Original Research

Journal of Endocrinology and Reproduction, *Vol 21(1), 27-34, 2017, DOI: 10.18311/jer/2017/20188*

ISSN (Print) : 0971-913X
ISSN (Online) : 2455–1244

Thiazolidinedione Class of Anti-Diabetic Drugs Modulate Nuclear Receptor CAR Function

Shashi Kala Singh and Rakesh K. Tyagi*

Special Centre for Molecular Medicine Jawaharlal Nehru University New Delhi – 110067, India;
rktyagi@yahoo.com, rkt2300@mail.jnu.ac.in

Abstract

The human body has developed a defence system to prevent the accumulation of endogenous (bile acids, steroids, cholesterol metabolites, neurotransmitters, etc.) as well as exogenous (xenobiotics, clinical drugs, etc.) small molecules at toxic levels. This task is accomplished by 'drug metabolism and disposition (DMD) machinery' which entails phase I and phase II enzymes, and phase III transporter proteins. The components of this machinery act in a coordinated manner to biotransform and facilitate the elimination of small toxic molecules from the cellular milieu. Constitutive androstane receptor (CAR), a member of the nuclear receptor superfamily, acts as one of the major transcriptional regulators of the DMD machinery. Prescription of combination therapy is a common regimen during the treatment of diverse metabolic disorders and infectious diseases. In such combination therapies one drug may modulate the expression of genes of DMD, influencing the metabolism of another co-administered drug. This leads to decreased bioavailability or increased toxicity of the latter. Evaluation of drug-drug interactions (DDIs) has now become a major safety concern during drug discovery and development processes. Pre-assessment of the small molecules for modulatory effects on CAR and induction of the components of DMD can resolve the safety concerns, treatment failures and drug withdrawals due to the harmful DDIs. In the present study, we have followed a 'reverse approach' to assess CAR activation by drugs previously withdrawn from clinical practices. We selected three redundant members of thiazolidinedione family of anti-diabetic drugs and examined their potential in regulation of CAR and its target gene CYP2B6. These drugs showed differential transcriptional activation of CAR. Two of the TZD i.e., rosiglitazone and pioglitazone enhanced CAR activity by behaving as receptor ligands while the other (troglitazone) did not influence the receptor function and was justly withdrawn since it inflicted cytotoxicity.

Keywords: Anti-Diabetic Drugs, Constitutive Androstane Receptor, Drug Metabolism and Disposition Machinery, Nuclear Receptor, Nuclear Translocation

1. Introduction

Nuclear Receptors (NRs) constitute a superfamily of ligand-modulated transcription factors with 48 members identified in the human genome [10, 23, 5]. Members of the NR superfamily are involved in almost all the major aspects of biological processes such as growth, development, metabolism, homeostasis, etc. They execute their transcriptional functions in response to small lipophilic ligands like hormones, xenobiotics, fatty acids, vitamins, all-trans retinoic acid (RA), 9-cis-retinoic acid (9-cis-RA) and diverse endogenous metabolites[5]. Constitutive androstane receptor (CAR: NR1I3) is one of the key members of the human NR superfamily. It was isolated for the first time in 1994 from human liver using degenerate oligonucleotide probes[1,4]. It is predominantly expressed in liver and intestine, the primary site of drug detoxification [21]. CAR acts as a promiscuous receptor as it gets activated by a broad range of structurally dissimilar xenobiotics[21]. CAR exhibits differential subcellular localization and transcription function behaviour depending on cell and tissue type[26]. Recently, unliganded red fluorescent protein-tagged CAR (RFP-CAR) was observed to shift preferentially to the cytoplasmic compartment making it amenable for nuclear translocation studies [6]. CAR has a high basal activity without the involvement of binding to

