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The oxysterome and its receptors as pharmacological targets in inflammatory diseases

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Abstract

Oxysterols have gained attention over the last decades and are now considered as full-fledged bioactive lipids. The study of their levels in several diseases, (e.g. atherosclerosis, obesity, neurodegenerative diseases) led to a better understanding of their involvement in (patho)physiological processes such as inflammation and immunity. For instance, the characterization of the cholesterol-7α,25-dihydroxycholesterol/GPR183 axis and its implication in immunity represents an important step in the oxysterome study. Besides this axis, others were identified as important in several inflammatory pathologies (such as colitis, lung inflammation, and atherosclerosis). However, the oxysterome is a complex system notably due to a redundancy of metabolic enzymes and a wide range of receptors. Indeed, deciphering oxysterol roles and identifying the potential receptor(s) involved in a given pathology remains challenging. Oxysterols properties are very diverse but most of them could be connected by a common component: inflammation. Here, we review the implication of oxysterol receptors in inflammatory diseases.

Abbreviation list

ALI	acute lung injury
apoE	apolipoprotein E
ARDS	acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
CD	Crohn's disease
CHD	coronary heart disease
Chol25OHase	cholesterol-25-hydroxylase
COPD	chronic obstructive pulmonary disease
CXCR2	C-X-C motif chemokine receptor 2
diOHC	dihydroxycholesterol
DSS	dextran sulphate sodium
EBI2	Epstein-Barr virus-induced G-protein coupled receptor 2 (also known as GPR183)
epoxychol	epoxycholesterol
ER	estrogen receptor
GPER	G protein-coupled estrogen receptor
GPR17	G protein-coupled receptor 17
GPR183	G protein-coupled receptor 183 (also known as EBI2)
GR	glucocorticoid receptor
HFD	high fat diet
LPS	lipopolysaccharides
LXR	liver X receptor
MPO	myeloperoxidase
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
OHC	hydroxycholesterol
ROR	retinoic acid receptor-related orphan receptors
SIRT-1	sirtuin 1
Smo	smoothened
T2D	type II diabetes
TNBS	trinitrobenzene sulfonic acid
UC	ulcerative colitis

Over the years, oxysterols were shown to be directly involved in the etiopathology of several inflammatory diseases and their levels have been put forth as diseases biomarker. Indeed, as we will illustrate in the following sections, in many pathological situations characterized by inflammation, oxysterol levels were altered greatly both in clinical and preclinical settings. Oxysterols are oxygenated species derived from cholesterol and their metabolism is quite complex and intricate as one given oxysterol can be formed through the action of more than one enzyme and/or through reactive oxygen species (ROS)-induced reactions (**Figure 1**). Moreover, one enzyme can have different oxysterols as substrates hence greatly complexifying oxysterol study (Mutemberezi et al., 2016). As for many endogenous metabolites, determining whether changes in their levels are simply consequences or causal factors (or merely associated with disease evolution) is no easy task.

Over the last decades, several receptors were found to be targets of oxysterols such as the liver X receptors (LXR) (Fu et al., 2001; Janowski et al., 1999), retinoic acid receptor-related orphan receptors (ROR) (Bitsch et al., 2003; Wang et al., 2010a), estrogen receptor α (ERα) (DuSell et al., 2008; Lappano et al., 2011), G protein-coupled receptor 183 (GPR183) (also known as Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2)) (Hannedouche et al., 2011; Liu et al., 2011), C-X-C motif chemokine receptor 2 (CXCR2) (Raccosta et al., 2013), G protein-coupled receptor 17 (GPR17) (Sensi et al., 2014), smoothened (smo) (Nachtergaele et al., 2012) or glucocorticoid receptor (GR) (Voisin et al., 2017). Interestingly, these receptors are involved in very different (patho)physiological processes and some of which do not seem to be related at first glance. These receptors also bind other endogenous ligands (some of which remain to be identified) and in (patho)physiological conditions it is difficult to distinguish which ligands is directly involved in a given process. However, because they share oxysterols as ligands, these receptors and the process they regulate can reveal new interesting interconnections. One such interconnection is the ability of the aforementioned receptors to

control inflammatory processes whether directly or indirectly and to a different extent. Another important connection lies in the fact that inflammation is also associated with an increase in ROS production that can lead to oxidative stress. This specific (patho)physiological process is of importance as it is responsible for the synthesis and degradation of several oxysterol species that do not rely on enzymatic reactions.

As we will illustrate in this review, one strategy to assess whether oxysterols are involved in the pathophysiology of diseases is to interfere with their signalling either by altering their metabolism or by interacting directly with their molecular targets. As targeting metabolism often lead to changes in several oxysterol levels and because different oxysterols bind a given receptor with different functionalities (agonist, antagonist…) (**Table 1**), in this review, we chose to focus on studies using a pharmacological approach to decipher the role(s) of oxysterol receptors in the etiopathology of several diseases with an inflammatory component (even though we do not discuss the inflammatory aspects of the diseases presented) (**Table 2**). In the present review, we will focus on four main systems (cardiovascular, respiratory, and enteroendocrine) and several associated inflammatory diseases, we will focus on oxysterol level variations in humans and on the effect(s) of pharmacological tools targeting oxysterol receptors in a preclinical and clinical settings.

1. CARDIOVASCULAR SYSTEM

Due to the associations found between plasma cholesterol and cardiovascular events, cardiovascular diseases were logically the first pathologies where oxysterols were studied.

Atherosclerosis and coronary heart disease

Atherosclerosis is an inflammatory pathology affecting the arteria wall and is characterized by a deposition of calcium, lipids and inflammatory cells forming a plaque that narrows the artery

lumen and decreases blood flow. Both innate and adaptive immunity were shown to be directly involved in its pathogenesis. Coronary heart disease (CHD), or coronary artery disease, is related to atherosclerosis but affecting coronal arteries and therefore can lead to a decrease in oxygen and nutrients supply and further cause myocardial infarction. Interestingly, the severity and the prognosis of coronary heart disease relies on inflammatory marker levels such as IL-6 or C-reactive protein.

In plasma from atherosclerotic patients, 5α,6α-Epoxychol, 5β,6β-Epoxychol, 7β-OHC, and 7-KC levels (abbreviations can be found in **Table 1**) were significantly increased compared to control patients while levels of 7α-OHC and 27-OHC remained unchanged (Zhou et al., 2000). Levels of oxysterols were measured in the plasma of patients with stable CHD compared to patients with a similar atherogenic profile but with normal coronary arteries and levels of 7-KC were found to be increased (Hitsumoto et al., 2009). Moreover, comparing patients with stable CHD compared to healthy patients most of the plasma oxysterol levels measured (i.e. 5α,6α-Epoxychol, 5β,6β-Epoxychol, 7α-OHC, 25-OHC, and 27-OHC) were not significantly altered while levels of the levels of 7β-OHC were found to be increased (Rimner et al., 2005). In another study, plasma levels of 7β-OHC, 25-OHC and 27-OHC were increased in patients displaying advanced CHD with an important stenosis (Yasunobu et al., 2001). Moreover, levels of 7-KC were found to be directly linked to an increase in fatal outcome in patients suffering from stable CHD (Song et al., 2017) and to both the progression of coronary atherosclerosis and the associated inflammation (Hitsumoto et al., 2009). Conversely, 27-OHC levels were not found to be linked with increased risk of CHD (Rossouw et al., 2012).

In coronary artery plaques, 5α,6α-Epoxychol, 5β,6β-Epoxychol, 7α-OHC, 7β-OHC, 7-KC and 27-OHC levels increased compared to a directly adjacent plaque free region (Vaya et al., 2001). Interestingly, the oxysterol profile was slightly different in atherosclerotic plaques coming from the carotid as the same variations were observed except for 7α-OHC that was not detected

and 5α,6α-Epoxychol that remained unaltered compared to a directly adjacent plaque-free region (Vaya et al., 2001).

According to an anatomopathological classification of atheroma plaques, 27-OHC levels increased with the progression in severity of the plaque which was not the case for 7β-OHC (Carpenter et al., 1995). For instance, 27-OHC was more abundant in advanced lesions compared to intermediate lesions. This oxysterol levels were also increased in macrophage-rich lesions compared to fibrous ones (Carpenter et al., 1995). Finally, a topographic analysis of plaque demonstrated a different repartition of oxysterols. Indeed, both 7β-OHC and 27-OHC were more abundant in the core compared to the fibrous cap (Garcia-Cruset et al., 1999).

Because LXRs are directly involved in cholesterol and lipid homeostasis, their involvement was studied in several animal model of atherosclerosis in order to explore new therapeutic avenues. In $LDLR^{-/-}$ mice fed a "western diet", administration of T0901317 (LXRα and β agonist and RORα and γ inverse agonist, see **Table 2**) significantly reduced atherosclerosis in two distinct arterial regions compared to the untreated group albeit to a different extent according to specific regions (Peng et al., 2009). In the same animal model, T0901317 was shown to decrease both lesion size and foam cell formation. In the aorta, the treatment decreased IL-6, TNFα and MCP-1 levels and significantly reduced the number of macrophages. Furthermore, the authors assessed the polarization of these plaque-associated macrophages and found that T0901317 converted them towards a M2-like polarization type (Dou et al., 2019). In a model of APOE*3 Leiden mice, two LXRα and β agonists, AZ876 and GW3965, both significantly decreased lesion area, the number of plaque-adhering monocytes, and IL-1β and TNFα plasma levels (van der Hoorn et al., 2011).

RORs have been much less studied in this context. However, Billon et al. administered the ROR inverse agonists SR1001 to $LDLR^{-/-}$ mice and showed that this treatment led to a

significant decrease in plaque formation and a reduction of the inflammatory profile of the animals. The treatment also decreased LDL levels, without affecting HDL, and enhanced cholesterol excretion by the intestine (Billon et al., 2016).

The protective effects of ERα has long been documented in this setting. For instance, in ApoE$^{-/-}$ mice, treatment with the ER agonist estradiol significantly decreased the size of atherosclerotic lesions and their histological complexity and decreased plasma total cholesterol. These beneficial effects were abolished for ApoE$^{-/-}$ mice lacking ERα supporting the role of this receptor (Hodgin et al., 2001). ERα can be located at either the cell membrane or in the nucleus. In an elegant study, Guivarc'h et al. used mice which ERα sequence was mutated for a key amino acid allowing for membrane anchoring and ERα mutated mice where nuclear action were disrupted. Using these two ERα mutant mice, they demonstrated that the ERα nuclear pathway was responsible for the vasculoprotective effects of ERα as mice mutated for the nuclear response were much more susceptible to atheroma (Guivarc'h et al., 2018).

Interestingly, the oxysterol 27-OHC has been directly implicated in atherosclerosis through its binding to ERα. Indeed, using the ApoE$^{-/-}$ model, the administration of 27-OHC increased atherosclerosis while no such effect was observed in ApoE$^{-/-}$; ERα$^{-/-}$ mice. The authors also showed that 27-OHC stimulated NF-κB activation and increased leukocyte-endothelial adhesion in vivo in an ERα-dependent manner as co-treatment with an ER antagonist (ICI 182780, **Table 2**) decreased the adhesion (Umetani et al., 2014).

In aorta plaque from LDLR$^{-/-}$ mice, the secretions of macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) (both endogenous ligands of CXCR2) were significantly increased compared to control animals (Mihara et al., 2007). Moreover, the administration of the CXCR2 antagonist SB225002 to ApoE$^{-/-}$ mice was able to decrease significantly aorta plaque formation (Wu et al., 2011). Finally, a human trial focusing on CHD

patients and investigating the effects of AZD5069 (a CXCR2 antagonist) is currently ongoing (eudraCT number 2016-000775-24).

Thrombosis

Platelets are key elements regulating haemostasis, thrombosis, and inflammation. They play an active role in immune responses and are responsible for the secretion of many pro- and anti-inflammatory mediators and several factors regulating thrombogenesis and vascular inflammation.

In a rabbit model of plaque erosion leading to atherothrombotic occlusions, treatment with ezetimibe (reducing cholesterol absorption by targeting the NPC1 Like Intracellular Cholesterol Transporter 1 (NPC1L1)) decreased plasma levels of 7α-OHC, 7-KC, 25-OHC and 27-OHC compared to vehicle-treated animals. This was concomitant with a reduction of circulating levels of IL-1β, IL-6 and TNFα and was accompanied by decreased thrombotic occlusion, an improved re-endothelialisation and an increase in survival (Honda et al., 2018). In human platelets, several oxysterols, i.e. 5α,6α-Epoxychol, 5β,6β-Epoxychol, 7α-OHC, 7-KC and 27-OHC, significantly increased thrombin- or ADP-induced aggregation and decreased aggregation lag time (Selley et al., 1996).

Despite being anucleate cells, platelets were shown to express LXRβ. The incubation of human platelets with T0901317 (LXRα and β agonist and RORα and γ inverse agonist) or GW3965 (LXRα and β agonist) led non-genomically to an inhibition of both platelet aggregation and calcium mobilization when stimulated with either collagen, C-reactive protein or thrombin. Using a tail bleeding assay in mice, GW3965 significantly increased the time needed to observe bleeding cessation. Moreover, using a laser-induced injury leading to thrombus formation in vivo, GW3965 displayed antithrombotic effects as a significantly lower number of platelets was recruited to the forming thrombus (Spyridon et al., 2011). Whether oxysterols (e.g. 27-

OHC) can modulate platelet aggregation through LXR remains to be determined. Thrombomodulin is an important vasoprotective effector acting on vascular endothelial cells by suppressing circulating cell adhesion and inflammatory response. In HUVEC, incubation with T0901317 increased the expression of thrombomodulin and decreased levels of IL-1β and TNFα (Ding et al., 2016).

