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The imidazoline-like drug S23515 affects lipid metabolism in hepatocyte by inhibiting the oxidosqualene: lanosterol cyclase activity

Nicolas Venteclef, Raphaelle Guillard, Marc Issandou *

GlaxoSmithKline, 25 Avenue du Québec, Les Ulis, Cedex 91951, France

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Abstract

Imidazoline-like drugs are centrally-acting antihypertensive agents that inhibit the activity of the sympathetic nervous system by interacting with the α2-adrenoreceptor and also with a non-adrenergic imidazoline binding site called the imidazoline 1 receptor. Recently, these molecules were proposed to play an additional role in cardiovascular diseases by acting on glucose and lipid metabolism. We used S23515, a potent imidazoline-like molecule acting selectively on blood pressure through the imidazoline 1 receptor, to decipher the effects of these drugs on lipid metabolism. We found that S23515 inhibited specifically and dose-dependently cholesterol synthesis in cultured rodent and primate hepatocytes. This hypocholesterolemic effect was likely due to the inhibition of the oxido:lanosterol cyclase (OSC), a rate-limiting enzyme in the cholesterol biosynthetic pathway. Partial OSC inhibition induced by S23515 led to the generation of 24(*S*),25-epoxycholesterol, a potent ligand for the liver X receptor (LXR). Furthermore, S23515 increased in human macrophages the expression of both ABCA1 and G1, the 2 ATP binding cassette transporters, which play a pivotal role in the reverse cholesterol transport. Thus, these results suggest that S23515, and potentially other imidazoline-like drugs, could exert hypolipidemic effects in addition to their hypotensive activities.

Keywords: Imidazoline; Oxydosquale:lanoterol cyclase; Lipid neo-synthesis

1. Introduction

Imidazoline-like drugs such as clonidine, benazoline or moxonidine are centrally-acting antihypertensive agents that inhibit the activity of the sympathetic nervous system. These molecules exert their hypotensive effects by interacting not only with the α2-adrenoreceptor (α2-AR) but also with a non-adrenergic imidazoline binding site (IBS) also called imidazoline 1 receptor (I1R) [1]. This IBS is now considered to be a receptor due to the fact that it fulfils the criteria for identification as receptor, including specificity in binding assay, association with physiological function and identification of endogenous ligands [2]. A cDNA corresponding to an imidazoline receptor was recently identified [3] but this result raised some controversy about the literature [4]. Identification of the transduction pathway associated with IBS has been partially demonstrated using PC12 cells, showing some coupling of IBS to the cAMP pathway [5]. In recent years, several groups have worked to identify new molecules that display higher affinity and selectivity for I1R imidazoline receptor [6] leading to the identification of S23515, a non-adrenergic imidazoline-like drug with high affinity for IBS (K_i = 6 nM) and negligible affinity and activity toward α2-AR (K_i > 10,000 nM) [7]. Recently, LNP 906, the first high-affinity photo-affinity ligand selective for I1R imidazoline receptor was described [8] and considered to be very useful for I1R purification and subsequent cloning.

Hypertension is one of the metabolic diseases described in the metabolic syndrome that frequently precedes the development of type II diabetes and atherosclerosis, in association with hyperlipidemia, insulin resistance and glucose intolerance [9]. Using the spontaneously

Abbreviations: IBS, imidazoline binding site; MOS, 2,3-monoepoxysqualene; DOS, 2,3 ;22,23-diepoxysqualene; OSC, oxidosqualene:lanosterol cyclase; α2-AR, α2-adrenoreceptor; I1R, imidazoline 1 receptor; LXR, liver X receptor; ABC-A1/G1, ATP-binding cassette transporter A1 and G1; HMGCoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A; LDL-r, low density lipoprotein receptor; SREBP, sterol regulatory element binding protein; FCS, fetal calf serum

* Corresponding author. Tel.: +33 1 69 29 60 69; fax: +33 1 69 07 48 92.
E-mail address: MI18785@GSK.com (M. Issandou).

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hypertensive, hyperlipidemic obese rat model, it was demonstrated that the imidazoline drug moxonidine, a non-selective I1R/α2-AR agonist, could affect glucose and lipid metabolism by stimulation of I1R imidazoline receptor [10–11], thus suggesting that the centrally-acting imidazoline-like drugs could also exert beneficial metabolic effects.

Cholesterol and triglyceride homeostasis in the hepatocyte are regulated by a complex set of mechanisms that include lipid biosynthesis, storage, catabolism, and lipoprotein assembly and secretion [12–13]. In addition to the statin class of lipid-lowering drugs, which affect cholesterol neo-synthesis [14], various pharmacologically acting molecules are under intensive exploratory development in order to find new therapies for dyslipidemia [15]. The fact that a single molecule such as an imidazoline-like drug could have the potential to exert a pharmacological effect on hypertension, hyperlipidemia and glucose homeostasis may represent an alternative for future therapy in cardiovascular area.