**Author for correspondence*

a ligand which makes it unique from other NRs. CITCO (6-[4-chlorophenyl] imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-[3,4-dichlorobenzyl]-oxime) was the first ligand reported to activate human CAR [26]. CAR heterodimerizes with RXR and binds with its response element PBREM (phenobarbital-responsive enhancer module) present in the target genes promoter [25,4]. Furthermore, for its action CAR recruits co-activators (SRC-1, Sp-1 and signal cointegrator-2 etc.), chromatin remodeling complexes and histone modifiers after binding with DNA response element [21]. This cascade of protein recruitment leads to transcriptional response. CAR acts as a 'xenosensor' and regulates phase I (CYP2B6, CYP2C9, CYP2C19 etc.), phase II (UGT1A1, SULT2A1, GSTA1 etc.) and phase III (ABCB1, OATP2, MRP-1 etc.) genes involved in biotransformation and transport of endogenous and exogenous compounds [2]. Therefore, modulation of CAR can alter the pharmacokinetic profile of drugs by affecting bioavailability and efficacy of drugs. CYP2B6 has been delineated as a prototypical target gene for CAR among Cytochrome P450 family members. CYP2B6 accounts for the metabolism of ~10% of the medicinally active compounds[2]. Thus, increased induction of CYP2B6 by one drug may decrease the efficacy or increase the toxicity of other co-administered drugs. Though the primary function of CAR is regulation of DMD machinery, it is also implicated in other physiological as well as pathophysiological conditions like obesity, diabetes and cancer by regulating energy homeostasis, insulin signaling pathways and cell proliferations[17]. CAR is also reported to inhibit gluconeogenesis genes in case of diabetes [7]. Diabetes mellitus is a complex metabolic disorder with type II diabetes (T2D), being the most prevalent form of diabetes in recent times. T2D is characterized by hyperglycemia resulting from insulin resistance at initial stage, followed by deficiency of insulin due to autoimmune destruction of β-cells in later stages[9]. There are different classes of oral anti-diabetic medications available for T2D management including sulfonylurea, biguanide (metformin), meglitinide analogs, dipeptidyl peptidase-4 inhibitors, α-glucosidase inhibitors, sodium glucose transporter 2 (SGLT) inhibitors and thiazolidinedione (TZD)[13]. Members of TZD class of drugs also act as PPAR-γ ligands[24]. TZD inhibit hepatic gluconeogenesis, improve insulin sensitivity and also execute anti-inflammatory activities[13]. Despite multitude of beneficial effects there are also some serious side effects reported for some members of TZD group of anti-diabetic drugs which raises safety concerns over their uses.

Troglitazone, one of the members of TZD, was reported to cause hepatocellular injury and thus removed from the clinical practice[16]. In 2012, another member of TZD, rosiglitazone was also banned as it was found to be associated with myocardial infarction[19-20]. Pioglitazone proved to be a good choice among TZD and also in current use as its benefits outweighs over side effects. However, its usage is again debatable over the development of bladder cancer among diabetic patients. Due to the occurance of bladder cancer its use is stopped in some countries, while in others it is prescribed under restricted use[15]. Mounting evidences suggest that some of the anti-diabetic drugs show DDIs with ones prescribed for combination therapy[27]. Phenytoin was the first drug reported to show DDIs by activating CAR[28]. It has been reported to show DDI with co-administered anti-neoplastic drugs cyclophosphamide and ifosfamide leading to the induction of CYP2B6[8,18]. To address the safety concern of new small molecules, assessing pharmacokinetic DDI potential during preclinical stages has gained importance[11]. In this context, studying CAR modulation by therapeutic drugs is important to understand the pharmacokinetic profile of drugs. In the present study, we have followed the 'reverse approach' to examine the modulation of CAR by the withdrawn category of TZD class of anti-diabetic drugs and revealed the importance of CAR in pre-validating the small molecules on such drug screens.

2. Materials and Methods

2.1 Chemicals and Reagents

Troglitazone was purchased from Santacruz Biotechnology (CA, USA). CITCO, rosiglitazone and pioglitazone were procured from Sigma-Aldrich (St. Louis, MO, USA). Mammalian cell culture medium Dulbecco's Modified Eagle's Medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and PAN Biotech (Germany) respectively. Antibiotic and anti-mycotic solution was a product from Himedia (India) while OptiMEM I was obtained from Gibco (USA). Plastic-wares used in mammalian cell culture experiments were obtained from Corning Costar Corp. (USA). Transfection reagents Escort III/IV were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luciferase assay kit was sourced from Promega (Madison, WI, USA). All other general chemicals and reagents used were of analytical grade and procured from different commercial sources.