Two oxysterols, 25-OHC and 27-OHC, are well known modulators of ERα (**Table 1**). Activation of this receptor, for instance using 17α-ethinylestradiol, results in a decrease of the activity and expression of most coagulating factors. Using either, $ER\alpha^{-/-}$ or $ER\beta^{-/-}$ mice, it was shown that ERα was necessary for the observed alterations of coagulation factor profile (Cleuren et al., 2010). Consistent with this finding, the administration of estetrol (ER agonist) protected wild-type but not $ER\alpha^{-/-}$ mice, from both venous and arterial thrombosis and led to a significant decrease in thromboembolism-induced mortality in the wild type mice (Valera et al., 2018). Using hematopoietic cell transfer lacking either membrane ERα or nuclear ERα, it was shown that either membrane or nuclear ERα could mediate estradiol effect on bleeding time, however with a prominence for hematopoietic nuclear ERα. Concerning thromboembolism-induced mortality, both knockout types led to a protection when treated with estradiol. However, it seemed that the protection from thromboembolism was mainly mediated through hematopoietic nuclear ERα compared with hematopoietic nuclear ERα (Valera et al., 2015). Whether these mechanisms can be affected by the oxysterols binding to ERα remains to be established.

2. RESPIRATORY SYSTEM

The respiratory system can be affected by several acute or chronic inflammatory conditions. As discussed here, several elements point to the oxysterols as impotent players in these diseases.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is defined as a chronic inflammation of lung parenchyma and of peripheral airways associated with airway obstruction and emphysema resulting in a severe decline in lung function. In clinical settings, patients suffering from COPD displayed increased levels of 27-OHC in their sputum compared to control patients. Moreover, two lung function parameters (forced vital capacity (FVC) % predicted and the forced expiratory volume 1 (FEV1) % predicted) used to diagnose the COPD severity were found to be inversely correlated with 27-OHC sputum levels (Kikuchi et al., 2012). In the lung parenchyma of COPD patients, cholesterol-25-hydroxylase (Chol25OHase) expression was found to be significantly higher compared to control (Jia et al., 2018; Sugiura et al., 2012) and this enzyme expression in the lungs was also directly correlated with two distinct markers of COPD severity (namely the FEV1% and the diffusing capacity of the lungs for carbon monoxide). Chol25OHase expression was further studied in human small airways and was found to be increased in COPD patients presenting emphysema compared to COPD patients without emphysema (Jia et al., 2018). Interestingly, sputum 25-OHC was significantly increased in COPD patients as well as IL-8 sputum levels and neutrophil count, while 25-OHC levels remained unchanged in the plasma of the same patients compared to control. Here again, 25-OHC sputum levels were directly correlated with three different parameters used to assess the disease severity and were also shown to correlate with IL-8 and neutrophil count in the sputum (Sugiura et al., 2012).

The interest of LXR in the context of COPD has not been studied extensively and remains to be fully explored. However, the expression of both LXRα and LXRβ was found to be increased in the lung parenchyma of COPD patients compared to control even though their expression remained unchanged in lung macrophage. In an ex vivo setting, the incubation of GW3965 (LXRα and β agonist) with lipopolysaccharides (LPS)-activated alveolar macrophage pooled from smoking patients and COPD patients was able to significantly decrease the expression of CXCL10, CCL5, and IL-6 (Higham et al., 2013).

In a mouse model of COPD using tobacco smoke exposure, Jia et al. put forth the GPR183-7α,25-diOHC axis as central in the disease pathogenesis. Indeed, they showed that wild-type mice exposed to cigarette smoke developed emphysema and inducible bronchus-associated lymphoid tissue (iBALT) (formation of a secondary lymphoid-like structure within the lung parenchyma). However, in either mice lacking Chol25OHase or mice lacking GPR183, both emphysema and iBALT were significantly decreased. Finally, using clotrimazole (a CYP450 inhibitor, used here to target Cyp7B1) to decrease the formation of 7α,25-diOHC in vivo, the authors evidenced also a significant decrease in emphysema and iBALT development along with a decrease in macrophages and neutrophils in the broncholaveolar lavage fluid (BALF) (Jia et al., 2018). However, the observed effects using clotrimazole could also result from the inhibition of other cytochromes.

In the context of pulmonary diseases, of the different identified oxysterol receptors, CXCR2 has clearly attracted most of the attention. In a clinical trial designed to assess the safety and tolerability of AZD5069 (a CXCR2 antagonist, see **Table 2**), blood neutrophil count was significantly decreased compared to placebo-treated patients. Despite the trial short duration and the small sample size, a trend towards an improvement of lung function was observed but still need to be confirmed (Kirsten et al., 2015). In another human study, healthy patients were treated with either AZD8309 (another CXCR2 antagonist) or a placebo for three days before a

LPS challenge and their sputum was recovered. The total cell number as well as the neutrophil count and inflammatory mediators (CXCL1, LTB4) were significantly decreased by the treatment. AZD8309-treated volunteers also displayed a quicker recovery of lung function as several markers started to normalize 6 hours following LPS challenge while this was not the case for placebo-treated patients (Leaker et al., 2013). Another study further demonstrated the interest of CXCR2 antagonists, this time using an ozone challenge in healthy volunteers. The sputum of SB-656933-treated volunteers showed a significant decrease in neutrophil count and myeloperoxidase activity compared to placebo-treated volunteers (Lazaar et al., 2011).

Acute lung injury and acute respiratory distress syndrome

Acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS) can occur as the result of direct or indirect insults to the lungs. These pathologies are characterized by lung oedema, alveolar and surfactant dysfunctions, and by inflammation that lead to severe hypoxia. Several pro-inflammatory cytokines (e.g. IL-6, TNFα, IL-8) are proposed as biomarkers predicting both morbidity and mortality in these pathologies. In patients, oxysterol levels were not assessed and, therefore, their variations could potentially become an interesting research topic in order to better decipher the pathogenesis of these diseases. In animal models, oxysterol effect(s) are starting to be unravelled. In a LPS-induced ALI mouse model, intraperitoneal pre-treatment with 25-OHC was shown to decrease both lung injury and mortality and to decrease pro-inflammatory mediator levels (e.g. IL-6, TNFα, KC) in the BALF (Ouyang et al., 2018). Beneficial effects on lung inflammation were also observed following intratracheal administration of 25-OHC (Bottemanne et al., 2021). In another study, the effects of 25-OHC and its two sulphated metabolites (25-hydroxycholesterol-3-sulfate and 25-hydroxycholesterol-3,25-disulphate) were studied in a LPS-induced systemic inflammation model. Histological assessment of lung injury and its scoring were much higher in LPS-treated mice compared to control animals and characterized

with neutrophil infiltration, oedema, and marked structural loss. Pre-treatment with 25-OHC or either of its sulphated forms led to a significant decrease in lung injury score. Interestingly, 25-hydroxycholesterol-3-sulfate and 25-hydroxycholesterol-3,25-disulfate (25-OHC-3-diS) were able to decrease further the lung injury score compared to treatment with 25-OHC. Indeed, mice treated with either the sulfate or the disulfate metabolite of 25-OHC displayed a normalized lung structure despite the presence of few neutrophil infiltrates (Ning et al., 2017).

In a rat model of LPS-induced ALI, BALF pro-inflammatory cytokines levels (IL-1β, IL-6, TNF) neutrophil count as well as the histological lung injury score were all significantly decreased with T0901317 (LXRα and β agonist and RORα and γ inverse agonist) (Wang et al., 2011). In a paraquat-induced ALI mouse model, T0901317 was able to dose-dependently reduce the histological lung injury score as well as both IL-1β and TNFα levels and myeloperoxidase (MPO) activity in the BALF at several time-points. The authors also studied the effects of T0901317 treatment on NF-κB pathway and found that T0901317 was able to dose-dependently decrease NF-κB and increase IκB-α lung levels (Hu et al., 2017). The protective effects of T0901317 were also confirmed in a carrageenan model of ALI where it reduced lung injury as well as cytokine levels and immune cell number in the pleural exudate (Crisafulli et al., 2010). Zhao et al. studied the effects of the activation of LXR either through pharmacological means or through genetic means (using a VP-LXRα knock-in, resulting in a constitutively active LXRα) in an oleic acid mouse model of ARDS. They found that both mice expressing the constitutively active LXRα or treated with GW3965 displayed a significant reduction in oleic acid-induced lung permeability and oedema. Both approaches (genetic and pharmacological) led to a significant decrease in neutrophil count in the BALF and to a decrease in IL-6 and TNFα levels in both the plasma and the BALF. Finally, both approaches aimed at increasing LXR signalling led to an increased activity of enzymes involved in ROS quenching (i.e. superoxide dismutase and catalase) in the lung (Zhao et al., 2016).

In the context of ALI or ARDS, the potential role(s) of ER remain largely to be explored. Nevertheless, in a rat model using laparotomy and/or controlled haemorrhagic shock in order to elicit ALI, the involvement of ER receptors was studied. The administration of estradiol or diarylpropionitrile (or DPN, a ERβ agonist) was able to fully reverse lung permeability, MPO levels in the BALF as well as iNOS expression in the lungs while propylpyrazoletriol (or PPT, a ERα agonist) was only partially protective (Doucet et al., 2010).

As for other inflammatory pathologies of the respiratory tract, CXCR2 represents one of the major targets compared to other oxysterol receptors. In an LPS-induced ALI mouse model, mice treated with the antagonist SB225002 displayed a decreased histological lung injury and decreased lung MPO levels. Moreover, the CXCR2 antagonist was able to reduce lung permeability assessed using Evan's blue. Pro-inflammatory cytokines and chemokines were studied in the BALF and SB225002 significantly reduced their levels compared to vehicle-treated animals (Cao et al., 2018). Reparixin (a CXCR2 allosteric modulator) was shown to significantly reduce CXCL1-induced leukocyte adherence in vivo. In a LPS-induced ALI model, this compound was also able to dose-dependently decrease neutrophil count in the BALF. Interestingly, within the lung parenchyma, the Reparixin-induced decrease in neutrophil recruitment was localized to the interstitial compartment while no such decrease was found in the intravascular compartment. Here again, animals treated with Reparixin showed a decrease in lung permeability compared to vehicle-treated (Zarbock et al., 2008). Overall similar results were also observed in a rat model using the antagonist AZD5069 (Nicholls et al., 2015).

Asthma

Asthma is characterized by bouts of coughing, wheezing and by breathing difficulties. This disease is accompanied by chronic inflammation of airways including during asymptomatic

phases. Its pathogenesis stems from an aberrant immune reaction towards allergens that leads to an airway hyper-responsiveness and to airway remodelling.

In patients with mild asthma, levels of several oxysterols were quantified before and 48 hours after an allergen challenge. 7α-OHC, 27-OHC, and 7α,27-diOHC were significantly increased in the BALF after the allergen challenge. Moreover, the levels of these oxysterols were correlated with immune cell counts in the BALF. More specifically, 7β,27-diOHC BALF levels correlated with the eosinophil count, 25-OHC and 27-OHC both correlated with neutrophil count and both 7α,27-diOHC and 27-OHC correlated with lymphocyte count in the BALF of asthmatics (Shen et al., 2017).

Regarding oxysterol receptors, only two were studied using a pharmacological approach in animal models of asthma. Conversely to the other respiratory tract inflammatory pathologies addressed here, LXR activation would seem to exert some detrimental effects. Indeed, in a house dust mite mouse model, oral administration of GW3965 led to an increase of the inflammatory response as total cell count and eosinophil count were significantly increased in the BALF of these mice. Within the lung parenchyma, GW3965 treatment led to a higher expression of IL-4, IL-5 and IL-13. The detrimental effects of LXR activation in this animal model were further confirmed with the use of LXRαβ$^{-/-}$ animals (Smet et al., 2016). On the other hand, in an ovalbumin asthma mouse model, T0901317 (LXRα and β agonist and RORα and γ inverse agonist) administration changed neither the immune cells present in the BALF nor IL-4 and IL-13 levels in the BALF compared to vehicle-treated mice. However, it was able to significantly decrease circulating ovalbumin-specific IgE as well as mucus hyper-production and peri-bronchial fibrosis (Shi et al., 2014). Finally, in a rat model of ovalbumin-induced asthma, GW3965 treatment failed to reduce eosinophil count in the BALF, increased airway responsiveness and increased smooth muscle thickness in both asthmatic and healthy rats even though no change was measured regarding lung inflammation (Birrell et al., 2008).

The interest of CXCR2 in the context of asthma has led to clinical trials. AZD5069 (CXCR2 antagonist) was used at three different doses in patients with uncontrolled persistent asthma. Despite the observed dose-dependent decrease in circulating neutrophils between placebo- or AZD5069-treated patients, no clinical benefits were observed regarding the number of asthma exacerbation or in key breathing parameters (O'Byrne et al., 2016). In another clinical trial, experimenters elected to study the effects of the antagonist SCH527123 in patients with severe asthma. Patients treated with this CXCR2 antagonist displayed a significant decrease in both neutrophil and eosinophil counts in the sputum at the end of the study while no change was found in placebo-treated patients. Blood neutrophil count was also significantly decreased in SCH527123-treated patients. However, despite the fact that the treatment induced fewer mild exacerbations compared to placebo patients, no other significant improvement was observed regarding lung function (Nair et al., 2012). Therefore, CXCR2 antagonism in the context of asthma seems to lead to some consistent effects regarding immune cells count but, so far, does not translate into strong clinical effects.

Obesity and metabolic syndrome

Obesity and metabolic syndrome are accompanied by a chronic low-grade inflammation that affects the entire organism and is directly linked to several associated comorbidities. The specific inflammatory state associated with obesity or metabolic syndrome is not as extreme as it can be observed in other diseases. However, this low-grade inflammation extends over a long period of time and is associated with the development of several comorbidities such as hypertension, type 2 diabetes or dyslipidaemias.

To date, contrary to the preclinical data, there are limited clinical reports on the consequences of obesity on oxysterol levels (Guillemot-Legris et al., 2016). In patients suffering from obesity

or metabolic syndrome, plasma levels of 4β-OHC were found to be significantly decreased compared to control patients for both female and male subjects. Levels of the isomer 4α-OHC were only significantly increased in patients suffering from metabolic syndrome compared to control patients for both female and male subjects. Interestingly, in female subjects, more oxysterols were altered by obesity or metabolic syndrome. Indeed, plasma levels of 5α,6α-epoxychol were decreased for obese and female patients with metabolic syndrome compared to healthy controls while levels of 24(*S*)-OHC were only decreased obese female patients. Finally, plasma levels of 7α-OHC, 7β-OHC, 7-KC and 25-OHC were only increased for female patients suffering from metabolic syndrome compared to control (Tremblay-Franco et al., 2015). Interestingly, patients suffering from anorexia nervosa displayed the opposite variation with regards to plasma levels of 4β-OHC as it was increased in such patients compared to normal weight control subjects (Hole et al., 2018).