In this study, we used the selective potent imidazoline-like drug S23515 to dissect the putative effects of this class of molecule on lipid metabolism. Using rat, cynomolgus and human primary hepatocytes as well as a human hepatoma cell line (HepG2), we found that S23515 inhibits cholesterol synthesis. This effect is mediated by a specific inhibition of the oxidosqualene:lanosterol cyclase (OSC), a rate-limiting enzyme in the cholesterol synthesis pathway. OSC inhibition translates into a reduction of cholesterol synthesis and secretion but also into the induction of the expression of ATP binding cassette protein (ABC) A1 and G1, two proteins involved in reverse cholesterol transport.

2. Materials and methods

2.1. Materials

S23515, BIBB515, GW965 and atorvastatin were synthesized at GlaxoSmithKline. 24(*S*),25-Epoxycholesterol was purchased from Steraloids Inc. [^{14}C]-oleate and [^{14}C]-mevalonolactone were purchased from Amersham while [^{14}C]-acetate was purchased from Perkin Elmer. Basal Medium Eagle (BME) medium, RPMI 1640 medium, penicillin, streptomycin and fetal calf serum (FCS) were obtained from Gibco. All other reagents were from Sigma.

2.2. Cell line

HepG2 and THP-1 cells were obtained from ATCC. HepG2 cells were maintained in a humidified incubator (5% CO_2) at 37 °C in BME medium containing 10% FCS, 100 U/ml penicillin, 100 U/ml streptomycin, 1 mM sodium pyruvate and 0.1 mM of non-essential amino acids. THP-1 were maintained in suspension in RPMI 1640 medium containing 2 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, 0.05 mM of β-mercaptoethanol and 10% FCS. Cells were differentiated into macrophages by incubation for 48 h with 160 nM phorbol-12-myristate-13-acetate.

Primary hepatocytes from rat, cynomolgus and humans were purchased from Biopredic and cultured following the supplier's instructions.

2.3. Lipid synthesis

HepG2 cells plated in 24-well plates were incubated in the presence of vehicle or compounds at the indicated doses for 2 h in RPMI 1640 medium supplemented with 1% lipoprotein-deficient serum. Primary hepatocytes plated in 24-well plates were incubated in the presence of vehicle or compounds at the indicated doses for 2 h in Williams E medium supplemented with 4 μg/ml insulin, 100 nM dexamethasone and 1% lipoprotein-deficient serum. Differentiated THP-1 cells in 6-well plates were incubated in the presence of vehicle or compounds for 24 h in RPMI 1640 medium supplemented with 1% lipoprotein-deficient serum. Both HepG2 and primary hepatocytes were labelled during the 2 h with 18.5 kBq (0.5 μCi) [^{14}C]-oleate or with 18.5 kBq (0.5 μCi) [^{14}C]-mevalonolactone or with 74 kBq (2 μCi) [^{14}C]-acetate. THP-1 cells were incubated for 24 h with 74 kBq (2 μCi) [^{14}C]-acetate. At the end of the incubation period, secreted and/or intracellular lipids were extracted using isopropanol and separated by thin-layer chromatography as previously described [16]. Lipids were identified by using purified standards and the radioactivity associated with each individual lipids was quantified using a Phosphorscreen (Storm, Molecular Dynamics).

2.4. Real-time PCR quantification of RNA

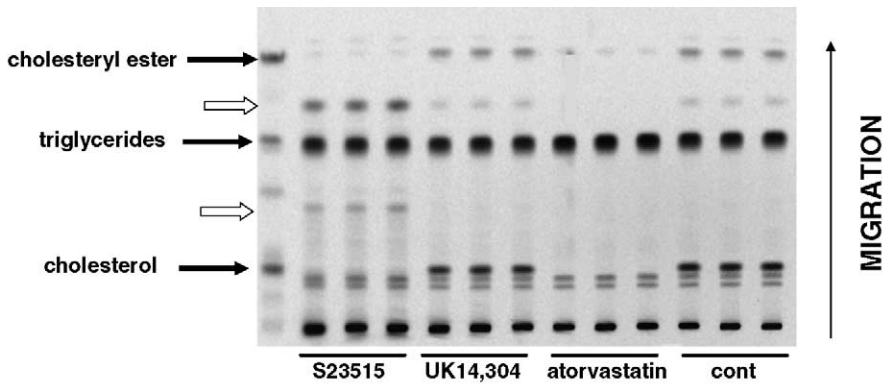
Differentiated THP-1 cells in 6-well plates were incubated in the presence of vehicle or compounds for 24 h in RPMI 1640 medium supplemented with 1% lipoprotein-deficient serum. Total RNA were prepared using the RNeasy Mini Kit (Qiagen). A 1 μg aliquot was used as a template for cDNA synthesis employing the Taqman Reverse Transcription Reagents kit (Applied Biosystems). Real-time PCR was performed in 384-well plates by using the ABI-PRISM 7900 sequence detection system (Applied Biosystems). The reactions contained, in a final volume of 20 μl, 4 μl of diluted (1:10) cDNA, a 300 nM concentration of the forward and reverse primers and 2× SYBR Green PCR Master Mix (Applied Biosystems). Primers were, respectively, GTTGCTGGCAGAGGAAATGAGAAG and CAAAGGAAGACGAGGAGCACGAT for LDLr, TGTCCAGTCCAGTAATGGTTCTGTGT and GCGAGATATGGTCCGGATTG for ABCA1, CATCTGCACTGCCAAGACTGA and CCACAATATTCATGCCTTCTTTCA for cyclophylin and CGACCGACGACACAGAGACT and GAGCACGAGACACCCACAAAC for ABCG1. Levels of

