in breast cancer patients: the first study of a steroid sulfatase inhibitor. Clin Cancer Res *12*: 1585-1590, 2006.

10 Purohit A, Fusi L, Brosens J, Woo LWL, Potter BVL and Reed MJ: Inhibition of steroid sulphatase activity in endometriotic implants by 667 COUMATE: a potential new therapy. Human Reprod *23*: 290-297, 2008

11 Hoffmann R, Rot A, Niiyama N and Billich A: Steroid sulfatase in the human hair follicle concentrates in the dermal papilla. J Invest Dermatol *117*: 1342-1348, 2001.

12 Billich A, Meingassner JG, Nussbaumer P, Desrayand S, Lam C, Winishi A and Schreiner E: 6-[2-(Adamantylidene)-hydroxy-benzoxazole]-*O*-sulfamate, a steroid sulfatase inhibitor for the treatment of androgen and estrogen-dependent diseases. J Steroid Biochem Mol Biol *92*: 29-37, 2004.

13 Chen WC, Thiboutot D and Zouboulis CC: Cutaneous androgen metabolism: research and clinical perspectives. J Invest Dermatol 119: 992-1007, 2002.

14 Battaglioli T and Martinelli I: Hormone therapy and thromboembolic disease. Curr Opin Hematol *14*: 488-493. 2007.

15 Ho YT, Purohit A, Vicker N, Newman SP, Robinson JJ, Leese MP, Ganeshapillai D, Woo LWL, Potter BVL and Reed MJ: Inhibition of CAII activity by steroidal and non-steroidal sulphamates. Biochem Biophys Res Commun *305*: 909-914, 2003.

16 Woo LWL, Purohit A, Malini B, Reed MJ and Potter BVL: Potent active site-directed inhibition of steroid sulfatase by tricyclic coumarin-based sulphamates. Chem Biol *7*: 773-791, 2000.

17 Duncan L, Purohit A, Howarth NM, Potter BVL and Reed MJ: Inhibition of estrone sulfatase activity by estrone-3-methylthiophosphonate – a potential therapeutic agent in breast cancer. Cancer Res *53*: 298-303, 1993.
18 Purohit A, Hejaz HAM, Woo LWL, van Strien AE, Potter BVL and Reed MJ: Recent advances in the development of steroid sulphatase inhibitors. J Steroid Biochem Mol Biol *69*: 227-238. 1999.
19 Malini B, Purohit A, Ganeshapillai D, Woo LWL, Potter BVL and Reed MJ: Inhibition of steroid sulphatase activity by tricyclic coumarin sulphamates. J Steroid Biochem Mol Biol *75*: 253-258, 2000.
20 Ireson CR, Parish D, Woo LWL, Potter BVL, Chander SK and Reed MJ: Development of a sensitive high-performance liquid chromatography method for the detection of 667 COUMATE *in vivo*. J Steroid Biochem Mol Biol *84*: 337-342, 2003.
21 Vicker N, Ho YT, Robinson J, Woo LWL, Purohit A, Reed MJ and Potter BVL: Docking studies of sulphamate inhibitors of estrone sulphatase in human carbonic anhydrase II. Bioorg Med Chem Lett *13*: 863-865, 2003.
22 Billich A, Rot A, Lam C, Schmidt JB and Schuster I: Immunohistochemical localization of steroid sulfatase in acne lesions: implications for the contribution of dehydroepiandrosterone sulfate to the pathogenesis of acne. Horm Res *532*: 99 (Abstract 22), 2000.
23 Namazi MR: Steroid sulfatase inhibitors as novel additions to the antipsoriatic armamentarium. Med Sci Monit *11*: HY7-9, 2005.
24 Daynes RA, Araneo BA, Dowell TA, Huang K and Dudley D: Regulation of murine lymphokine production *in vivo*. J Exp Med *171*: 979-996, 1990.
25 Rook GAW, Hernandez-Pando R and Lightman S: Hormones, peripherally activated hormones and regulation of Th1/Th2 balance. Immunology Today *15*: 301-303, 1994.
26 Reed MJ: The discriminant function test: a marker of Th1/Th2 cytokine secretion and breast tumour oestrogen synthesis. Mol Med Today *1*: 98-103, 1995.

Received January 10, 2008
Revised February 27, 2008
Accepted March 10, 2008