In this context, pharmacological tools were employed to target LXR in animal models of obesity. The administration of T0901317 (LXRα and β agonist and RORα and γ inverse agonist) resulted in decreased body weight gain and decreased fat mass development in mice fed a high fat diet (HFD). Interestingly, while the epididymal and visceral adipose depots displayed a decreased expansion, the brown adipose tissue depot remained unaltered by T0901317 compared to vehicle-animals (Gao et al., 2013). Besides an increase in fat mass, obesity is also characterized by macrophage and lymphocyte infiltration of the adipose tissue. In the ob/ob mice, the LXRα and β agonist GW3965 decreased the number of macrophages and lymphocytes in the visceral and subcutaneous adipose tissue, respectively. The treatment also decreased the inflammatory tone of both fat depots (Archer et al., 2013).

Contrary to the LXR agonist, administration of the ROR agonist SR1078 led to an increase in plasma and epididymal adipose tissue expression of IL-6 and TNFα (Liu et al., 2017). Conversely, treatment with a RORγ inverse agonists exerted beneficial effects in this context.

Indeed, SR1555 administration or SR1903 administration to mice in a diet-induced obesity model led to a significant decrease in weight gain and fat mass development as well as circulating lipid levels (Chang et al., 2019; Chang et al., 2015). The important role of RORα in the context of obesity was also confirmed by the fact that RORα knockout mice were partly protected against obesity and display decreased weight gain and fat mass (Lau et al., 2008).

The potential role of smoothened and of CXCR2 in obesity was only recently addressed. In a study using db/db mice, treatment with the CXCR2 antagonist G31P (antagonist of both CXCR1 and CXCR2) decreased body weight and fat mass. It also reduced the weight of different white adipose tissue depots and circulating levels of IL-6, TNFα and CXCL8 compared to vehicle-treated animals (Cui et al., 2019). G31P administration also significantly reduced the expression of MCP-1 and TNFα in the subcutaneous adipose tissue and reduced the presence of crown-like structures (Cui et al., 2019). Finally, using a genetic construct leading to the constitutive activation of adipose tissue smoothened, the authors found that activation of smoothened reduced body weight gain under HFD (Shi et al., 2017). Conversely, mice lacking smoothened in the myeloid cell line displayed a higher body weight gain compared to wild type animals and an increased recruitment of macrophages in the adipose tissue further supporting the role of this receptor in the context of obesity (Braune et al., 2017).

NAFLD and NASH

Obesity and metabolic syndrome are often accompanied by severe alterations of the liver. Non-alcoholic fatty liver disease (NAFLD) aetiology is still not fully understood however it is linked to obesity, high circulating triglycerides and insulin resistance. NAFLD is characterized by an ectopic deposition of fat within hepatocytes that leads to lipotoxicity and inflammation alterations of hepatic functions. Non-alcoholic steatohepatitis (or NASH) represents a more

serious pathology as hepatocytes are not only inflamed but dying and liver fibrosis can also be observed.

Levels of oxysterols were measured directly in the liver of NASH patients. Levels of 7α-OHC, 7β-OHC, 7-hydroxycholestenone, 7α,27-diOHC and 24(*S*)-OHC were increased compared to healthy subjects while levels of 25-OHC and 27-OHC remained unchanged. Furthermore, correlations were also established between 7α-OHC, 7β-OHC, 24(*S*)-OHC and 7-hydroxycholestenone levels and the anatomopathological score of patient's livers (Raselli et al., 2019a).

As the liver represent a key organ with regards to lipid and glucose metabolisms, the role of LXR was investigated. Patients with NAFLD were shown to display higher levels of LXRα in the liver compared to healthy subjects and these levels were associated with increased inflammation and fibrosis (Ahn et al., 2014).

In a NASH mouse model, SR9238 administration led to a significant decrease in liver weight and steatosis. Treatment with the LXR agonist also decreased the hepatic expression of pro-inflammatory markers as well as the expression of CD68 supporting a decrease in macrophage infiltration (Griffett et al., 2015). Interestingly, in a mouse model of NAFLD, the LXR inverse agonist SR9238 decreased liver steatosis, macrophage recruitment, and levels of inflammation-associated hepatic enzymes (ALT, ALP and AST). This LXR antagonist also decreased the expression of IL-1β and TNFα in the liver (Griffett et al., 2013). Finally, oral administration of the oxysterol 22(*S*)-OHC (reported to be a LXR antagonist) to HFD fed rats led to a decrease in hepatic triglycerides compared to vehicle-treated animals (Tranheim Kase et al., 2012). As both LXR agonism and antagonism seem to exert some beneficial effects in the context of NAFLD and NASH, further studies are clearly needed to decipher the underlying mechanisms at play. However, one pharmacological avenue that is still being explored relies on the

development of isoform-specific drugs. Indeed, LXRα is the major LXR isoform expressed in hepatocytes. Therefore, selectively targeting LXRβ would allow to circumvent lipogenesis induction.

In a diet-induced NASH mouse model, treatment with the RORα activator JC1-40 significantly reduced hepatic triglyceride levels and increased liver expression of antioxidant enzymes (superoxide dismutase 2 and Glutathione peroxidase 1). The treatment also decreased liver inflammation (i.e. expression of IL-1β and TNFα) and macrophage infiltration (Han et al., 2014). In a follow up study, the authors found that JC1-40 administration was able to decrease circulating GPT and GOT along with liver triglyceride levels. It also decreased the expression of IL-1β, IL-6 and TNFα in Kupffer cells and further skewed Kupffer cell polarization towards a M2-like phenotype (Han et al., 2017).

The involvement of the 7α,25-diOHC receptor GPR183 has been studied only through genetic approaches. Mice with genetic deletion of either GPR183, or Chol25OHase or Cyp7b1 (enzymes involved in 7α,25-diOHC production) were studied in a diet-induced NASH model. Wild-type mice fed a HFD developed NASH as they displayed increased NAFLD activity score along with fibrosis compared standard diet-fed animals. Interestingly, no effect of either GPR183 deletion, Chol25OHase deletion or Cyp7b1 deletion was observed on these parameters suggesting that GPR183 or the enzymes responsible for the metabolism of its endogenous ligands are involved in NAFLD and NASH pathogenesis in this animal model (Raselli et al., 2019a). Of note, of these three genetic deletions, only $GPR183^{-/-}$ mice displayed increased serum AST and ALT levels compared to wild type animals for mice fed a HFD (Raselli et al., 2019a).

In patients, ERα positive immunostaining in liver biopsies of NASH patients was significantly lower compared to patients with simple steatosis and with healthy subjects. ERα positive

staining was also found to be lower in patients with simple steatosis compared with healthy patients (Erkan et al., 2013). In diet-induced NAFLD, treatment of mice with the selective oestrogen receptor modulator tamoxifene decreased circulating levels of ALT and AST and decreased liver steatosis. Hepatic inflammatory marker (TNFα and MCP-1) expression and levels of endoplasmic reticulum stress markers (Bip and eIF2α) were also significantly reduced in the liver of mice treated with tamoxifen (Miyashita et al., 2012). In a NAFLD mice model, Raloxifene, another selective estrogen receptor modulator, decreased circulating levels of ALT and free fatty acids. Even though this treatment did not change liver steatosis score or liver triglycerides levels, it was able to decrease the lobular inflammation scores, liver fibrosis as well as the expression of IL-1β and IL-6 (Luo et al., 2015). Finally, the interest of hepatic ERα was addressed using liver-specific ERα knockout mice. The mice were fed a HFD and were treated with either estradiol (ER agonist), tamoxifen (selective estrogen receptor modulator) or vehicle. Wild-type mice receiving estradiol or tamoxifen displayed decreased liver steatosis and decreased liver triglyceride and cholesterol ester liver contents. In liver-specific ERα knockout mice, no beneficial effect was observed for these molecules supporting a central role for liver ERα in mediating estradiol and tamoxifen effects in the context of diet induced NAFLD. Interestingly, vehicle treated mice presenting the liver specific deletion of ERα did not display any alteration in triglyceride and cholesterol ester liver contents, compared to wild type animals, showing that liver ERα deletion alone is not sufficient to modify these parameters in the context of diet-induced obesity (Guillaume et al., 2019).

Using mice with a conditional hepatocyte-specific deletion of Smo, Matz-Soja et al. assessed the potential role of this receptor in NAFLD. Such mice developed a liver steatosis under normal diet as well as increased VLDL levels compared to wild type mice. Smoothened hepatocyte-specific deletion also led to a disruption in both expression and levels of key lipogenic transcription factors and enzymes, namely by up-regulating among others

carbohydrate response element binding protein (ChREBP), peroxisome proliferator-activated receptor (PPAR) α, PPARγ or sterol regulatory element-binding protein 1 (SREBP1) (Matz-Soja et al., 2016). Another research group also used liver-specific Smo knockout mice and fed them a HFD. No change was observed for several NAFLD parameters (e.g. liver triglycerides, hepatic enzymes) compared to wild-type animals. However, hepatocyte-specific Smo knockout mice displayed a lower macrophage recruitment in the liver as well as lower pro-inflammatory cytokine and chemokine expression compared to wild-type animals when fed a HFD. The authors also explored the effects of smo pharmacological inhibition in HFD induced NAFLD. The two smo antagonists tested, GDC-0449 or LDE225, were able to decrease hepatic steatosis and circulating cholesterol levels compared to vehicle-treated mice. GDC-0449 was able to decrease circulating triglyceride levels but not liver triglyceride levels and the opposite was true for LDE225. Finally, both smo antagonists inhibited macrophage recruitment and decreased proinflammatory cytokine and chemokine expression in the liver (Kwon et al., 2016).

Type II diabetes

Type II diabetes is a chronic condition characterized by insulin resistance that leads to profound alterations in glucose metabolism. T2D is often associated with overweight or obesity. It has been demonstrated that chronic inflammation plays a central role in T2D onset and in the alteration of insulin secretion.

Type II diabetes patients were shown to have increased plasma and serum levels of 7-KC compared to healthy subjects (Abo et al., 2000; Samadi et al., 2019). Moreover, a difference in plasma 7-KC levels between patients suffering from type I diabetes and type II diabetes was also put forth. This oxysterol levels were increased in diabetic patients of both types compared

to control patients and 7-KC levels were significantly higher in type II diabetes patients compared to patients with type I diabetes. Furthermore, correlations were found between 7-KC levels and glycemia, HbA1c, or total cholesterol levels in both types of diabetes (Samadi et al., 2019). Finally, 7-KC levels in erythrocytes were increased in T2D patients compared to control subjects and were found to be negatively correlated with HDL levels (Abo et al., 2000). Concerning other oxysterols, plasma levels of 7β-OHC, 5α,6α-epoxychol and 5β,6β-epoxychol were found to be increased in T2D patients compared to lean subjects (Ferderbar et al., 2007). In the omental visceral adipose tissue, levels of both 25-OHC and 7α,25-diOHC remained unchanged between obese T2D patients and obese non-diabetic male patients. Despite this absence of variation, Chol25OHase expression was found to be increased in obese T2D patients compared to obese non-diabetic controls (Russo et al., 2020).

In a HFD mouse model, deletion of Chol25OHase led to an improvement in several parameters associated with insulin resistance such as insulin levels, post-prandial glucose levels and in the insulin tolerance test (Russo et al., 2020). In another study, a large cohort of mice was fed a HFD and was further divided between insulin-sensitive or insulin-resistant groups. Hepatic levels of Chol25OHase were decreased in insulin-resistant mice compared to insulin-sensitive animals. Interestingly, adenovirus-mediated hepatic Chol25OHase overexpression resulted in lower glucose and insulin levels during oral glucose and insulin tolerance tests. (Noebauer et al., 2017).

The involvement of LXR was studied in the context of insulin resistance and T2D using different pharmacological tools. In a diet induced obesity model, treatment with GW3965 improved glucose tolerance in mice compared to vehicle-treated HFD fed animals. In this study, the authors dissected further the LXR-dependent mechanisms leading to this beneficial effect. Indeed, they demonstrated that GW3965 treatment inhibited the gluconeogenic response in the liver and induced glucose utilization. Furthermore, the authors evidenced hallmarks of

improved peripheral glucose uptake in the adipose tissue (Laffitte et al., 2003). In ob/ob female mice, treatment with GW3965 also improved insulin tolerance and reduced insulin levels albeit no change in fasted glycaemia were measured in mice (Archer et al., 2013). Interestingly, the LXR inverse agonist SR9238 did not alter insulin or glucose levels in ob/ob mice compared to vehicle-treated ob/ob animals (Griffett et al., 2015).

Treatment of ob/ob mice with the ROR inverse agonist SR1001 improved both glucose and insulin tolerance tests. These beneficial effects were also accompanied by decreased endoplasmic reticulum stress in the epididymal adipose tissue (Liu et al., 2017). Using a diet-induced obesity model, the administration of either SR1555 or SR1903, two RORγ inverse agonists, was shown to reduce fasting insulin levels and to significantly improve both glucose and insulin tolerance tests (Chang et al., 2019; Chang et al., 2015).

In male patients, low testosterone levels alone were not associated with an increased risk of T2D. However, individuals presenting low total or free testosterone and presenting the single nucleotide polymorphism rs2207396 of the ESR1 gene (ERα) displayed an increase of 7.3 or 15.9 times of odds ratio of presenting T2D (Linner et al., 2013). A meta-analysis of genetic studies examining two ERα polymorphisms found that the PvuII polymorphism (intron 1 polymorphism PvuII-rs2234693: T.C) was associated with a decreased risk of T2D in the Chinese population while the XbaI polymorphism (intron 1 polymorphism XbaI-rs9340799: A.G) was linked to a reduction in T2D susceptibility in Caucasians (Yang et al., 2018). Besides these genetic studies implicating ERα and T2D risks, animal studies using pharmacological modulation targeting this receptor were conducted. Using a diet induced obesity model, treatment with either estradiol (an ER agonist), PPT (ERα agonist) or DPN (an ERβ agonist) did not change insulin levels or glucose levels (Tang et al., 2019). However, in a study using Zucker diabetic rats, treatment with estradiol led to a significant decrease in blood glucose at several time points (from 7 to 16 weeks). It also led to a clear improvement in glucose tolerance

and was accompanied by a normalization of β-cells mass and in insulin pancreatic levels (Tiano et al., 2011). In mice fed a standard diet, treatment with estradiol led to a significant decrease in blood glucose levels for both male and female animals. This treatment was also able to improve both glucose and insulin tolerance test for animals of both sexes (Yan et al., 2019). Finally, estradiol or tamoxifen (selective estrogen receptor modulator) administration in a diet-induced obesity mouse model was able to improve glucose tolerance and insulin resistance. However, in liver-specific ERα knockout mice fed a HFD, only estradiol treatment was still able to improve glucose and insulin levels while tamoxifen treatment no longer improved these parameters (Guillaume et al., 2019).