2.2 Maintenance of Cell Lines

HepG2 (human hepatocellular carcinoma cell line) and HEK293T (human embryonic kidney cell line) cells were obtained from National Centre for Cell Science (NCCS) National Repository (Pune, India). Both of these cell lines were cultured and routinely maintained in complete DMEM supplemented with 10% FBS, 100 µg/mL of penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin. The cultures were maintained in a humidified incubator at 5% CO_2 and 95% air atmosphere at 37°C.

2.3 Plasmid Constructs

Mammalian expression plasmid for human CAR (splice variant1; pcDNA3-CAR1) and CYP2B6-PBREM-Luc were kind gifts from Dr. Oliver Burk (Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Auerbachstrasse, Stuttgart, Germany) and Dr. Hongbing Wang (Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599-7360), respectively. Construct XREM-Luc and plasmid for pCMV-β galactosidase (β-gal) were described previously [22]. RFP-hCAR plasmid was generated in our laboratory[6]. All the experiments were conducted on CAR of human origin and thus denote the same used wherever.

2.4 Transient Transfection and Luciferase Reporter Assay

HepG2 cells were seeded at ~60% confluency a day before transfection in 12-well cell culture plates in DMEM media containing 10% FBS with antibiotics. Cells were transfected with DNA-liposome complex using Escort III and plasmid DNA (wt CAR and CYP2B6-Luc) along with β-gal plasmid (for normalization) in optiMEM I. Subsequent to transfection period of 12 hr, the medium was replaced with DMEM having 5% steroid-stripped serum without antibiotics followed by treatment with drugs for 24 hr. After drug treatment period the cells were washed with PBS and lysed in reporter lysis buffer to measure the luciferase and β-gal activity according to manufacturer's protocol (Promega, Madison, WI, USA). Luciferase activities were measured by TD-20/20 DLReady™ luminometer (Turner Designs, USA). Individual luciferase activities were normalized with β-gal value.

2.5 Live Cell Imaging by Fluorescence Microscopy

HEK293T cells were seeded in 35 mm plate to ~60% confluency in complete DMEM containing 10% FBS with antibiotics. Next day, the cells were transfected with DNA-liposome complex of Escort IV and RFP-CAR plasmid in optiMEM I. Following 12 hr transfection period the cells were treated with drugs in DMEM supplemented with 5% steroid-stripped serum without antibiotics for 24 hr. After drug treatment period, Hoechst dye was added at least two hours before visualizing the nucleus followed by imaging under a Nikon upright fluorescence microscope model 80i equipped with water immersion objectives and connected to a cooled-CCD digital camera (model Evolution VF, Media Cybernetics, USA). For subcellular localization purposes we counted 100 cells under different experimental conditions. When the receptor fluorescence was exclusively or predominantly nuclear it was considered as 'N'. When it was exclusively or predominantly cytoplasmic or uniformly distributed between nucleus and cytoplasm it was considered as 'C'.

2.6 Statistical Analysis

All experiments were performed for at least three times and values represent the means ± SE of three independent experiments. Statistical analysis was done by unpaired Student's t-test, and asterisks (** and ***) signify values that differed significantly from the control experiments with p-value less than 0.01 and 0.001, respectively ($p<0.01$ and $p<0.001$).

3. Results

3.1 Modulation of CAR Transcriptional Activity by Thiazolidinedione (TZD)

To study the regulatory effect of selected TZD on CAR transcriptional activity, transient transfections were performed in HepG2 cells. The cells were seeded and transfected with CAR expression plasmid and CAR-responsive promoter-reporter construct CYP2B6-Luc as mentioned *vide supra*. Rosiglitazone and pioglitazone were used at final concentration of 20µM, while troglitazone was used at 10µM. We predetermined these concentrations to be optimum during initial experiments. CITCO, a well known standard activator of CAR, was used at 10µM as reported in literature [12].

It was observed that pioglitazone induced maximum transcriptional activity in CAR, while rosiglitazone activated CAR as potently as CITCO. Transcriptional activity of CAR remained uninfluenced by troglitazone (**Figure 1**).