ABCA1, ABCG1 and LDLr were normalized to cyclophylin to compensate for variations in input RNA amounts (cyclophylin levels were unaffected by the treatments). Relative abundance of mRNAs were calculated from the cycle threshold (C_t) using the formula $2^{-\Delta C_t}$ and expressed as arbitrary units.

3. Results

3.1. Effects of S23515 on lipid metabolism in primary rat hepatocytes

We used primary rat hepatocytes to explore the potential of the imidazoline-like drug S23515 to modify lipid metabolism. UK14,304 was used as a specific α2C adrenoreceptor agonist [17] and the well-known HMGCoA reductase inhibitor, atorvastatin, was added as a positive control to specifically inhibit both cholesterol and cholesteryl ester synthesis in hepatocytes [18]. Cells were incubated for 2 h with the different drugs and labelled with [^{14}C]-mevalonolactone to specifically label both cholesterol and cholesteryl ester, [^{14}C]-oleate to label both cholesteryl ester and triglycerides and finally [^{14}C]-acetate to label the full panel of lipids. As shown in Fig. 1, incubation of the cells with 1 μM atorvastatin in the presence of [^{14}C]-acetate strongly inhibits cholesterol but not triglyceride synthesis. A similar profile is observed with 25 μM S23515, which induces a strong decrease in cholesterol synthesis (87%) compared to untreated cells, without affecting triglyceride synthesis. This effect is not mediated by activation of the α2-AR since 25 μM UK14,304 is inactive on both parameters. The inhibition of cholesterol synthesis induced by S23515 is also observed upon [^{14}C]-mevalonate labelling of the cells (84% compared to untreated cells) while, as expected, atorvastatin is rendered totally inactive using this labelling. This result demonstrates that S23515 affects cholesterol synthesis at a stage beyond mevalonate in the cholesterol biosynthetic pathway. The absence of effect of S23115 on triglyceride synthesis was confirmed using [^{14}C]-oleate labelling. Parallel to the inhibition of cholesterol, we also obtained with S23515 and atorvastatin a similar inhibition of cholesteryl ester synthesis (Fig. 1). Thus, these results indicate that S23515 is able to inhibit specifically cholesterol and cholesteryl ester synthesis in rat hepatocyte, to a similar degree as the statins do, but on a different step in the cholesterol biosynthetic pathway.



	[^{14}C]-acetate		[^{14}C]-mevalonate	[^{14}C]-oleate
	cholesterol	TG	cholesterol	TG
Control	100 +/- 2%	100 +/- 5%	100 +/- 3%	100 +/- 2%
S23515 25 μM	13 +/- 2%	95 +/- 6%	16 +/- 1%	97 +/- 10%
Atorvastatin 1 μM	4 +/- 0.5%	129 +/- 8%	92 +/- 5%	109 +/- 6%
UK14,304 25 μM	86 +/- 6%	92 +/- 4%	81 +/- 5%	111 +/- 6%

Fig. 1. Inhibition of cholesterol and triglycerides synthesis induced by S23515, atorvastatin and UK14,304 in primary rat hepatocytes. Cells were incubated without (control) or with the indicated drugs for 2 h in presence of the different radioisotopes. The top panel shows the autoradiograph of synthetized intracellular lipids separated by thin layer chromatography after labelling of cells with [^{14}C]-acetate. The positions of the different lipids have been determined using [^{14}C] standards. The bottom panel shows the quantification using a Phosphorimager of the intensity of the bands corresponding to cholesterol and triglycerides (TG) synthesis following labelling of the cells with, respectively [^{14}C]-acetate, [^{14}C]-mevalonate and [^{14}C]-oleate. Values are expressed as percentage of control and are the mean ± S.D. of triplicates. The relative amounts of radioactivity measured in control were, respectively, 11,660 ± 232 a.u. for cholesterol and 11,325 ± 272 a.u. for triglycerides using [^{14}C]-acetate labelling, 223,800 ± 6581 a.u. using [^{14}C]-mevalonate labelling and 159,030 ± 2748 a.u. using [^{14}C]-oleate labelling.