In db/db mice, G31P (antagonist of both CXCR1 and CXCR2) administration was able to reduce fasting insulin levels but not fasting glucose levels. This treatment also significantly improved glucose and insulin tolerance tests. The authors further demonstrated that G31P administration improved hepatic insulin sensitivity through the modulation of gluconeogenesis and the Akt pathway (Cui et al., 2019).

Using a genetic construct leading to the constitutive activation of smoothened in the adipose tissue, mice fed a HFD displayed significant improvements in both glucose and insulin tolerance tests (Shi et al., 2017). The implication of smoothened expressed in myeloid cells was also investigated using specific myeloid smoothened knockout mice fed a HFD. These mice displayed a significant decrease in insulin sensitivity (Braune et al., 2017).

Inflammatory bowel diseases

Crohn's disease (CD) and ulcerative colitis (UC) represent the two major inflammatory bowel diseases. Their etiopathologies are still not fully understood but they are characterized by chronic inflammation and by periods of flares and remissions. In CD, ulcerations can extend

to all layers of the bowel wall and may occur in any part of the gastrointestinal tract. Ulcerations in UC are localised to the colon and rectum.

Oxysterol levels were measured in patients suffering from CD or UC in colon biopsies. Most of the variations were very similar in the two pathologies compared to colon biopsies from non-inflammatory bowel disease subjects. Levels of 7α-OHCnone and 25-OHC were increased in UC and CD patients compared to control while levels of 27-OHC were decreased. 4β-OHC levels were significantly decreased for CD patients only compared to control. More marked differences between these two types of IBD were found in the colon expression of enzymes involved in oxysterol metabolism. While the expression of CYP3A4, CYP3A5 and CYP27A1 were decreased in colon biopsies from both pathologies compared to control, the expression of CYP7A1, CYP8B1 and Chol25OHase were increased only for patients suffering from UC (Guillemot-Legris et al., 2019). Interestingly, in another study focusing on the ileum of CD patients, the expression of Chol25OHase was increased compared to non-IBD patients and further increased in fibrotic ileum tissue from CD patients compared to non-fibrotic ileum tissue from CD patients (Raselli et al., 2019b).

In a dextran sulphate sodium (DSS) mouse model of colitis, oral administration of GW3965 was able to significantly decrease body weight loss. The authors also characterized the recruitment of immune cells in the lamina propria and found that CD11b+ cells as well as $Ly6C/G^{+}/CD11b^{+}$ cells were less recruited when mice were treated with the LXR agonist. Finally, the use of LXR knockout mice allowed to further implicate them in the pathogenesis of the DSS mouse model of colitis as the loss of either LXR isoforms proved to be deleterious in this context (Jakobsson et al., 2014). Another study used three different pharmacological tools in the DSS mouse model: T0901317 (LXRα and β agonist and RORα and γ inverse agonist), DMHCA (LXR agonist) and MePipHCA (LXR agonist). Interestingly, in this study, T0901317 administration exerted no beneficial effects. However, DMHCA and MePipHCA

decreased the expression of IL-1β and MCP-1 in the colon compared to vehicle-treated DSS mice and only MePipHCA was able to significantly reduce the histological score (Yu et al., 2016).

In a model of colon bacterial infection using mice infected with *C. rodentium*, oral GSK805 (RORγ inverse agonist) administration led to a reduction in IL-17A- and IL-22-producing TH17 cells in the lamina propria and mesenteric lymph node compared with vehicle-treated animals. However, GSK805 administration did not alter the number of IL-17A- and IL-22 type 3 innate lymphoid cell (immune cells notably important in the maintenance and protection of the intestinal epithelial barrier) in these two tissues. The authors also tested GSK805 effects in another model of colitis (IL-$10^{-/-}$ mouse) and evidenced a significant reduction in colon shortening, reduced histologic hallmarks of inflammation as well as a reduction in IL-17A- and IL-22-producing TH17 cells in the lamina propria and mesenteric lymph node but not in type 3 innate lymphoid cells (Withers et al., 2016).

In a trinitrobenzene sulfonic acid (TNBS) mouse model of colitis, estradiol administration led to a protection against weight loss and to a reduction of the colon histological inflammatory score. Estradiol treatment also significantly reduced colon levels of several proinflammatory cytokines (Armstrong et al., 2017). In another study, administration of 17β-estradiol significantly reduced both macroscopic and histological scores and MPO activity while 17α-estradiol exerted no such effects (Verdu et al., 2002). Administration of a novel selective estrogen receptor modulator (named SERM2) significantly reduced weight loss, the disease activity index and rectal bleeding compared with vehicle-treated DSS mice. However, this treatment did not alter the expression of pro-inflammatory cytokines or chemokines in the colon or their circulating levels (Polari et al., 2019).

CXCR2 implication in animal models of colitis has been studied through the use of CXCR2$^{-/-}$ mice. Such animals were shown to be protected from DDS-induced colitis as they displayed a reduced weight loss, a significant decrease of the histological score index as well as lower pro-inflammatory cytokines and chemokines in colon and in plasma compared to wild type animals (Ranganathan et al., 2013). In a TNBS colitis mouse model, treatment with SB225002 (CXCR2 antagonist) was shown to rescue weight loss, increase survival, and decrease colon macroscopic damage. SB225002 administration was also able to decrease colon MPO activity and histological damage. Finally, this treatment decreased colon levels of pro-inflammatory cytokines and chemokines and increased anti-inflammatory cytokine levels (i.e. IL-4 and IL-10) (Bento et al., 2008).

Conclusions and perspectives

Oxysterols are a complex yet very interesting family of bioactive lipids. As discussed in this review, they appear to be involved in key inflammatory processes in several pathologies. However, concerning their level quantification, opposite variations are sometimes reported for the same oxysterol in the same pathology. Some potential explanations could reside in differences in the analytical methodology employed or to the fact that the sampling is not always performed at a similar stage of the disease. Besides, for some diseases, it is not always easy to exclude potential confounding factors, such as drugs affecting oxysterol metabolism or the presence of unknown comorbidities. Moreover, in human most of the oxysterol quantification is performed in the blood (plasma or serum) as tissues are never easily accessible. However, oxysterol levels at the site of action (e.g. specific tissue, intracellular,…) could be very different than in the systemic circulation. Indeed, depending on the main site of production, barrier crossing, or local metabolism the local oxysterol content could be higher or lower compared to the one found in the systemic circulation.

As outlined in this review, ascribing an effect to a given oxysterol and a specific receptor is extremely complex. For instance, the use of animal models where one metabolic enzyme has been invalidated will most likely lead to changes in multiple oxysterol levels. If we consider cyp27a1, Cyp27a1$^{-/-}$ mouse could see the levels of 27-OHC, 7α,27-diOHC, 7β,27-diOHC, 7-KC,27-OHC, 24(*S*),27-diOHC altered. Similarly, the genetic invalidation of Cyp7b1 could result in altered levels of 7α,25-diOHC, 7α,27-diOHC or 27-cholestenoic acid. Besides, as multiple enzymes can catalyse similar reactions, enzyme invalidation could result in only mild changes in lipid levels. Case in point, if we consider specifically the Chol25OHase, its invalidation can obviously lead to a decrease in 25-OHC levels but Cyp27a1 has been shown as being able to produce 25-OHC as well which could explain that 25-OHC is still detected in these animals. These difficulties are also true for inhibitors of enzymes involved in oxysterol metabolism such as clotrimazole used in some animal experiments as a Cyp inhibitor.

Moreover, one oxysterol can often act on several receptors therefore increasing the complexity of their study. Furthermore, it is not easy to relate oxysterol levels at the site of action and the affinity of the oxysterol for a given receptor. To date, one of the approaches used to study oxysterol impact in pathophysiological conditions relies mostly on the use of knockout models for an enzyme responsible for its metabolism or on the use of knockout models for one of the oxysterol receptors. This approach is clearly helpful and provides an interesting framework to decipher further the roles of these bioactive lipids. However, the use of such models is not devoid of limitations, for instance compensatory mechanisms could take place, and can only rarely lead to the identification of the oxysterol - receptor(s) pathway(s) at play. A pharmacological approach can clearly help underline the existing pathophysiological mechanisms involving the oxysterome. Using highly selective antagonists/agonists of oxysterols' target receptors allow for pinpointing which one is involved in the pathophysiological process studied. However, identifying the actual oxysterol responsible is

more delicate. One potential way to address this point could be to administer the oxysterol of interest along with a receptor antagonist (or agonist). Thus, the pharmacological profile of the pharmacological tools is of great importance in order to draw the proper conclusions. Case in point, the widely used T0901317 is able to act as a LXR agonist and as a ROR inverse agonist. However, its actions on ROR are very often overlooked despite being almost equipotent on ROR and LXR. In specific settings, the use of both pharmacological tools and oxysterol receptor knockout models are extremely useful as it allows for the unequivocal identification of the specific receptor isoform(s) (LXRα or LXRβ, ERα or ERβ…) involved in the observed effects when the molecule used does not show enough specificity towards one specific isoform.

Hence, in (patho)physiological conditions, identifying which component of the oxysterome is directly involved is no easy task and represents a challenging aspect of future studies. However, by combining different approaches (quantification methods, genetic, and particularly pharmacological approaches) and bearing in mind their respective limitations, the identification of the oxysterol and receptor at play becomes within reach.

The studies we discussed in this review point to interconnections between different pharmacological systems thought to be completely unrelated (for some of them) thanks to the oxysterols. While much remains to be explored within the oxysterome in the context of inflammatory pathologies, one of its receptors seems to be quite promising but remains much less studied. Indeed, GPR17 was shown to be implicated (through knockout models) in inflammatory pathologies such as asthma (Maekawa et al., 2010). However, in order to gain more insights in its function(s), the development of more specific pharmacological tools will be necessary.

In conclusion, it appears clearly that oxysterols, by binding to multiple molecular targets, are important lipid mediators in the context of inflammation and inflammatory diseases. We

discussed the effects of exogenous compounds in the context of diseases characterized by an inflammatory component which allow for a better understanding of the receptors' pathophysiological implications. However, the role(s) of several oxysterols in these pathologies remain(s) to be fully and the receptor(s) mediating their effects remain(s) to be investigated

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

Author contribution

O G-L and GGM both wrote and approved the final version of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

REFERENCES

Abo K, Mio T, & Sumino K (2000). Comparative analysis of plasma and erythrocyte 7-ketocholesterol as a marker for oxidative stress in patients with diabetes mellitus. Clin Biochem 33**:** 541-547.

Ahn SB, Jang K, Jun DW, Lee BH, & Shin KJ (2014). Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease. Dig Dis Sci 59**:** 2975-2982.

Alexander SPH, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, *et al.* (2019). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Introduction and Other Protein Targets. Br J Pharmacol 176 Suppl 1**:** S1-S20.

Archer A, Stolarczyk E, Doria ML, Helguero L, Domingues R, Howard JK, *et al.* (2013). LXR activation by GW3965 alters fat tissue distribution and adipose tissue inflammation in ob/ob female mice. J Lipid Res 54**:** 1300-1311.

Armstrong CM, Allred KF, Weeks BR, Chapkin RS, & Allred CD (2017). Estradiol Has Differential Effects on Acute Colonic Inflammation in the Presence and Absence of Estrogen Receptor beta Expression. Dig Dis Sci 62**:** 1977-1984.

Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, *et al.* (2014). International Union of Basic and Clinical Pharmacology. [corrected]. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors. Pharmacol Rev 66**:** 1-79.

Baron BM, Harrison BL, McDonald IA, Meldrum BS, Palfreyman MG, Salituro FG, *et al.* (1992). Potent indole- and quinoline-containing N-methyl-D-aspartate antagonists acting at the strychnine-insensitive glycine binding site. J Pharmacol Exp Ther 262**:** 947-956.

Bento AF, Leite DF, Claudino RF, Hara DB, Leal PC, & Calixto JB (2008). The selective nonpeptide CXCR2 antagonist SB225002 ameliorates acute experimental colitis in mice. J Leukoc Biol 84**:** 1213-1221.

Berrodin TJ, Shen Q, Quinet EM, Yudt MR, Freedman LP, & Nagpal S (2010). Identification of 5alpha, 6alpha-epoxycholesterol as a novel modulator of liver X receptor activity. Mol Pharmacol 78**:** 1046-1058.

Bertini R, Allegretti M, Bizzarri C, Moriconi A, Locati M, Zampella G, *et al.* (2004). Noncompetitive allosteric inhibitors of the inflammatory chemokine receptors CXCR1 and CXCR2: prevention of reperfusion injury. Proc Natl Acad Sci U S A 101**:** 11791-11796.

Billon C, Sitaula S, & Burris TP (2016). Inhibition of RORalpha/gamma suppresses atherosclerosis via inhibition of both cholesterol absorption and inflammation. Mol Metab 5**:** 997-1005.

Birrell MA, De Alba J, Catley MC, Hardaker E, Wong S, Collins M, *et al.* (2008). Liver X receptor agonists increase airway reactivity in a model of asthma via increasing airway smooth muscle growth. J Immunol 181**:** 4265-4271.

Bitsch F, Aichholz R, Kallen J, Geisse S, Fournier B, & Schlaeppi JM (2003). Identification of natural ligands of retinoic acid receptor-related orphan receptor alpha ligand-binding domain expressed in Sf9 cells--a mass spectrometry approach. Anal Biochem 323**:** 139-149.

Bottemanne P, Paquot A, Ameraoui H, Guillemot-Legris O, Alhouayek M, & Muccioli GG (2021). 25-Hydroxycholesterol metabolism is altered by lung inflammation, and its local administration modulates lung inflammation in mice. FASEB J 35**:** e21514.