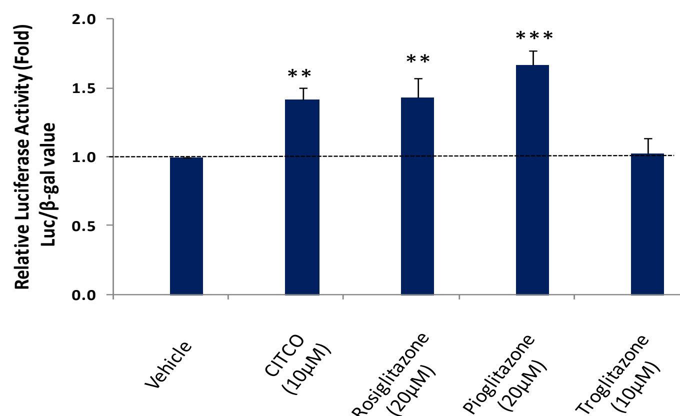
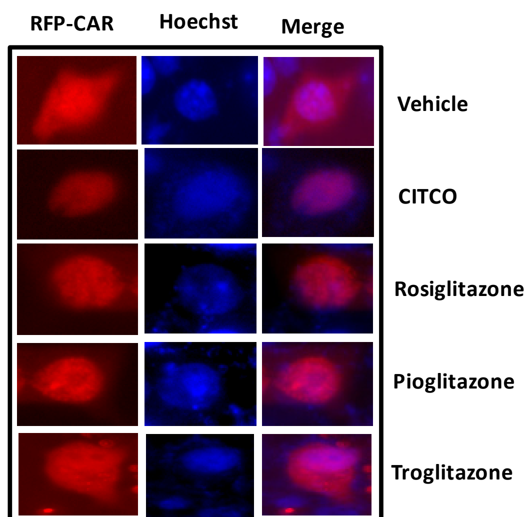


Figure 1: Effect of anti-diabetic drugs (TZD) on CAR-mediated transactivation of CYP2B6-promoter. *HepG2 cells were co-transfected with expression plasmid pcDNA3.1-CAR and construct for CYP2B6 promoter-reporter in 1:6 ratio. Plasmid encoding β-gal enzyme was also co-transfected for normalization purposes. Similarly, carrier DNA was included to maintain equal concentration of total DNA in each well. After transfection period drug treatments were given for 24 hr. Rosiglitazone and pioglitazone were used at 20µM and troglitazone was used at 10µM, respective final concentrations. CITCO, the standard ligand for CAR, was also used at 10µM final concentration. After 24hr of treatment period, luciferase activity was determined and normalized with β-gal value. Data represent the mean ± S.E. of three independent experiments. Asterisks **and *** signify luciferase values that differed significantly from the scores of vehicle treated control (P<0.01, P<0.001) in Student's t-test.*