3.2. Effects of S23515 on the oxidosqualene:lanosterol cyclase

Using the protocol described in Fig. 1, we observed on the TLC plate, in addition to the well-known bands corresponding to cholesterol, triglyceride and cholesteryl ester, two additional bands that were induced by S23515 but not by atorvastatin (Fig. 1, white arrow). We postulated that one of the potential enzymes in the cholesterol pathway that could be inhibited by S23515 was the oxidosqualene:lanosterol cyclase (OSC), a rate-limiting enzyme that catalyzes the conversion of 2,3-monoepoxysqualene (MOS) to lanosterol. MOS accumulation that results from OSC inhibition can be visualized by thin layer chromatography using the appropriate standard [16].

Primary rat hepatocytes were incubated for 2 h with increasing doses of S23515 or with 1 μM of BIBB 515 a potent and selective inhibitor of OSC [19–20]. As shown in Fig. 2A, BIBB 515 induced a concomitant decrease in cholesterol and cholesteryl ester synthesis and an increase

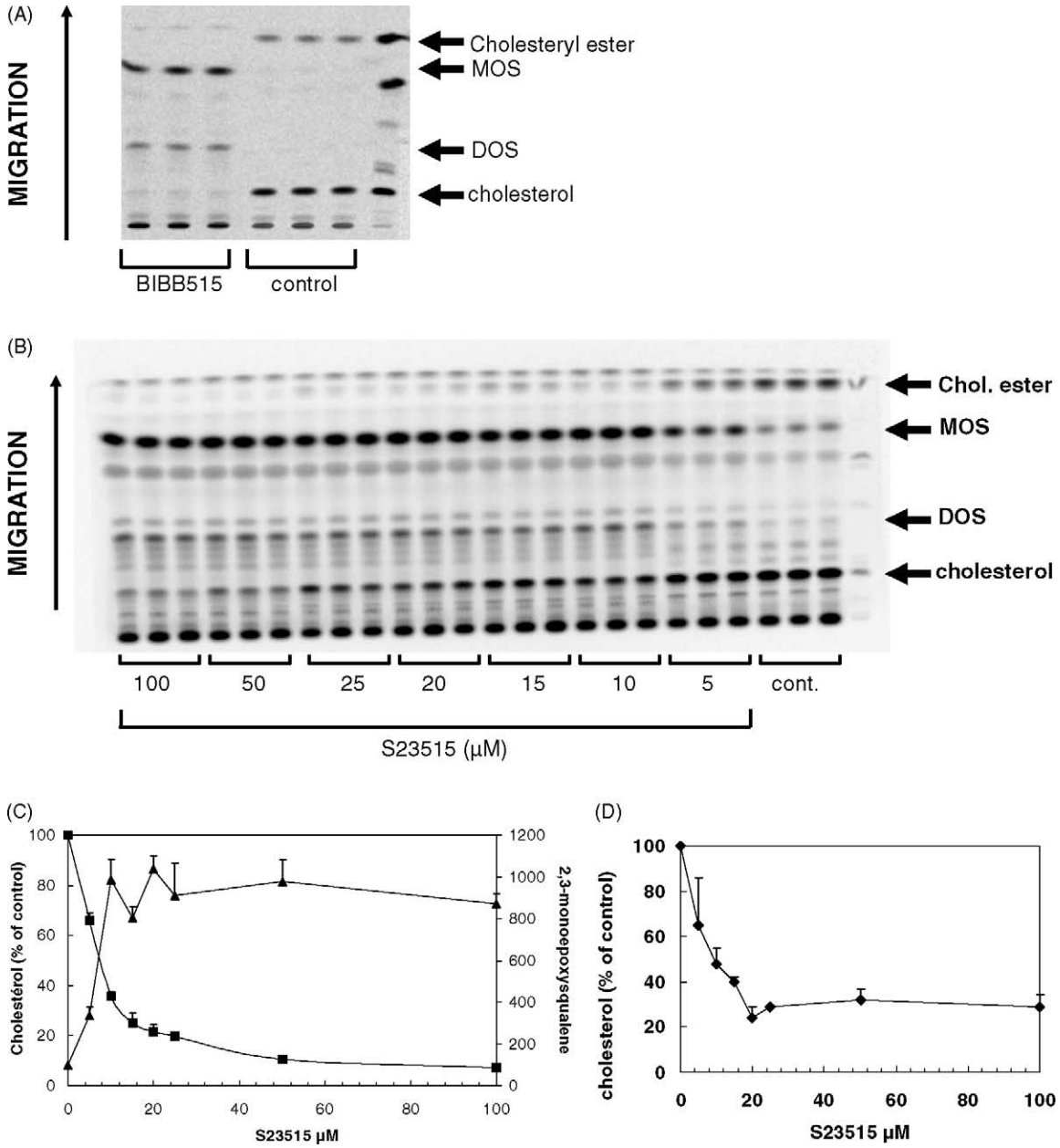


Fig. 2. S23515 decreases cholesterol synthesis by inhibiting the oxidosqualene:lanosterol cyclase in primary rat hepatocytes. Cells were incubated for 2 h without (control) or with BIBB 515 at 1 μM or S23515 at the indicated doses in presence of [^{14}C]-mevalonate. Secreted and intracellular lipids were extracted and analyzed as described in Section 2. (A) and (B) represent autoradiographs of synthetized intracellular lipids separated by thin layer chromatography. The positions of the different lipids have been determined using radioactive (cholesterol, cholesteryl ester) and non-radioactive standards (MOS, DOS). The intensity of the bands corresponding to the intracellular cholesterol (C, black square), the intracellular MOS (C, black triangle) or the secreted cholesterol (D, black diamond) was quantified. Values are expressed as percentage of control and are the mean ± S.D. of triplicates.

in MOS synthesis. S23515 induced a similar profile to BIBB 515 with a dose-dependent decrease in both cholesterol and cholesteryl ester synthesis which correlated with an increase in MOS synthesis (Fig. 2B). This result demonstrates that S23515 likely affects cholesterol synthesis by inhibiting the enzyme OSC in intact cells. The IC_{50} obtained for S23515 on cholesterol synthesis and MOS formation was 8 μM (Fig. 2C). One of the characteristics of the hepatocyte is its ability to secrete neo-synthesized lipids. We then analyzed the neo-synthesized lipids secreted by the cells into the medium and found that S23515 was able to inhibit the secretion of cholesterol with an IC_{50} = 10 μM (Fig. 2D), similar to that obtained for the intracellular lipids.