Braune J, Weyer U, Matz-Soja M, Hobusch C, Kern M, Kunath A, *et al.* (2017). Hedgehog signalling in myeloid cells impacts on body weight, adipose tissue inflammation and glucose metabolism. Diabetologia 60**:** 889-899.

Cao Q, Li B, Wang X, Sun K, & Guo Y (2018). Therapeutic inhibition of CXC chemokine receptor 2 by SB225002 attenuates LPS-induced acute lung injury in mice. Arch Med Sci 14**:** 635-644.

Carpenter KL, Taylor SE, van der Veen C, Williamson BK, Ballantine JA, & Mitchinson MJ (1995). Lipids and oxidised lipids in human atherosclerotic lesions at different stages of development. Biochim Biophys Acta 1256**:** 141-150.

Chang MR, Ciesla A, Strutzenberg TS, Novick SJ, He Y, Garcia-Ordonez RD, *et al.* (2019). Unique Polypharmacology Nuclear Receptor Modulator Blocks Inflammatory Signaling Pathways. ACS Chem Biol 14**:** 1051-1062.

Chang MR, He Y, Khan TM, Kuruvilla DS, Garcia-Ordonez R, Corzo CA, *et al.* (2015). Antiobesity Effect of a Small Molecule Repressor of RORgamma. Mol Pharmacol 88**:** 48-56.

Ciana P, Fumagalli M, Trincavelli ML, Verderio C, Rosa P, Lecca D, *et al.* (2006). The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. EMBO J 25**:** 4615-4627.

Cleuren AC, Van der Linden IK, De Visser YP, Wagenaar GT, Reitsma PH, & Van Vlijmen BJ (2010). 17alpha-Ethinylestradiol rapidly alters transcript levels of murine coagulation genes via estrogen receptor alpha. J Thromb Haemost 8**:** 1838-1846.

Collins JL, Fivush AM, Watson MA, Galardi CM, Lewis MC, Moore LB, *et al.* (2002). Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. J Med Chem 45**:** 1963-1966.

Crisafulli C, Mazzon E, Paterniti I, Galuppo M, Bramanti P, & Cuzzocrea S (2010). Effects of Liver x receptor agonist treatment on signal transduction pathways in acute lung inflammation. Respir Res 11**:** 19.

Cui S, Qiao L, Yu S, Men L, Li Y, Li F, *et al.* (2019). The antagonist of CXCR1 and CXCR2 protects db/db mice from metabolic diseases through modulating inflammation. Am J Physiol Endocrinol Metab 317**:** E1205-E1217.

Ding H, Li Y, Feng Y, Chen J, Zhong X, Wang N, *et al.* (2016). LXR agonist T0901317 upregulates thrombomodulin expression in glomerular endothelial cells by inhibition of nuclear factorkappaB. Mol Med Rep 13**:** 4888-4896.

Dou F, Chen J, Cao H, Jia Q, Shen D, Liu T, *et al.* (2019). Anti-atherosclerotic effects of LXRalpha agonist through induced conversion of M1 macrophage to M2. Am J Transl Res 11**:** 3825-3840.

Doucet D, Badami C, Palange D, Bonitz RP, Lu Q, Xu DZ, *et al.* (2010). Estrogen receptor hormone agonists limit trauma hemorrhage shock-induced gut and lung injury in rats. PLoS One 5**:** e9421.

DuSell CD, Umetani M, Shaul PW, Mangelsdorf DJ, & McDonnell DP (2008). 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator. Mol Endocrinol 22**:** 65-77.

Dwyer MP, Yu Y, Chao J, Aki C, Chao J, Biju P, *et al.* (2006). Discovery of 2-hydroxy-N,N-dimethyl-3-{2-[[(R)-1-(5- methylfuran-2-yl)propyl]amino]-3,4-dioxocyclobut-1-enylamino}benzamide (SCH 527123): a potent, orally bioavailable CXCR2/CXCR1 receptor antagonist. J Med Chem 49**:** 7603-7606.

Erkan G, Yilmaz G, Konca Degertekin C, Akyol G, & Ozenirler S (2013). Presence and extent of estrogen receptor-alpha expression in patients with simple steatosis and NASH. Pathol Res Pract 209**:** 429-432.

Ferderbar S, Pereira EC, Apolinario E, Bertolami MC, Faludi A, Monte O, *et al.* (2007). Cholesterol oxides as biomarkers of oxidative stress in type 1 and type 2 diabetes mellitus. Diabetes Metab Res Rev 23**:** 35-42.

Fu X, Menke JG, Chen Y, Zhou G, MacNaul KL, Wright SD, *et al.* (2001). 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. J Biol Chem 276**:** 38378-38387.

Gan X, Kaplan R, Menke JG, MacNaul K, Chen Y, Sparrow CP, *et al.* (2001). Dual mechanisms of ABCA1 regulation by geranylgeranyl pyrophosphate. J Biol Chem 276**:** 48702-48708.

Gao M, & Liu D (2013). The liver X receptor agonist T0901317 protects mice from high fat diet-induced obesity and insulin resistance. AAPS J 15**:** 258-266.

Garcia-Cruset S, Carpenter KL, Guardiola F, & Mitchinson MJ (1999). Oxysterols in cap and core of human advanced atherosclerotic lesions. Free Radic Res 30**:** 341-350.

Gessier F, Preuss I, Yin H, Rosenkilde MM, Laurent S, Endres R, *et al.* (2014). Identification and characterization of small molecule modulators of the Epstein-Barr virus-induced gene 2 (EBI2) receptor. J Med Chem 57**:** 3358-3368.

Griffett K, Solt LA, El-Gendy Bel D, Kamenecka TM, & Burris TP (2013). A liver-selective LXR inverse agonist that suppresses hepatic steatosis. ACS Chem Biol 8**:** 559-567.

Griffett K, Welch RD, Flaveny CA, Kolar GR, Neuschwander-Tetri BA, & Burris TP (2015). The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. Mol Metab 4**:** 353-357.

Guillaume M, Riant E, Fabre A, Raymond-Letron I, Buscato M, Davezac M, *et al.* (2019). Selective Liver Estrogen Receptor alpha Modulation Prevents Steatosis, Diabetes, and Obesity Through the Anorectic Growth Differentiation Factor 15 Hepatokine in Mice. Hepatol Commun 3**:** 908-924.

Guillemot-Legris O, Mutemberezi V, Buisseret B, Paquot A, Palmieri V, Bottemanne P, *et al.* (2019). Colitis Alters Oxysterol Metabolism and is Affected by 4beta-Hydroxycholesterol Administration. J Crohns Colitis 13**:** 218-229.

Guillemot-Legris O, Mutemberezi V, & Muccioli GG (2016). Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids. Trends Mol Med 22**:** 594-614.

Guivarc'h E, Buscato M, Guihot AL, Favre J, Vessieres E, Grimaud L, *et al.* (2018). Predominant Role of Nuclear Versus Membrane Estrogen Receptor alpha in Arterial Protection: Implications for Estrogen Receptor alpha Modulation in Cardiovascular Prevention/Safety. J Am Heart Assoc 7.

Han YH, Kim HJ, Kim EJ, Kim KS, Hong S, Park HG, *et al.* (2014). RORalpha decreases oxidative stress through the induction of SOD2 and GPx1 expression and thereby protects against nonalcoholic steatohepatitis in mice. Antioxid Redox Signal 21**:** 2083-2094.

Han YH, Kim HJ, Na H, Nam MW, Kim JY, Kim JS, *et al.* (2017). RORalpha Induces KLF4-Mediated M2 Polarization in the Liver Macrophages that Protect against Nonalcoholic Steatohepatitis. Cell Rep 20**:** 124-135.

Hannedouche S, Zhang J, Yi T, Shen W, Nguyen D, Pereira JP, *et al.* (2011). Oxysterols direct immune cell migration via EBI2. Nature 475**:** 524-527.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S, *et al.* (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucleic Acids Res 46**:** D1091-D1106.

Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, *et al.* (2000). Characterization of the human cysteinyl leukotriene 2 receptor. J Biol Chem 275**:** 30531-30536.

Hennen S, Wang H, Peters L, Merten N, Simon K, Spinrath A, *et al.* (2013). Decoding signaling and function of the orphan G protein-coupled receptor GPR17 with a small-molecule agonist. Sci Signal 6**:** ra93.

Hessvik NP, Bakke SS, Smith R, Ravna AW, Sylte I, Rustan AC, *et al.* (2012). The liver X receptor modulator 22(S)-hydroxycholesterol exerts cell-type specific effects on lipid and glucose metabolism. J Steroid Biochem Mol Biol 128**:** 154-164.

Higham A, Lea S, Plumb J, Maschera B, Simpson K, Ray D, *et al.* (2013). The role of the liver X receptor in chronic obstructive pulmonary disease. Respir Res 14**:** 106.

Hitsumoto T, Takahashi M, Iizuka T, & Shirai K (2009). Clinical significance of serum 7-ketocholesterol concentrations in the progression of coronary atherosclerosis. J Atheroscler Thromb 16**:** 363-370.

Hodgin JB, Krege JH, Reddick RL, Korach KS, Smithies O, & Maeda N (2001). Estrogen receptor alpha is a major mediator of 17beta-estradiol's atheroprotective effects on lesion size in Apoe-/- mice. J Clin Invest 107**:** 333-340.

Hole K, Heiberg PL, Gjestad C, Mehus LL, Ro O, & Molden E (2018). Elevated 4beta-hydroxycholesterol/cholesterol ratio in anorexia nervosa patients. Pharmacol Res Perspect 6**:** e00430.

Honda K, Matoba T, Antoku Y, Koga JI, Ichi I, Nakano K, *et al.* (2018). Lipid-Lowering Therapy With Ezetimibe Decreases Spontaneous Atherothrombotic Occlusions in a Rabbit Model of Plaque Erosion: A Role of Serum Oxysterols. Arterioscler Thromb Vasc Biol 38**:** 757-771.

Hu X, Shen H, Wang Y, & Zhao M (2017). Liver X Receptor Agonist TO901317 Attenuates Paraquat-Induced Acute Lung Injury through Inhibition of NF-kappaB and JNK/p38 MAPK Signal Pathways. Biomed Res Int 2017**:** 4652695.

Jain N, Kanojia RM, Xu J, Jian-Zhong G, Pacia E, Lai MT, *et al.* (2006). Novel chromene-derived selective estrogen receptor modulators useful for alleviating hot flushes and vaginal dryness. J Med Chem 49**:** 3056-3059.

Jakobsson T, Vedin LL, Hassan T, Venteclef N, Greco D, D'Amato M, *et al.* (2014). The oxysterol receptor LXRbeta protects against DSS- and TNBS-induced colitis in mice. Mucosal Immunol 7**:** 1416-1428.

Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, *et al.* (1999). Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. Proc Natl Acad Sci U S A 96**:** 266-271.

Jia J, Conlon TM, Sarker RS, Tasdemir D, Smirnova NF, Srivastava B, *et al.* (2018). Cholesterol metabolism promotes B-cell positioning during immune pathogenesis of chronic obstructive pulmonary disease. EMBO Mol Med 10.

Jin L, Martynowski D, Zheng S, Wada T, Xie W, & Li Y (2010). Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORgamma. Mol Endocrinol 24**:** 923-929.

Kase ET, Andersen B, Nebb HI, Rustan AC, & Thoresen GH (2006). 22-Hydroxycholesterols regulate lipid metabolism differently than T0901317 in human myotubes. Biochim Biophys Acta 1761**:** 1515-1522.

Kikuchi T, Sugiura H, Koarai A, Ichikawa T, Minakata Y, Matsunaga K, *et al.* (2012). Increase of 27-hydroxycholesterol in the airways of patients with COPD: possible role of 27-hydroxycholesterol in tissue fibrosis. Chest 142**:** 329-337.

Kim EJ, Yoon YS, Hong S, Son HY, Na TY, Lee MH, *et al.* (2012). Retinoic acid receptor-related orphan receptor alpha-induced activation of adenosine monophosphate-activated protein kinase results in attenuation of hepatic steatosis. Hepatology 55**:** 1379-1388.

Kirsten AM, Forster K, Radeczky E, Linnhoff A, Balint B, Watz H, *et al.* (2015). The safety and tolerability of oral AZD5069, a selective CXCR2 antagonist, in patients with moderate-to-severe COPD. Pulm Pharmacol Ther 31**:** 36-41.

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, *et al.* (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138**:** 863-870.

Kumar N, Solt LA, Conkright JJ, Wang Y, Istrate MA, Busby SA, *et al.* (2010). The benzenesulfoamide T0901317 [N-(2,2,2-trifluoroethyl)-N-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethy l]phenyl]-benzenesulfonamide] is a novel retinoic acid receptor-related orphan receptor-alpha/gamma inverse agonist. Mol Pharmacol 77**:** 228-236.

Kwon H, Song K, Han C, Chen W, Wang Y, Dash S, *et al.* (2016). Inhibition of hedgehog signaling ameliorates hepatic inflammation in mice with nonalcoholic fatty liver disease. Hepatology 63**:** 1155-1169.

Laffitte BA, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, *et al.* (2003). Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A 100**:** 5419-5424.

Lappano R, Recchia AG, De Francesco EM, Angelone T, Cerra MC, Picard D, *et al.* (2011). The cholesterol metabolite 25-hydroxycholesterol activates estrogen receptor alpha-mediated signaling in cancer cells and in cardiomyocytes. PLoS One 6**:** e16631.

Lau P, Fitzsimmons RL, Raichur S, Wang SC, Lechtken A, & Muscat GE (2008). The orphan nuclear receptor, RORalpha, regulates gene expression that controls lipid metabolism: staggerer (SG/SG) mice are resistant to diet-induced obesity. J Biol Chem 283**:** 18411-18421.

Lazaar AL, Sweeney LE, MacDonald AJ, Alexis NE, Chen C, & Tal-Singer R (2011). SB-656933, a novel CXCR2 selective antagonist, inhibits ex vivo neutrophil activation and ozone-induced airway inflammation in humans. Br J Clin Pharmacol 72**:** 282-293.