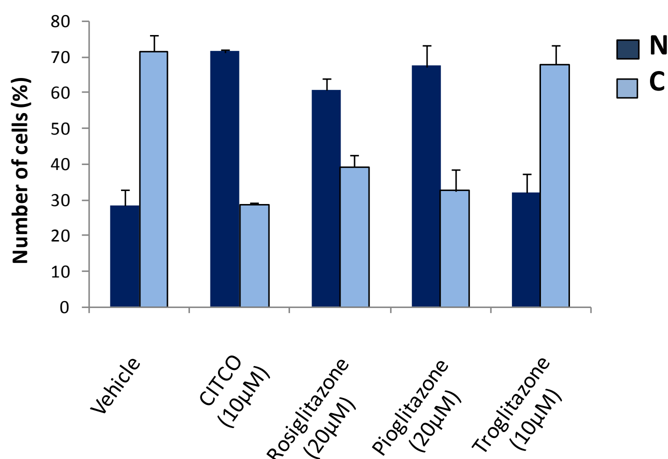


Figure 2: Effect of anti-diabetic drugs (TZD) on subcellular localization of RFP-CAR. *HEK293T cells were transiently transfected with RFP-CAR for 12 hr. Following tranfection period cells were treated with vehicle, CITCO (10 µM), rosiglitazone (20 µM), pioglitazone (20 µM) and troglitazone (10µM) for 24 hr and visualized by fluorescence microscope. Two hours before imaging, Hoechst was added to the cells to visualize the nucleus.* ***(A)*** *Live cell images were captured for RFP-CAR localization after treatment with the drugs. The left panel shows the subcellular localization of RFP-CAR while middle panel shows the corresponding nuclei of transfected cell and the right panel represents merged images of the two fluorescences.* ***(B)*** *Graph represents the average number of cells (in percentage) of three independent experiments with ± S.E. for the cytosolic and nuclear localization of RFP-CAR under different treatment conditions as indicated.*

3.2 Altered Localization of CAR by Thiazolidinedione (TZD)

As evident from Figure 1, rosiglitazone and pioglitazone induced CYP2B6-promoter by activating CAR. Activation of a nuclear receptor by its ligand or activators is known to be reflected on its translocation from cytoplasm to nucleus [14,3,6]. To confirm receptor-drug interactions we performed nuclear translocation studies using fluorescent protein tagged CAR and live cell imaging. However, until recently it remained a challenge to perform CAR translocation experiments as the receptor was reported to remain predominantly nuclear in immortalized cell lines. The difficulty to perform nuclear translocation experiments was recently resolved in our laboratory after tagging CAR with red fluorescent protein (RFP)[6]. This helped us to probe and identify CAR ligands which can shift the receptor into the nucleus. To explore the translocation of CAR, HEK293T cells were transfected with RFP-CAR and visualized for dynamic