The inhibition of OSC by a pharmacological drug represents an attractive target in dyslipidemia due to the induction of an alternate pathway. When the conversion of MOS to lanosterol is inhibited, part of the MOS that accumulates can be converted into 2,3;22,23-diepoxysqualene (DOS). As shown in Fig. 2, it was possible to identify the synthesis of DOS induced by both BIBB 515 and S23515. It has been demonstrated that the enzyme OSC can also convert DOS into 24(*S*),25-epoxylanosterol to ultimately form 24(*S*),25-epoxycholesterol [21], a natural ligand for the liver X receptor (LXR) [22–23]. It was postulated that OSC has a higher affinity for DOS than MOS thus indicating that partial inhibition of the enzyme will increase the conversion of MOS to DOS and subsequently will increase the synthesis of 24(*S*),25-epoxycholesterol [19–20]. In contrast, full OSC inhibition using high doses of inhibitor will completely block the synthesis of 24(*S*),25-epoxycholesterol. This biphasic effect on the synthesis of 24(*S*),25-epoxycholesterol, depending on the dose of OSC inhibitor, is another way to determine if a drug affects OSC activity. Using a commercial source of 24(*S*),25-epoxycholesterol, we identified by thin layer chromatography the band corresponding to this sterol intermediate. Fig. 3 shows that S23515 induces a biphasic increase in 24(*S*),25-epoxycholesterol synthesis with a maximal effect at 12–25 μM followed by a decrease at the higher doses, thus confirming that S23515 inhibits the oxidosqualene:lanosterol cyclase.

3.3. Effects of S23515 and benazoline on cholesterol synthesis in hepatocytes from various species

We wanted to determine whether the effects of S23515 on OSC inhibition could be observed in hepatocytes from non-rodent species including human. We also wanted to know whether other imidazoline-like drugs could reproduce the profile induced by S23515. We compared the ability of S23515 and benazoline to inhibit cholesterol synthesis and to induce MOS synthesis in primary hepatocytes obtained from rat, cynomolgus and humans as well as in the human hepatoma cell line HepG2 cells. Table 1 shows that both imidazoline-like drugs significantly inhibit cholesterol synthesis in all cell types used. The magnitude of the inhibition is greater for S23515 than for benazoline suggesting that S23515 is more potent. As expected, the synthesis of MOS is induced in a parallel manner, thus confirming that the 2 compounds inhibit OSC in rodent as well as in non-rodent hepatocytes. As previously observed, the α2-AR agonist UK14,304 is totally inactive in all cell types used. We also used both guanabenz as another α2-AR agonist and two α2-AR antagonists namely rauwolscine and efaroxan [11]. When added to rat primary hepatocytes for 2 h, we found that these compounds were totally inactive on OSC activity such as UK14,304 (data not shown). Moreover, neither rauwolscine nor efaroxan, antagonized the effects of S23515 on OSC activity when