Leaker BR, Barnes PJ, & O'Connor B (2013). Inhibition of LPS-induced airway neutrophilic inflammation in healthy volunteers with an oral CXCR2 antagonist. Respir Res 14**:** 137.

Li F, Zhang X, & Gordon JR (2002). CXCL8((3-73))K11R/G31P antagonizes ligand binding to the neutrophil CXCR1 and CXCR2 receptors and cellular responses to CXCL8/IL-8. Biochem Biophys Res Commun 293**:** 939-944.

Linner C, Svartberg J, Giwercman A, & Giwercman YL (2013). Estrogen receptor alpha single nucleotide polymorphism as predictor of diabetes type 2 risk in hypogonadal men. Aging Male 16**:** 52-57.

Liu C, Yang XV, Wu J, Kuei C, Mani NS, Zhang L, *et al.* (2011). Oxysterols direct B-cell migration through EBI2. Nature 475**:** 519-523.

Liu Y, Chen Y, Zhang J, Liu Y, Zhang Y, & Su Z (2017). Retinoic acid receptor-related orphan receptor alpha stimulates adipose tissue inflammation by modulating endoplasmic reticulum stress. J Biol Chem 292**:** 13959-13969.

Luo F, Ishigami M, Achiwa K, Ishizu Y, Kuzuya T, Honda T, *et al.* (2015). Raloxifene Ameliorates Liver Fibrosis of Nonalcoholic Steatohepatitis Induced by Choline-Deficient High-Fat Diet in Ovariectomized Mice. Dig Dis Sci 60**:** 2730-2739.

Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, *et al.* (1999). Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 399**:** 789-793.

Ma Y, Xu L, Rodriguez-Agudo D, Li X, Heuman DM, Hylemon PB, *et al.* (2008). 25-Hydroxycholesterol-3-sulfate regulates macrophage lipid metabolism via the LXR/SREBP-1 signaling pathway. Am J Physiol Endocrinol Metab 295**:** E1369-1379.

Maekawa A, Xing W, Austen KF, & Kanaoka Y (2010). GPR17 regulates immune pulmonary inflammation induced by house dust mites. J Immunol 185**:** 1846-1854.

Malamas MS, Manas ES, McDevitt RE, Gunawan I, Xu ZB, Collini MD, *et al.* (2004). Design and synthesis of aryl diphenolic azoles as potent and selective estrogen receptor-beta ligands. J Med Chem 47**:** 5021-5040.

Matz-Soja M, Rennert C, Schonefeld K, Aleithe S, Boettger J, Schmidt-Heck W, *et al.* (2016). Hedgehog signaling is a potent regulator of liver lipid metabolism and reveals a GLI-code associated with steatosis. Elife 5.

Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, & Katzenellenbogen JA (2001). Estrogen receptor-beta potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. J Med Chem 44**:** 4230-4251.

Mihara K, Spansier M, Rooseboom M, Smit MJ, & Dokter W (2007). Functional replacement of murine CXCR2 by its human homologue in the development of atherosclerosis in LDLR knockout mice. Biol Pharm Bull 30**:** 1231-1236.

Miyashita T, Toyoda Y, Tsuneyama K, Fukami T, Nakajima M, & Yokoi T (2012). Hepatoprotective effect of tamoxifen on steatosis and non-alcoholic steatohepatitis in mouse models. J Toxicol Sci 37**:** 931-942.

Mutemberezi V, Guillemot-Legris O, & Muccioli GG (2016). Oxysterols: From cholesterol metabolites to key mediators. Prog Lipid Res 64**:** 152-169.

Nachtergaele S, Mydock LK, Krishnan K, Rammohan J, Schlesinger PH, Covey DF, *et al.* (2012). Oxysterols are allosteric activators of the oncoprotein Smoothened. Nat Chem Biol 8**:** 211-220.

Nair P, Gaga M, Zervas E, Alagha K, Hargreave FE, O'Byrne PM, *et al.* (2012). Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. Clin Exp Allergy 42**:** 1097-1103.

Nicholls DJ, Wiley K, Dainty I, MacIntosh F, Phillips C, Gaw A, *et al.* (2015). Pharmacological characterization of AZD5069, a slowly reversible CXC chemokine receptor 2 antagonist. J Pharmacol Exp Ther 353**:** 340-350.

Ning Y, Kim JK, Min HK, & Ren S (2017). Cholesterol metabolites alleviate injured liver function and decrease mortality in an LPS-induced mouse model. Metabolism 71**:** 83-93.

Noebauer B, Jais A, Todoric J, Gossens K, Sutterluty-Fall H, & Einwallner E (2017). Hepatic Cholesterol-25-Hydroxylase Overexpression Improves Systemic Insulin Sensitivity in Mice. J Diabetes Res 2017**:** 4108768.

Nury T, Samadi M, Varin A, Lopez T, Zarrouk A, Boumhras M, *et al.* (2013). Biological activities of the LXRalpha and beta agonist, 4beta-hydroxycholesterol, and of its isomer, 4alpha-hydroxycholesterol, on oligodendrocytes: effects on cell growth and viability, oxidative and inflammatory status. Biochimie 95**:** 518-530.

O'Byrne PM, Metev H, Puu M, Richter K, Keen C, Uddin M, *et al.* (2016). Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 4**:** 797-806.

Ouyang W, Zhou H, Liu C, Wang S, Han Y, Xia J, *et al.* (2018). 25-Hydroxycholesterol protects against acute lung injury via targeting MD-2. J Cell Mol Med 22**:** 5494-5503.

Paul SM, Doherty JJ, Robichaud AJ, Belfort GM, Chow BY, Hammond RS, *et al.* (2013). The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. J Neurosci 33**:** 17290-17300.

Peng D, Hiipakka RA, Reardon CA, Getz GS, & Liao S (2009). Differential anti-atherosclerotic effects in the innominate artery and aortic sinus by the liver X receptor agonist T0901317. Atherosclerosis 203**:** 59-66.

Podolin PL, Bolognese BJ, Foley JJ, Schmidt DB, Buckley PT, Widdowson KL, *et al.* (2002). A potent and selective nonpeptide antagonist of CXCR2 inhibits acute and chronic models of arthritis in the rabbit. J Immunol 169**:** 6435-6444.

Polari L, Anttila S, Helenius T, Wiklund A, Linnanen T, Toivola DM, *et al.* (2019). Novel Selective Estrogen Receptor Modulator Ameliorates Murine Colitis. Int J Mol Sci 20.

Raccosta L, Fontana R, Maggioni D, Lanterna C, Villablanca EJ, Paniccia A, *et al.* (2013). The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. J Exp Med 210**:** 1711-1728.

Ranganathan P, Jayakumar C, Manicassamy S, & Ramesh G (2013). CXCR2 knockout mice are protected against DSS-colitis-induced acute kidney injury and inflammation. Am J Physiol Renal Physiol 305**:** F1422-1427.

Raselli T, Hearn T, Wyss A, Atrott K, Peter A, Frey-Wagner I, *et al.* (2019a). Elevated oxysterol levels in human and mouse livers reflect nonalcoholic steatohepatitis. J Lipid Res 60**:** 1270-1283.

Raselli T, Wyss A, Gonzalez Alvarado MN, Weder B, Mamie C, Spalinger MR, *et al.* (2019b). The Oxysterol Synthesising Enzyme CH25H Contributes to the Development of Intestinal Fibrosis. J Crohns Colitis 13**:** 1186-1200.

Revankar CM, Cimino DF, Sklar LA, Arterburn JB, & Prossnitz ER (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307**:** 1625-1630.

Rimner A, Al Makdessi S, Sweidan H, Wischhusen J, Rabenstein B, Shatat K, *et al.* (2005). Relevance and mechanism of oxysterol stereospecifity in coronary artery disease. Free Radic Biol Med 38**:** 535-544.

Rossouw JE, Prentice RL, Manson JE, Aragaki AK, Hsia J, Martin LW, *et al.* (2012). Relationships of coronary heart disease with 27-hydroxycholesterol, low-density lipoprotein cholesterol, and menopausal hormone therapy. Circulation 126**:** 1577-1586.

Russo L, Muir L, Geletka L, Delproposto J, Baker N, Flesher C, *et al.* (2020). Cholesterol 25-Hydroxylase (CH25H) as a promoter of adipose tissue inflammation in obesity and diabetes. Mol Metab**:** 100983.

Samadi A, Gurlek A, Sendur SN, Karahan S, Akbiyik F, & Lay I (2019). Oxysterol species: reliable markers of oxidative stress in diabetes mellitus. J Endocrinol Invest 42**:** 7-17.

Schmidt JM, Mercure J, Tremblay GB, Page M, Kalbakji A, Feher M, *et al.* (2003). De novo design, synthesis, and evaluation of novel nonsteroidal phenanthrene ligands for the estrogen receptor. J Med Chem 46**:** 1408-1418.

Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, *et al.* (2000). Role of LXRs in control of lipogenesis. Genes Dev 14**:** 2831-2838.

Selley ML, McGuiness JA, & Ardlie NG (1996). The effect of cholesterol oxidation products on human platelet aggregation. Thromb Res 83**:** 449-461.

Sensi C, Daniele S, Parravicini C, Zappelli E, Russo V, Trincavelli ML, *et al.* (2014). Oxysterols act as promiscuous ligands of class-A GPCRs: in silico molecular modeling and in vitro validation. Cell Signal 26**:** 2614-2620.

Shen ZJ, Hu J, Kashi VP, Kelly EA, Denlinger LC, Lutchman K, *et al.* (2017). Epstein-Barr Virus-induced Gene 2 Mediates Allergen-induced Leukocyte Migration into Airways. Am J Respir Crit Care Med 195**:** 1576-1585.

Shi Y, & Long F (2017). Hedgehog signaling via Gli2 prevents obesity induced by high-fat diet in adult mice. Elife 6.

Shi Y, Xu X, Tan Y, Mao S, Fang S, & Gu W (2014). A liver-X-receptor ligand, T0901317, attenuates IgE production and airway remodeling in chronic asthma model of mice. PLoS One 9**:** e92668.

Smet M, Van Hoecke L, De Beuckelaer A, Vander Beken S, Naessens T, Vergote K, *et al.* (2016). Cholesterol-sensing liver X receptors stimulate Th2-driven allergic eosinophilic asthma in mice. Immun Inflamm Dis 4**:** 350-361.

Solt LA, Kumar N, He Y, Kamenecka TM, Griffin PR, & Burris TP (2012). Identification of a selective RORgamma ligand that suppresses T(H)17 cells and stimulates T regulatory cells. ACS Chem Biol 7**:** 1515-1519.

Solt LA, Kumar N, Nuhant P, Wang Y, Lauer JL, Liu J, *et al.* (2011). Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. Nature 472**:** 491-494.

Song J, Wang D, Chen H, Huang X, Zhong Y, Jiang N, *et al.* (2017). Association of Plasma 7-Ketocholesterol With Cardiovascular Outcomes and Total Mortality in Patients With Coronary Artery Disease. Circ Res 120**:** 1622-1631.

Soroosh P, Wu J, Xue X, Song J, Sutton SW, Sablad M, *et al.* (2014). Oxysterols are agonist ligands of RORgammat and drive Th17 cell differentiation. Proc Natl Acad Sci U S A 111**:** 12163-12168.

Spyridon M, Moraes LA, Jones CI, Sage T, Sasikumar P, Bucci G, *et al.* (2011). LXR as a novel antithrombotic target. Blood 117**:** 5751-5761.

Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, *et al.* (2000). Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. J Med Chem 43**:** 4934-4947.

Sugiura H, Koarai A, Ichikawa T, Minakata Y, Matsunaga K, Hirano T, *et al.* (2012). Increased 25-hydroxycholesterol concentrations in the lungs of patients with chronic obstructive pulmonary disease. Respirology 17**:** 533-540.

Tang SS, Ren Y, Ren XQ, Cao JR, Hong H, Ji H, *et al.* (2019). ERalpha and/or ERbeta activation ameliorates cognitive impairment, neurogenesis and apoptosis in type 2 diabetes mellitus mice. Exp Neurol 311**:** 33-43.

Thomas P, Pang Y, Filardo EJ, & Dong J (2005). Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146**:** 624-632.

Tiano JP, Delghingaro-Augusto V, Le May C, Liu S, Kaw MK, Khuder SS, *et al.* (2011). Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents beta cell failure in rodent models of type 2 diabetes. J Clin Invest 121**:** 3331-3342.

Tranheim Kase E, Nikolic N, Pettersen Hessvik N, Fjeldheim AK, Jensen J, Thoresen GH, *et al.* (2012). Dietary supplementation with 22-S-hydroxycholesterol to rats reduces body weight gain and the accumulation of liver triacylglycerol. Lipids 47**:** 483-493.

Tremblay-Franco M, Zerbinati C, Pacelli A, Palmaccio G, Lubrano C, Ducheix S, *et al.* (2015). Effect of obesity and metabolic syndrome on plasma oxysterols and fatty acids in human. Steroids 99**:** 287-292.

Umetani M, Ghosh P, Ishikawa T, Umetani J, Ahmed M, Mineo C, *et al.* (2014). The cholesterol metabolite 27-hydroxycholesterol promotes atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha. Cell Metab 20**:** 172-182.

Valera MC, Fontaine C, Lenfant F, Cabou C, Guillaume M, Smirnova N, *et al.* (2015). Protective Hematopoietic Effect of Estrogens in a Mouse Model of Thrombosis: Respective Roles of Nuclear Versus Membrane Estrogen Receptor alpha. Endocrinology 156**:** 4293-4301.

Valera MC, Noirrit-Esclassan E, Dupuis M, Fontaine C, Lenfant F, Briaux A, *et al.* (2018). Effect of estetrol, a selective nuclear estrogen receptor modulator, in mouse models of arterial and venous thrombosis. Mol Cell Endocrinol 477**:** 132-139.

van der Hoorn J, Linden D, Lindahl U, Bekkers M, Voskuilen M, Nilsson R, *et al.* (2011). Low dose of the liver X receptor agonist, AZ876, reduces atherosclerosis in APOE*3Leiden mice without affecting liver or plasma triglyceride levels. Br J Pharmacol 162**:** 1553-1563.

Vaya J, Aviram M, Mahmood S, Hayek T, Grenadir E, Hoffman A, *et al.* (2001). Selective distribution of oxysterols in atherosclerotic lesions and human plasma lipoproteins. Free Radic Res 34**:** 485-497.