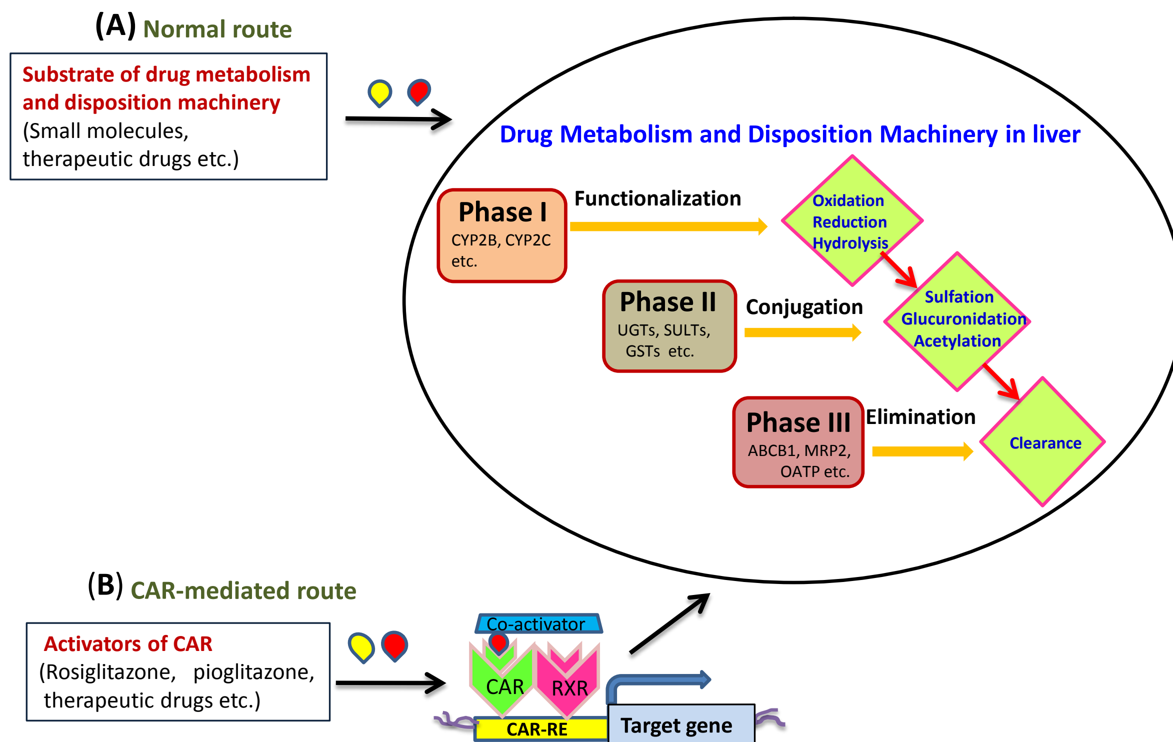


Figure 3: Schematic illustration of the fate of small / drug molecules via 'drug detoxification and disposition machinery' in liver under normal physiological control and accelerated conditions. *Bioavailability of therapeutic molecules in blood plasma depends on the rate of their metabolism and disposition in liver. Under normal physiological conditions, any therapeutic drug acts as a substrate for 'drug metabolism and disposition machinery' and, consequently, gets activated or deactivated as per the nature of the molecule (**A**). Thus, in view of this cascade therapeutic blood plasma levels of drugs are achieved through defined drug doses. (**B**) On the contrary, a fast elimination of therapeutic drugs will be undertaken if molecule(s) act as activator(s) of CAR. CAR has long been known as one of the major regulators of this machinery and may enhance metabolism of drugs, if activated. This upregulated machinery consequently enhances the metabolism of not only the activator but also the co-administered drugs, leading to undesired effects. Based on the observations from our study we suggest that rosiglitazone and pioglitazone act as CAR activators, resulting in rapid self-elimination and decreased therapeutic efficacy. Troglitazone, though not an activator of CAR, had earlier shown general cytotoxicity and was withdrawn.*

movement. A major fraction of unliganded RFP-CAR was observed to reside within the cytoplasm. RFP-CAR was ~75% cytosolic and ~25% nuclear in unliganded state (vehicle treated). CITCO, which acts as a ligand of CAR dramatically, shifted cytoplasmic RFP-CAR into the nuclear compartment of the cell. Similarly, rosiglitazone and pioglitazone also shifted about 60% and 65% of RFP-CAR to the nuclear compartment, respectively. Conversely, troglitazone was unable to translocate CAR into the nucleus (**Figure 2**). These observations suggest that the two drugs i.e., rosiglitazone and pioglitazone act as CAR activating ligands.

4. Discussion

Nuclear receptor CAR is well-documented for sensing and responding against chemical insults by regulating 'drug metabolism and disposition (DMD) machinery' in liver. Down-regulation or inhibition of 'drug metabolizing enzymes and elimination machinery' leads to the accumulation of small molecules which may be harmful. Conversely, upregulation or activation of this machinery may cause reduced bioavailability/efficacy of small drug molecules. In the present study we have assessed the effect of TZD class of anti-diabetic drugs i.e., rosiglitazone, pioglitazone and troglitazone on the activation of CAR. Rosiglitazone and pioglitazone were observed to enhance the transcriptional activity of CAR similar to standard ligand, CITCO. The enhanced activity of CAR was reflected in the increased induction of CYP2B6 gene. The third TZD, troglitazone, had no influence on CAR activity.

Unliganded CAR is reported to be a predominantly nuclear protein in immortalized cell lines which has hindered studies on ligand-mediated nuclear translocation of this receptor. Recently, our laboratory had overcome this limitation [6]. It was observed that unliganded RFP-tagged CAR shifted preferentially towards the cytoplasmic compartment extending opportunity for ligand-mediated nuclear translocation [6]. In view of this opportunity we performed nuclear translocation experiments using RFP-CAR and live cell imaging with three members of TZD drugs. We observed that rosiglitazone and pioglitazone acted on CAR and shifted it to the nuclear compartment similar to CITCO, a standard activator of CAR. Troglitazone remained ineffective in nuclear translocation assays in agreement to transcription assays. Based on these data we hypothesize that troglitazone, like non-activators of CAR, gets eliminated from the body through normal route of DMD machinery **(Figure 3)**. On the contrary, rosiglitazone and pioglitazone serve as CAR activators thereby enhancing the DMD machinery that results in rapid self-elimination. In view of the observations herein it appears that these two drugs i.e., rosiglitazone and pioglitazone have failed clinically owing to their being CAR activators. However, other contributory factors may have added to their clinical failure. Therefore, it is advisable that when advocating for novel small therapeutic molecules they must be screened and validated for their nature as activators of CAR or similars.