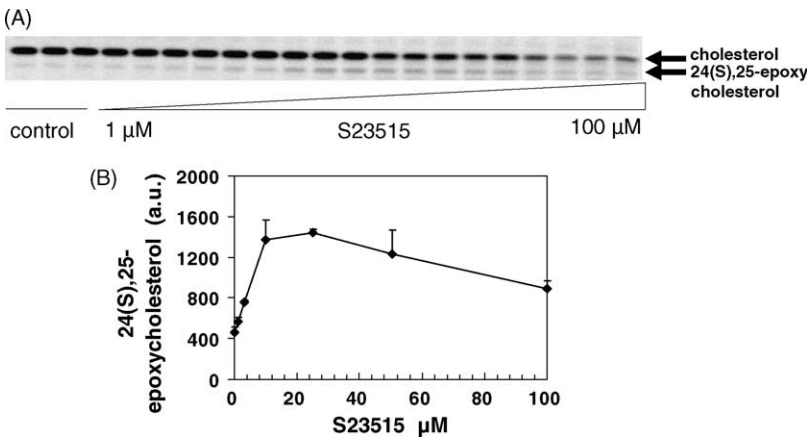


Fig. 3. S23515 stimulates the synthesis of 24(*S*),25-epoxycholesterol in primary rat hepatocytes. Cells were incubated for 6 h without (control) or with increasing doses of S23515 in presence of [^{14}C]-mevalonate. Intracellular lipids were extracted and separated as described in Section 2. (A) Represents part of the autoradiograph of synthetized cholesterol and 24(*S*),25-epoxycholesterol separated by thin layer chromatography. The positions of both lipids have been determined using standard. (B) Represents the quantification of the band corresponding to 24(*S*),25-epoxycholesterol. Values are expressed as percentage of control and are the mean ± S.D. of triplicates.

Table 1
Inhibition of OSC induced by S23515, benazoline and UK14,304 in hepatocytes from various species

	Rat	Cynomolgus	Human	HepG2
Cholesterol (% of control)				
S23515 (100 μM)	4 ± 1	33 ± 8	34 ± 2	25 ± 3
S23515 (25 μM)	17 ± 1	69 ± 2	71 ± 2	46 ± 3
Benazoline (100 μM)	26 ± 6	48 ± 4	61 ± 11	69 ± 9
UK14,304 (25 μM)	90 ± 1	103 ± 2	90 ± 8	95 ± 11
2,3-Monoepoxysqualene (% of control)				
S23515 (100 μM)	1446 ± 167	17981 ± 3642	2085 ± 107	939 ± 93
S23515 (25 μM)	1387 ± 160	2730 ± 456	649 ± 24	750 ± 54
Benazoline (100 μM)	339 ± 30	407 ± 56	173 ± 41	240 ± 68
UK14,304 (25 μM)	118 ± 6	119 ± 3	110 ±13	44 ± 31

Hepatocytes from various species were incubated without (control) or with the indicated drugs for 2 h in presence of [^{14}C]-mevalonate. Cholesterol and 2,3-monoepoxysqualene synthesis were assessed as indicated in Section 2. Values are expressed as percentage of control and are the mean ± S.D. of triplicates.

added in combination with S23515 (data not shown). All these data suggest that the inhibitory effects on OSC activity observed with S23515 cannot be attributed to activation of α2-AR.

3.4. Activation by S23515 of LXR target genes in human macrophages

Activation of LXR leads to the induction of expression of a set of genes involved in cholesterol efflux from peripheral tissues, a mechanism considered as the initial step in HDL formation and reverse cholesterol transport [24]. The ATP binding cassette proteins (ABC) A1 and G1 constitute the main transmembrane proteins responsible for cholesterol efflux from macrophages and their expressions are transcriptionally regulated by activation of LXR [25]. We wanted to know if OSC inhibition induced by S23515 and the subsequent generation of 24(*S*),25-epoxycholesterol could translate into ABCA1 and ABCG1 up-regulation. We used the human macrophage cell line THP-1 as a responsive cell line to synthetic LXR ligands [21]. We first wanted to know if S23515 was able to inhibit OSC activity in THP-1 cells. Differentiated THP-1 cells were incubated for 24 h with S23515 in presence of [^{14}C]-acetate to label the full panel of lipids. As shown in Fig. 4A, S23515 inhibits cholesterol synthesis (39%)