Verdu EF, Deng Y, Bercik P, & Collins SM (2002). Modulatory effects of estrogen in two murine models of experimental colitis. Am J Physiol Gastrointest Liver Physiol 283**:** G27-36.

Voisin M, de Medina P, Mallinger A, Dalenc F, Huc-Claustre E, Leignadier J, *et al.* (2017). Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor. Proc Natl Acad Sci U S A 114**:** E9346-E9355.

Wang D, Liu M, Wang Y, Luo M, Wang J, Dai C, *et al.* (2011). Synthetic LXR agonist T0901317 attenuates lipopolysaccharide-induced acute lung injury in rats. Int Immunopharmacol 11**:** 2098-2103.

Wang J, Mook RA, Jr., Lu J, Gooden DM, Ribeiro A, Guo A, *et al.* (2012). Identification of a novel Smoothened antagonist that potently suppresses Hedgehog signaling. Bioorg Med Chem 20**:** 6751-6757.

Wang Y, Cai W, Cheng Y, Yang T, Liu Q, Zhang G, *et al.* (2015). Discovery of Biaryl Amides as Potent, Orally Bioavailable, and CNS Penetrant RORgammat Inhibitors. ACS Med Chem Lett 6**:** 787-792.

Wang Y, Kumar N, Crumbley C, Griffin PR, & Burris TP (2010a). A second class of nuclear receptors for oxysterols: Regulation of RORalpha and RORgamma activity by 24S-hydroxycholesterol (cerebrosterol). Biochim Biophys Acta 1801**:** 917-923.

Wang Y, Kumar N, Nuhant P, Cameron MD, Istrate MA, Roush WR, *et al.* (2010b). Identification of SR1078, a synthetic agonist for the orphan nuclear receptors RORalpha and RORgamma. ACS Chem Biol 5**:** 1029-1034.

Wang Y, Kumar N, Solt LA, Richardson TI, Helvering LM, Crumbley C, *et al.* (2010c). Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands. J Biol Chem 285**:** 5013-5025.

White JR, Lee JM, Young PR, Hertzberg RP, Jurewicz AJ, Chaikin MA, *et al.* (1998). Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. J Biol Chem 273**:** 10095-10098.

Withers DR, Hepworth MR, Wang X, Mackley EC, Halford EE, Dutton EE, *et al.* (2016). Transient inhibition of ROR-gammat therapeutically limits intestinal inflammation by reducing TH17 cells and preserving group 3 innate lymphoid cells. Nat Med 22**:** 319-323.

Wu D, Nishimura N, Kuo V, Fiehn O, Shahbaz S, Van Winkle L, *et al.* (2011). Activation of aryl hydrocarbon receptor induces vascular inflammation and promotes atherosclerosis in apolipoprotein E-/- mice. Arterioscler Thromb Vasc Biol 31**:** 1260-1267.

Xu H, Lu H, Xu Z, Luan L, Li C, Xu Y, *et al.* (2016). Discovery of CNS Penetrant CXCR2 Antagonists for the Potential Treatment of CNS Demyelinating Disorders. ACS Med Chem Lett 7**:** 397-402.

Yan H, Yang W, Zhou F, Li X, Pan Q, Shen Z, *et al.* (2019). Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1. Diabetes 68**:** 291-304.

Yang J, Han R, Chen M, Yuan Y, Hu X, Ma Y, *et al.* (2018). Associations of Estrogen Receptor Alpha Gene Polymorphisms with Type 2 Diabetes Mellitus and Metabolic Syndrome: A Systematic Review and Meta-Analysis. Horm Metab Res 50**:** 469-477.

Yasunobu Y, Hayashi K, Shingu T, Yamagata T, Kajiyama G, & Kambe M (2001). Coronary atherosclerosis and oxidative stress as reflected by autoantibodies against oxidized low-density lipoprotein and oxysterols. Atherosclerosis 155**:** 445-453.

Yu S, Li S, Henke A, Muse ED, Cheng B, Welzel G, *et al.* (2016). Dissociated sterol-based liver X receptor agonists as therapeutics for chronic inflammatory diseases. FASEB J 30**:** 2570-2579.

Zarbock A, Allegretti M, & Ley K (2008). Therapeutic inhibition of CXCR2 by Reparixin attenuates acute lung injury in mice. Br J Pharmacol 155**:** 357-364.

Zhao Z, Xu D, Li S, He B, Huang Y, Xu M, *et al.* (2016). Activation of Liver X Receptor Attenuates Oleic Acid-Induced Acute Respiratory Distress Syndrome. Am J Pathol 186**:** 2614-2622.

Zhou Q, Wasowicz E, Handler B, Fleischer L, & Kummerow FA (2000). An excess concentration of oxysterols in the plasma is cytotoxic to cultured endothelial cells. Atherosclerosis 149**:** 191-197.

Zhu BT, Han GZ, Shim JY, Wen Y, & Jiang XR (2006). Quantitative structure-activity relationship of various endogenous estrogen metabolites for human estrogen receptor alpha and beta subtypes: Insights into the structural determinants favoring a differential subtype binding. Endocrinology 147**:** 4132-4150.

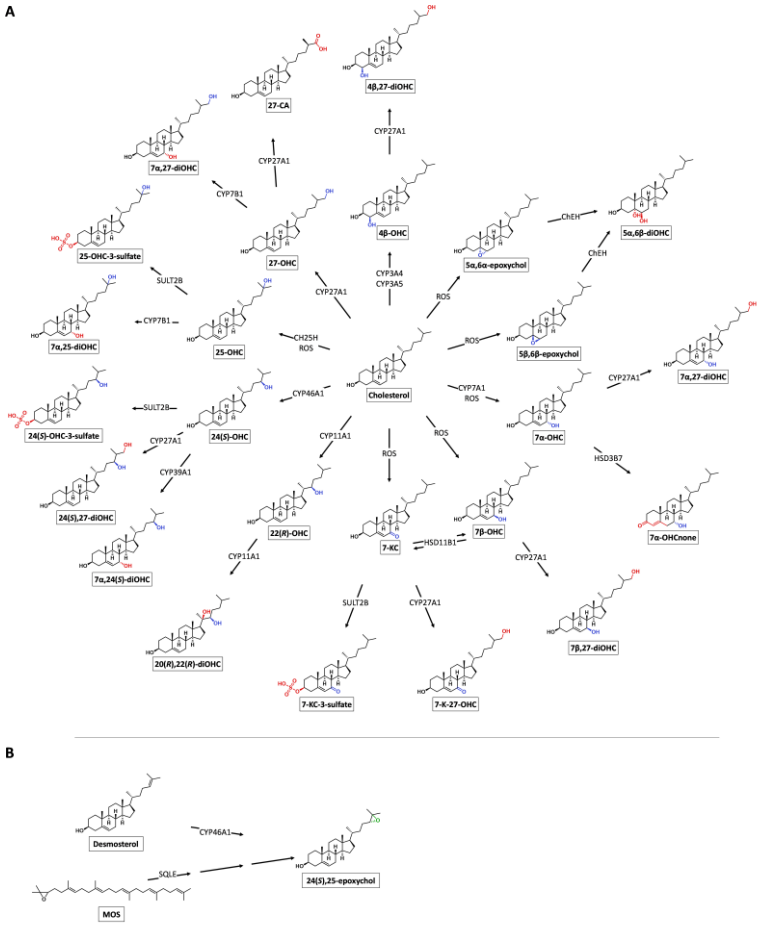

Figure 1. Simplified overview of oxysterol metabolism.
Oxysterol can be formed from cholesterol or from cholesterol precursors such as desmosterol or 2,3(*S*)-monooxidosqualene (MOS). Oxysterol metabolism relies on enzymatic reactions catalysed by cytochromes P450 (CYP), cholesterol 25-hydroxylase (CH25H), squalene epoxidase (SQLE), hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7 (HSD3B7), hydroxysteroid 11-beta dehydrogenase 1 (HSD11B1), cholesterol epoxide hydrolase (ChEH) and on chemical reactions mediated by reactive oxygen species (ROS). Sulfation reactions are mediated mainly by enzymes from the sulfotransferase 2B family (SULT2B). For oxysterol deriving directly from cholesterol, the result of the oxidation process is indicated in blue. These oxysterols can be further oxidized and the new chemical function is indicated in red. Most of the enzymes and reactions depicted are similar between human and rodent. However, human CYP3A4 and CYP3A5 do not exist in mice but the same reaction can be catalysed by their orthologues Cyp3a11 and Cyp3a13 respectively. 7α,27-diOHC is shown twice as it can be formed from two distinct pathways.
4β-OHC: 4β-hydroxycholesterol; 5α,6α-epoxychol: 5α,6α-epoxycholesterol; 5β,6β-epoxychol: 5β,6β-epoxycholesterol; 7α-OHC: 7α-hydroxycholesterol; 7β-OHC: 7β-hydroxycholesterol; 7-KC: 7-ketocholesterol; 22(*R*)-OHC: 22(*R*)-hydroxycholesterol; 24(*S*)-OHC: 24(*S*)-hydroxycholesterol; 25-OHC: 25-hydroxycholesterol; 27-OHC: 27-hydroxycholesterol; 4β,27-diOHC: 4β,27-dihydroxycholesterol; 5α,6β-diOHC: 5α,6β-dihydroxycholesterol; 7α,27-diOHC: 7α,27-dihydroxycholesterol; 7α-OHCnone: 7α-hydroxy-4-cholesten-3-one; 7β,27-diOHC: 7β,27-dihydroxycholesterol; 7-K-27-OHC: 7-keto-27-hydroxycholesterol; 7-KC-3-sulfate: 7-ketocholesterol-3-sulfate; 7α,24(*S*)-diOHC: 7α,24(S)-dihydroxycholesterol; 24(*S*),27-diOHC: 24(*S*),27-dihydroxycholesterol; 24(*S*)-OHC-3-sulfate: 24(*S*)-hydroxycholesterol-3-sulfate; 7α,25-diOHC: 7α,25-dihydroxycholesterol; 25-OHC-3-sulfate: 25-hydroxycholesterol-3-sulfate; 27-CA: 3β-hydroxy-(25R)-cholest-5-en-27-oic acid

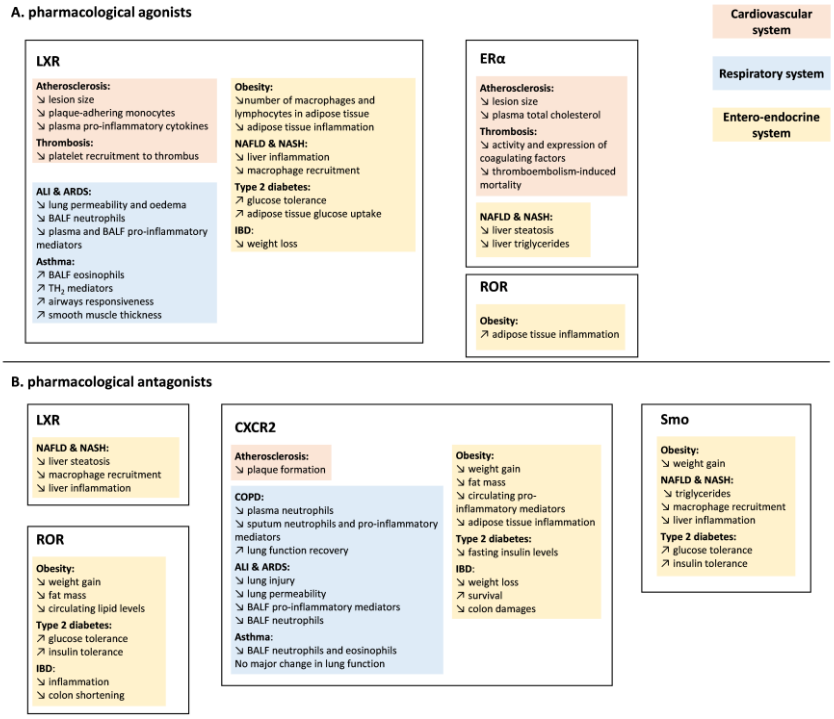

Figure 2. Oxysterol receptor pharmacomodulations in inflammatory diseases

Effects of pharmacological (**A**) agonists or (**B**) antagonists on different oxysterol receptors in inflammatory diseases of the cardiovascular system (salmon), the respiratory system (yellow), and the entero-endocrine system (green). Despite being involved in immune and inflammatory processes, the GPR183 receptor is not reviewed here as no pharmacological tools has been fully characterized yet.