5. Acknowledgement

The research work presented in this manuscript was financially supported by a research grant to RKT from the UGC (major) and UPE-II. Infrastructural facilities supported by UGC-SAP (DRS-I), ICMR-CAR, and DST-PURSE phase II to our Centre are gratefully acknowledged. SK acknowledges ICMR for the grant of research fellowship for Ph.D.

6. References

1. Baes M, Gulick T, Choi H-S, Martinoli MG, Simha D, Moore DD. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol Cell Biol. 1994; 14(3):1544-52. PMid:8114692 PMCid:PMC358513
2. Chang TKH. Activation of pregnane x receptor (PXR) and constitutive androstane receptor (CAR) by herbal medicines. AAPS J. 2009; 11(3):590–601. PMid:19688601. Available from: https://doi.org/10.1208/s12248-009-9135-y.
3. Chaturvedi NK, Kumar S, Negi S, Tyagi RK. Endocrine disruptors provoke differential modulatory responses on androgen receptor and pregnane and xenobiotic receptor:potential implications in metabolic disorders. Mol Cell Biochem. 2010; 345(1-2):291–308. PMid:20830510. Available from: https://doi.org/10.1007/s11010-010-0583-6.

4. Dash AK, Yende AS, Kumar S, Singh, SK, Kotiya D, Rana M et al. The Constitutive Androstane Receptor (CAR): a nuclear receptor in health and disease. J Endocrinol Reprod. 2014; 18(2):59 – 74.
5. Dash AK, Tyagi RK. A compendium of nuclear receptors: The superfamily of ligand-modulated transcription factors. J Endocrinol Reprod. 2016; 20(1):1-27.
6. Dash AK, Yende AS, Tyagi RK. Novel application of red fluorescent protein (DsRed-Express) for the study of functional dynamics of nuclear receptors. J Fluoresc. 2017; 27(4):1225-1231. PMid:28470379. Available from: https://doi.org/10.1007/s10895-017-2109-z.
7. Dong B, Saha PK, Huang W, Chen W, Abu-Elheiga LA, Wakil SJ et al. Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. Proc Natl Acad Sci USA. 2009; 106(44):18831-6. PMid:19850873. Available from: https://doi.org/ 10.1073/pnas.0909731106.
8. Ducharme MP, Bernstein ML, Granvil CP, Gehrcke B, Wainer IW. Phenytoin-induced alteration in the N-dechloroethylation of ifosfamide stereoisomers. Cancer Chemother Pharmacol. 1997; 40(6):531–533. PMid:9332469. Available from: https://doi.org/ 10.1007/s002800050698.
9. Gao J, Xie W. Targeting xenobiotic receptors PXR and CAR for metabolic diseases. Trends Pharmacol Sci. 2012; 33(10):552-8. PMid:22889594. Available from: https://doi.org/ 10.1016/j.tips.2012.07.003.
10. Gronemeyer H, Gustafsson JA, Laudet V. Principles for modulation of the Nuclear Receptor superfamily. Nat Rev Drug Discov. 2004; 3(11):950-64. PMid:15520817. Available from: https://doi.org/10.1038/nrd1551.
11. Huang SM, Temple R, Throckmorton DC, Lesko LJ. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clin Pharmacol Ther. 2007; 81(2):298– 304. PMid: 17259955. Available from: https://doi.org/10.1038/sj.clpt.6100054.
12. Huang W, Zhang J, Wei P, Schrader WP, Moore DD. Meclizine is an agonist ligand for mouse constitutive androstane receptor (CAR) and an inverse agonist for human CAR. Mol Endocrinol. 2004; 18(10):2402–2408. PMid:15272053. Available from: https://doi.org/10.1210/me.2004-0046.
13. Kaiser D, Oetjen E. Something old, something new and something very old: drugs for treating type 2 diabetes. Br J Pharmacol. 2014; 171(12):2940-50. PMid:24641580. Available from: https://doi.org/10.1111/bph.12624.