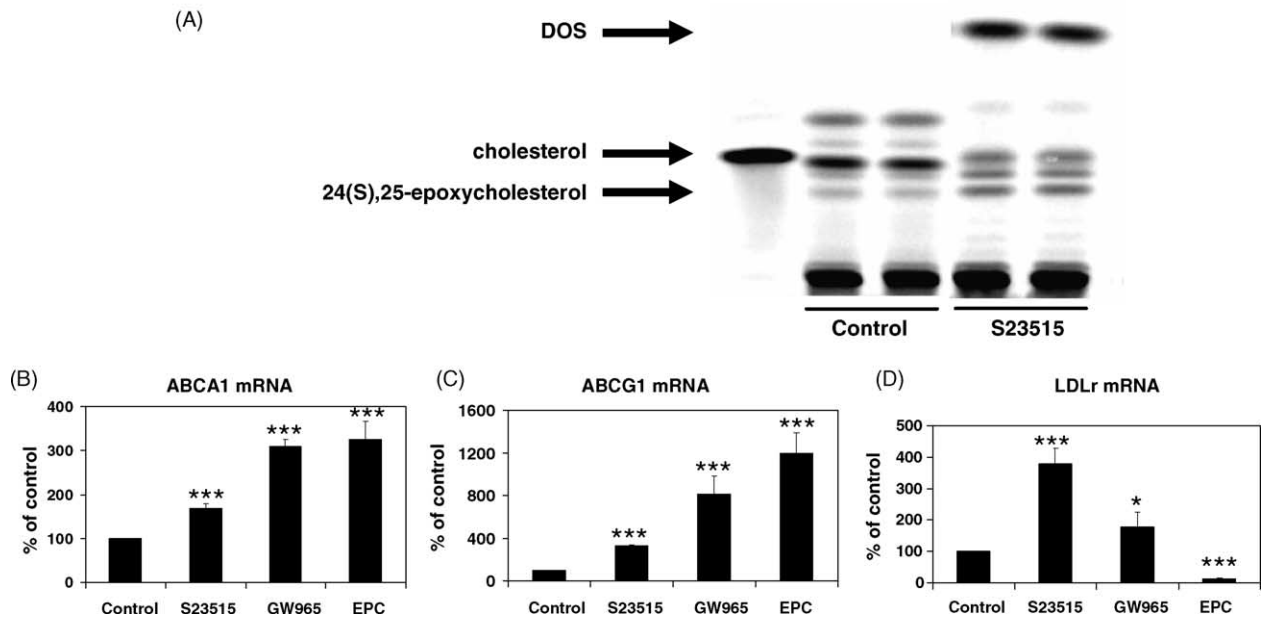


Fig. 4. S23515 inhibits OSC activity and increases the expression of ABCA1, ABCG1 and LDLr mRNA in human macrophages. (A) Differentiated THP-1 cells were incubated for 24 h without (control) or with 100 μM S23515 in presence of [^{14}C]-acetate. Intracellular lipids were extracted and separated as described in Section 2. The figure represents part of the autoradiograph showing the synthetized cholesterol, 24(*S*),25-epoxycholesterol and DOS separated by thin layer chromatography. The positions of lipids have been determined using radioactive and non-radioactive standards. (B–D) Differentiated THP-1 cells were incubated for 24 h without (control) or with 100 μM S23515, 1 μM GW965 or 4 μM 24(*S*),25-epoxycholesterol (EPC). At the end of the incubation period, mRNA were extracted and analyzed by quantitative real-time PCR. RNA amounts for ABCA1 (B), ABCG1 (C) and LDLr (D) were normalized with cyclophilin. Values are expressed as percentage of control and are the mean ± S.D. of triplicates ($^{\star}p < 0.05$, $^{\star\star\star}p < 0.001$).

and increases DOS synthesis (2580%) as well as it induces the synthesis of 24(*S*),25-epoxycholesterol (86%). This lipid profile corresponds exactly to that obtained in the same cell line using the OSC inhibitor BIBB 515 (data not shown) or with other OSC inhibitors [21,26] and suggests that S23515 is able to inhibit OSC activity and to generate the endogenous LXR ligand 24(*S*),25-epoxycholesterol in human macrophages. Then, using the same conditions, we analyzed the expression of LXR-regulated genes. Differentiated THP-1 cells were incubated for 24 h with S23515 or with the exogenous 24(*S*),25-epoxycholesterol or with the synthetic LXR ligand GW3965 and ABCA1 and ABCG1 mRNA were quantified by RT-PCR. Fig. 4B shows that S23515 induces a significant increase in ABCA1 (68%) and ABCG1 (230%) expression such as 24(*S*),25-epoxycholesterol or GW3965, thus confirming the activation of the LXR pathway in human macrophages. Parallel to this, we also quantified the mRNA for the low-density lipoprotein receptor (LDL-r), a gene known to be finely regulated by cholesterol [27], through the transcription factor called sterol regulatory element binding protein (SREBP) [28–29]. As observed in Fig. 4D, the exogenous oxysterol 24(*S*),25-epoxycholesterol completely decreased LDL-r expression by a process independent of LXR activation since the synthetic LXR ligand GW3965 did not decrease LDL-r mRNA. This effect of the oxysterol on LDLr expression could be attributed to the suppression of SREBP activation as previously described [28,30]. In contrast to 24(*S*),25-epoxycholesterol, S23515 induced a strong increase in LDL-r expression (280% compared to untreated cells) resulting from the inhibition of cholesterol synthesis. Thus, the different profile obtained in THP-1 cells between S23515, 24(*S*),25-epoxycholesterol and the exogenous LXR ligand GW965 suggests that S23515 could modulate gene expression through inhibition of cholesterol synthesis on one hand and through activation of the LXR pathway on the other.