ALI: acute lung injury; ARDS: acute respiratory distress syndrome; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; IBD: inflammatory bowel disease; BALF: bronchoalveolar lavage fluid; LXR: liver X receptor; ROR: Retinoic acid receptor-related orphan receptor; Erα: estrogen receptor α; CXCR2: CXC chemokine receptor 2; GPR17: G protein-coupled receptor 17; Smo: smoothened

Table 1: Oxysterols and their receptors. This table presents the main endogenous oxysterols and their receptors. The pharmacological parameters are presented as pKi (i.e. -log Ki), pEC_{50} (i.e. -log EC_{50}), or pIC_{50} (i.e. -log IC_{50}) depending on the data reported in the literature. ND: not determined; SERM: selective estrogen receptor modulator

Oxysterol		Target	Functionality	Pharmacological parameters	References
4β-hydroxycholesterol	4β-OHC	LXRα	agonist	ND	(Nury et al., 2013)
		LXRβ	agonist	ND	(Nury et al., 2013)
5α,6α-epoxycholesterol	5α,6α-epoxychol	LXRα	antagonist	pIC_{50}=7.1 pEC_{50}=5.8	(Berrodin et al., 2010)
		LXRβ	/		(Berrodin et al., 2010)
6-oxo-cholestan-3β,5α-diol	OCDO	GR	ligand *	pKi=6.5	(Voisin et al., 2017)
		LXRα	ligand	pK_D=4.1	
		LXRβ	ligand	pK_D=4.9	
7α-hydroxycholesterol	7α-OHC	GPR17	agonist	pIC_{50}=9.1 pEC_{50}=9.2	(Sensi et al., 2014)
		GPR183	agonist	pIC_{50}=5.9	(Liu et al., 2011)
				pIC_{50}=5.5	(Hannedouche et al., 2011)
		LXRα	/	pKi <5	(Janowski et al., 1999)
		LXRβ	/	pKi<5	(Janowski et al., 1999)
		RORα	inverse agonist	pIC_{50}=5.9	(Wang et al., 2010c)
		RORγ	inverse agonist	pKi ~ 7.6 $pEC_{\beta 0}$=5.8	(Wang et al., 2010c)
7β-hydroxycholesterol	7β-OHC	RORα	inverse agonist	pKi ~ 7.7 pEC_{50}=6.2	(Wang et al., 2010c)
		RORγ	inverse agonist	pKi ~ 7.6 pEC_{50}=5.7	(Wang et al., 2010c)
7-ketocholesterol	7-KC	LXRα	/	pKi<5.3	(Janowski et al., 1999)
		LXRβ	/	pKi<5.3	(Janowski et al., 1999)

		RORα	inverse agonist	pKi ~ 7.7 pEC_{50}=5.9	(Wang et al., 2010c)
		RORγ	inverse agonist	pKi ~ 7.6 pEC_{50}=6.2	(Wang et al., 2010c)
22(*R*)-hydroxycholesterol	22(*R*)-OHC	CXCR2	agonist	pEC_{50}=5.9	(Raccosta et al., 2013)
		GPR17	agonist	pIC_{50}=9.0 pEC_{50}=9.7	(Sensi et al., 2014)
		LXRα	agonist	pKi=6.4	(Janowski et al., 1999)
				pEC_{50}=5.4-5.6	(Berrodin et al., 2010)
		LXRβ	agonist	pKi=6.9	(Janowski et al., 1999)
				pEC_{50}=5.9-6.2	(Berrodin et al., 2010)
		RORγ	agonist	pEC_{50}=7.4-7.7	(Jin et al., 2010)
24(*S*)-hydroxycholesterol	24(*S*)-OHC	LXRα	agonist	pKi=7.0 pEC_{50}=5.4	(Janowski et al., 1999)
		LXRβ	agonist	pKi=7 pEC_{50}=5.5	(Janowski et al., 1999)
		NMDAR	allosteric modulator		(Paul et al., 2013)
		RORα	inverse agonist	pKi=7.6 pEC_{50}=6.2	(Wang et al., 2010a)
		RORγ	inverse agonist	pKi=7.6 pEC_{50}=5.9	(Wang et al., 2010a)
24(*S*),25-epoxycholesterol	24(*S*),25-epoxychol	LXRα	agonist	pKi=6.4-6.7	(Janowski et al., 1999)
				pEC_{50}=5.4-6.1	(Berrodin et al., 2010)
		LXRβ	agonist	pKi=6.4-6.7	(Janowski et al., 1999)
				pEC_{50}=5.5-6.1	(Berrodin et al., 2010)
		RORγ	agonist	pEC_{50}=5.8	(Soroosh et al., 2014)
			Inverse agonist	pKi=7.7 pEC_{50}=6.6	(Wang et al., 2010a)
25-hydroxycholesterol	25-OHC	Erα	agonist	pIC_{50} ~6.8	(Lappano et al., 2011)

		GPR183	agonist	pIC_{50}=5.9	(Liu et al., 2011)
				pIC_{50}<5	(Hannedouche et al., 2011)
		LXRα	agonist	pKi=6.7	(Janowski et al., 1999)
				pEC_{50}=4.7-5.2	(Berrodin et al., 2010)
		LXRβ	agonist	pKi=6.5	(Janowski et al., 1999)
				pEC_{50}=5.5	(Berrodin et al., 2010)
		RORα	ligand	pKd=8.5	(Kumar et al., 2010)
		RORγ	agonist	pKi=6.6	(Soroosh et al., 2014)
				pEC_{50}=6.0-7.7	(Jin et al., 2010)
		Smoothened	allosteric modulator	ND	(Nachtergaele et al., 2012)
25-hydroxycholesterol-3-sulfate	25-OHC-3-sulfate	LXRα	antagonist	ND	(Ma et al., 2008)
		LXRβ	antagonist	ND	(Ma et al., 2008)
27-hydroxycholesterol	27-OHC	Erα	SERM	pKi=5.9	(DuSell et al., 2008)
		GPR17	agonist	pIC_{50}=9.2 pEC_{50}=8.3	(Sensi et al., 2014)
		LXRα	partial agonist	pEC_{50}=7.0	(Fu et al., 2001)
		LXRβ	partial agonist	pEC_{50}=7.2	(Fu et al., 2001)
		RORγ	agonist	pKd= 7.1 pEC_{50}=6.1	(Soroosh et al., 2014)
7α,25-dihydroxycholesterol	7α,25-diOHC	GPR183	agonist	pIC_{50}=9.6	(Liu et al., 2011)
				pIC_{50}=7.2	(Hannedouche et al., 2011)
		RORγ	/	pKd= 6.5	(Soroosh et al., 2014)
7α,27-dihydroxycholesterol	7α,27-diOHC	GPR183	agonist	pIC_{50}=9.1	(Liu et al., 2011)
				pIC_{50}=6.4	(Hannedouche et al., 2011)
		RORγ	agonist	pKd= 6.34	(Soroosh et al., 2014)

				pEC_{50}=6.1	
7β,25-dihydroxycholesterol	7β,25-diOHC	GPR183	agonist	pIC_{50}=8.4	(Liu et al., 2011)
				pIC_{50}=5.6	(Hannedouche et al., 2011)
7β,27-dihydroxycholesterol	7β,27-diOHC	GPR183	agonist	pIC_{50}=6.5	(Liu et al., 2011)
				pIC_{50}<5	(Hannedouche et al., 2011)
		RORγ	agonist	pKd= 7.6 pEC_{50}=6.2	(Soroosh et al., 2014)
7-keto,27-hydroxycholesterol	7-keto,27-OHC	RORγ	**	pKd= 7.1	(Soroosh et al., 2014)

* OCDO and glucocorticoids act through GR but have different mechanisms of action on GR transcriptional targets (Voisin et al., 2017) ** The functional activity is unclear as different results are obtained depending on the construct used to measure RORγ activity (LBD vs full length ROR γ) (Soroosh et al., 2014)

Table 2: Pharmacological tools used to interact with oxysterol receptors. The table presents several exogenous compounds used to target specific oxysterol receptors. The pharmacological parameters are presented as p (-log) of Ki EC_{50}, IC_{50}. GPR183 does not appear in the table as no synthetic ligand have been developed to date (7α,25-dihydroxycholesterol or 7α,27-dihydroxycholesterol (endogenous ligands, refer to table 1) are sometimes used to this end.

Target	Compound	CAS registry number	Other name(s)	Target(s)	Functionality	Pharmacological parameters	Other described targets	References
LXR	22(*S*)-OHC	22348-64-7	/	LXRα	antagonist	ND	/	(Kase et al., 2006) (Hessvik et al., 2012)
				LXRβ	antagonist	ND		
	AZ876	898800-26-5	/	LXRα	agonist	pKi=8.1 pEC_{50}=8.2	/	(van der Hoorn et al., 2011)
				LXRβ	agonist	pKi=8.0 pEC_{50}=7.2		
	DMHCA	79066-03-8	/	LXRα	agonist	pEC_{50}=7.6	/	(Yu et al., 2016)
				LXRβ	agonist	pEC_{50}=8.2		
	Geranylgeranyl pyrophosphate	6699-20-3	GGPP	LXRα	antagonist	pIC_{50}=9.2	/	(Gan et al., 2001)
				LXRβ	antagonist	pIC_{50}=9.1		
	GW3965	405911-09-3	C473027; GW-3965	LXRα	agonist	pEC_{50}=6.7	/	(Collins et al., 2002)
				LXRβ	agonist	pEC_{50}=7.5		
	Methylpiperidinyl-3β-hydroxycholenamide	/	MePipHCA	LXRα	agonist	pEC_{50}=7.0	/	(Yu et al., 2016)
				LXRβ	agonist	pEC_{50}=7.4		
	SR9238	1416153-62-2	SR-9238	LXRα	inverse agonist	pIC_{50}=6.7	/	(Griffett et al., 2013)
				LXRβ	inverse agonist	pIC_{50}=7.4		
	T0901317	293754-55-9	T-0901317	LXRα	agonist	pEC_{50}=7.3	RORα inverse agonist: pKi=6.9; RORγ inverse agonist: pKi=7.3	(Schultz et al., 2000) (Kumar et al., 2010)
				LXRβ	agonist	pEC_{50}=7.3		

ROR	GSK805	1426802-50-7	/	RORγ	inverse agonist	pIC_{50}=8.3	/	(Wang et al., 2015)
	JC1-40	/	/	RORα	activator	/	/	(Kim et al., 2012)
	SR1001	1335106-03-0	/	RORα	inverse agonist	pKi=6.8	/	(Solt et al., 2011)
				RORγ	inverse agonist	pKi=6.9		
	SR1078	1246525-60-9	/	RORα	agonist	/	/	(Wang et al., 2010b)
				RORγ	agonist	/		
	SR1555	1386439-51-5	/	RORγ	inverse agonist	pIC_{50}=6	/	(Solt et al., 2012)
	SR1903	1414248-06-8	/	RORγ	inverse agonist	pIC_{50}=7.0	LXRα agonist: pEC_{50}=8.8	(Chang et al., 2019)
ER	17α-estradiol	57-91-0	α-estradiol	Erα	agonist	pIC_{50}=7.3		(Zhu et al., 2006)
				ERβ	agonist	pIC_{50}=6.6		
	17β-estradiol	50-28-2	E2	Erα	agonist	pKi=9.8	GPER agonist: pKi~8.3	(Kuiper et al., 1997) (Revankar et al., 2005)
				ERβ	agonist	pKi=9.3		
	Diarylpropionitrile	1428-67-7	DPN	Erα	agonist	pEC_{50}=7.2	/	(Meyers et al., 2001; Stauffer et al., 2000)
				ERβ	agonist	pEC_{50}=9.1; pKi=8.6		
	Ethinylestradiol	57-63-6	17α-ethynylestradiol	Erα	agonist	pIC_{50}=8.7	/	(Jain et al., 2006)
				ERβ	agonist	pIC_{50}=8.1		
	Estetrol	15183-37-6	15-Hydroxyestriol	Erα	agonist	pIC_{50}=6.6	/	(Zhu et al., 2006)
				ERβ	agonist	pIC_{50}=6.5		
	ICI 182780	129453-61-8	Fulvestrant; ZD-9238	Erα	antagonist	pKi=9.0	GPER agonist: pKi=7.0	(Schmidt et al., 2003) (Thomas et al., 2005)
				ERβ	antagonist	pKi=8.9		
	Propylpyrazoletriol	263717-53-9	PPT	Erα	agonist	pKi=9.6	/	(Stauffer et al., 2000)
	Raloxifene	84449-90-1	LY-156758	Erα	modulator	pKi=9.5	GPER agonist: pKi=7.0	(Kuiper et al., 1997) (Stauffer et al., 2000)
				ERβ	modulator	pKi=8.0		
	Tamoxifen	10540-29-1	ICI-46474	Erα	modulator	pKi=7.2-7.8	GPER agonist: pKi=6.0	

				ERβ	modulator	pKi=7.2-7.8		(Kuiper et al., 1997) (Thomas et al., 2005)
	WAY-202041	524684-52-4	Prinaberel; ERB-041	Erα	agonist	pIC_{50}=5.9	/	(Malamas et al., 2004)
				Erβ	agonist	pIC_{50}=8.3		
CXCR2	AZD5069	878385-84-3	/	CXCR2	antagonist	pIC_{50}=9.1	CXCR1 antagonist: pIC_{50}=6.9; CCR2: pEC_{50}=5.2; CCR5: pEC_{50}=4.9	(Nicholls et al., 2015)
	AZD8309	333742-48-6	/	CXCR2	antagonist	pIC_{50}=9.0	CXCR1 antagonist: pIC_{50}=6.6	(Nicholls et al., 2015)
	Compound 2	/	/	CXCR2	antagonist	pIC_{50}=8.1; pIC_{50}=6.8 or 9.2	/	(Xu et al., 2016)
	Compound 22	/	/	CXCR2	antagonist	pIC_{50}=8.8	/	(Xu et al., 2016)
	G31P	/	CXCL8(3-72) K11R/G31P	CXCR2	antagonist	/	CXCR1 antagonist	(Cui et al., 2019) (Li et al., 2002)
	Reparixin	266359-83-5	DF 1681Y	CXCR2	allosteric modulator	pIC_{50}=6.4	CXCR1 allosteric modulator: pIC_{50}=9.0	(Bertini et al., 2004)
	SB225002	182498-32-4	/	CXCR2	antagonist	pIC_{50}=7.7	/	(White et al., 1998)
	SB332235	276702-15-9	/	CXCR2	antagonist	pIC_{50}=8.0	CXCR1: pIC_{50}=5.0	(Podolin et al., 2002)
	SB656933	688763-64-6	Elubirixin; SB-656933	CXCR2	antagonist	pIC_{50}=7.7	/	(Bachelerie et al., 2014)
	SCH527123	473727-83-2	Navarixin; MK-7123	CXCR2	antagonist	pIC_{50}=10.3	CXCR1 antagonist: pIC_{50}=8.4	(Bachelerie et al., 2014) (Dwyer et al., 2006)
GPR17	MDL29951	130798-51-5	/	GPR17	agonist	pEC_{50}=6.3-7.3	NMDAR antagonist: pKi=6.9	(Hennen et al., 2013) (Baron et al., 1992)
	Pranlukast	103177-37-3	ONO RS-411	GPR17	antagonist	pIC_{50}=7.5-8	CysLT1R antagonist: pIC_{50}=8.1-10.0; CysLT2R antagonist: pIC_{50}=5.4	(Ciana et al., 2006) (Lynch et al., 1999) (Heise et al., 2000)
GPR183	NIBR189	1599432-08-2	/	GPR183	antagonist	pIC_{50}=7.8-8	/	(Gessier et al., 2014)
Smo	GDC-0449	879085-55-9	Vismodegib	smo	antagonist	pKi=7.8	/	(Wang et al., 2012)
	LDE225	1218778-77-8	Sonidegib; Erismodegib	smo	antagonist	pKi=8.2	/	(Wang et al., 2012)