14. Kumar S, Saradhi M, Chaturvedi NK, Tyagi RK. Intracellular localization and nucleocytoplasmic trafficking of steroid receptors: An overview. Mol Cell Endocrinol. 2006; 246(1-2):147-56. PMid:16388893. Available from: https://doi.org/10.1016/j.mce.2005.11.028.
15. Levin D, Bell S, Sund R, Hartikainen SA, Tuomilehto J, Pukkala E et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. Diabetologia. 2015; 58(3):493–504. PMid:25481707. Available from: https://doi.org/10.1007/s00125-014-3456-9.
16. Lim PLK, Liu J, Go ML, Boelsterli UA. The mitochondrial superoxide/thioredoxin-2/ask1 signaling pathway is critically involved in troglitazone-induced cell injury to human hepatocytes. Toxicol Sci. 2008; 101(2):341–349. PMid:17975114. Available from: https://doi.org/10.1093/toxsci/kfm273.
17. Mackowiak B, Wang H. Mechanisms of xenobiotic receptor activation: Direct vs. indirect. Biochim Biophys Acta. 2016; 1859(9):1130–1140. PMid:26877237. Available from: https://doi.org/10.1016/j.bbagrm.2016.02.006.
18. Nakajima M, Komagata S, Fujiki Y, Kanada Y, Ebi H, Itoh K. Genetic polymorphisms of CYP2B6 affect the pharmacokinetics/pharmacodynamics of cyclophosphamide in Japanese cancer patients. Pharmacogenet Genom. 2007; 17(6):431–45. PMid:17502835. Available from: https://doi.org/10.1097/FPC.0b013e328045c4fb.
19. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med. 2007; 356(24):2457–2471. PMid: 17517853. Available from: https://doi.org/10.1056/NEJMoa072761.
20. Nissen SE, Wolski K. Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. Arch Intern Med. 2010; 170(14):1191–1201. PMid:20656674. Available from: https://doi.org/ 10.1001/archinternmed.2010.207.
21. Prakash C, Zuniga B, Song CS, Jiang S, Cropper J, Park S et al. Nuclear receptors in drug metabolism, drug response and drug interactions. Nucl Receptor Res. 2015; 2:101178. PMCid:PMC4963026.
22. Rana M, Devi S, Gourinath S, Goswami R, Tyagi RK. A comprehensive analysis and functional characterization of naturally occurring non-synonymous variants of nuclear receptor PXR. Biochim Biophys Acta. 2016; 1859(9):1183-97. PMid:26962022. Available from: https://doi.org/10.1016/j.bbagrm.2016.03.001.
23. Staudinger JL, Lichti K. Cell signaling and nuclear receptors: New opportunities for molecular pharmaceuticals in liver disease. Mol Pharm. 2008; 5(1):17–34. PMid:18159925. Available from: https://doi.org/10.1021/mp700098c.
24. Taylor C, Hobbs FR. Type 2 diabetes, thiazolidinediones and cardiovascular risk. Br J Gen Pract. 2009; 59(564):520-524. PMid:19567003. Available from: https://doi.org/10.3399/bjgp09X453440.

25. Timsit YE, Negishi M. CAR and PXR: the xenobiotic-sensing receptors. Steroids. 2007; 72(3):231–46. PMid:17284330. Available from: https://doi.org/10.1016/j.steroids.2006.12.006.
26. Tolson AH, Wang H. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. Adv Drug Deliv Rev. 2010; 62(13):1238-1249. PMid:20727377. Available from: https://doi.org/10.1016/j.addr.2010.08.006.
27. Tornio A, Niemi M, Neuvonen PJ, Backman JT. Drug interactions with oral antidiabetic agents: pharmacokinetic mechanisms and clinical implications. Trends Pharmacol Sci. 2012; 33(6):312-322. PMid:22475684. Available from: https://doi.org/10.1016/j.tips.2012.03.001.
28. Wang H, Faucette S, Moore R, Sueyoshi T, Negishi M, LeCluyse E. Human constitutive androstane receptor mediates induction of cyp2b6 gene expression by Phenytoin. J Biol Chem. 2004; 279(28):29295–29301. PMid:15123723. Available from: https://doi.org/10.1074/jbc.M400580200.