4. Discussion

The presence of the statins as major drugs on the market, to reduce significantly plasma LDL cholesterol in human, demonstrates that inhibition of cholesterol synthesis represents a very attractive pathway for the treatment of dyslipidemia [31–33]. In this paper, we have demonstrated that the imidazoline-like drug S23515, originally developed as a hypotensive agent acting selectively through a non-adrenergic imidazoline binding site, can also inhibit cholesterol synthesis in hepatocytes. S23515 acts by inhibiting the activity of the enzyme oxidosqualene:lanosterol cyclase, a pivotal enzyme involved in cholesterol biosynthetic pathway but also in an alternate pathway that leads to the generation of 24(*S*),25-epoxycholesterol, a natural ligand of the nuclear receptor LXR. Inhibition of OSC activity is demonstrated by the concomitant inhibition of cholesterol synthesis and generation of monoepoxysqualene, the substrate of the enzyme. In addition, we observed the synthesis in a biphasic manner of 24(*S*),25-epoxycholesterol, the product of the alternate pathway. Activation of LXR triggers modulation of the expression of several genes involved in lipid metabolism such as SREBP1c and the ABC transporters ABCA1 and G1 [21–23,26]. Recently, a gene-selective modulation of LXR was observed with a synthetic oxysterol ligand showing no modulation of SREBP1c expression in hepatocytes and an increase in ABCA1 and G1 expression in macrophages [34]. Thus, inhibition of OSC could have the potential to inhibit cholesterol synthesis in hepatocytes and to stimulate cholesterol efflux from peripheral tissues. In the present study, we show that S23515 is able to inhibit OSC activity and to produce 24(*S*),25-epoxycholesterol, which in macrophages, translates into activation of LXR with the subsequent up-regulation of the two ATP-binding cassette ABCA1 and ABCG1. Increase in the expression of ABCA1 and ABCG1 has been demonstrated to be beneficial as these two proteins can promote cellular cholesterol efflux thus facilitating the removal of excess cholesteryl ester from cells including macrophages present in atherosclerotic lesion sites. Thus, we demonstrated that S23515, albeit at high dose, is able to inhibit cholesterol synthesis and secretion in human hepatocytes and to activate the expression of proteins involved in cholesterol efflux in human macrophages. Combination of these two pathways can represent a very attractive approach to the treatment of dyslipidemia.

It remains to be shown whether S23515 is a direct or an indirect inhibitor of the enzyme. The inability to obtain a labelled monoepoxysqualene to assess directly the effect of the drug on a microsomal fraction does not allow us to conclude on this point. We cannot rule out the possibility that the effect of S23515 could be mediated by the imidazoline binding site I1R. However, the fact that S23515 acts at the micromolar range to inhibit cholesterol synthesis in hepatocytes while it displaces the binding of [^{3}H] clonidine for I1R in calf frontal cortex membranes in the nanomolar range [7] does not support a receptor-mediated effect. Moreover, a great controversy exists in the literature whether or not the IBS can be considered as the receptor for imidazoline or if an unknown receptor is involved in this process. Once the I1R and the pathway associated with it will be fully characterized, it will be possible to determine if the receptor is expressed and is functional in hepatocytes. In addition, the high doses of S23515 or benazoline required to act on lipid metabolism did not allow us to try to antagonize these effects with I1R antagonists such as S23757 [7,35]. It will be necessary to find an imidazoline-like drug acting on cholesterol synthesis below the micromolar range to study the effect of an excess of I1R antagonists such as described in PC12 cells on cell apoptosis [35]. However, since we never observed any effects of UK14,304 or guanabenz, two α2-AR agonists, nor antagonized the effects of S23515 with the

α2-AR antagonists rauwolscine and efaroxan, we can totally exclude the contribution of the α2-AR in the inhibition of cholesterol synthesis induced by S23515.

Another imidazoline-like drug called moxonidine has been described to affect glucose and lipid metabolism when administrated orally to a spontaneously hypertensive obese rat model [10–11]. However, we cannot conclude from our results that the effect observed in the rat with moxonidine could be attributed to the inhibition of OSC and the subsequent inhibition of cholesterol synthesis. It will be necessary to perform in vivo studies with S23515 or other imidazoline-like drugs to determine definitively the profile of this class of molecules on cardiovascular functions.

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