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Identification of 20(*R*, *S*)-protopanaxadiol and 20(*R*, *S*)-protopanaxatriol for potential selective modulation of glucocorticoid receptor

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ABSTRACT

Although glucocorticoids (GCs) are widely used as anti-inflammatory drugs, they are often accompanied by adverse effects, which are mainly due to the transactivation of glucocorticoid receptor (GR) target genes. In order to screen novel plant-derived GR ligands (phytocorticoids) capable of separating transrepression from transactivation, this work focuses on the estimation of 20(*R*, *S*)-protopanaxadiol [PPD(*R*, *S*)] and 20(*R*, *S*)-protopanaxatriol [PPT(*R*, *S*)] for their dissociated characteristics. The reporter gene assay shows that ginsenosides cannot enhance glucocorticoid-responsive element-driven genes. The cytotoxicity assay shows that PPT(*S*), PPT(*R*), and PPD(*S*) can inhibit cell proliferation while PPD(*R*) does not suppress cell growth at available concentration. Further analysis of transactivation and transrepression activities indicates that PPD(*R*) can repress the transcription of GR target transrepressed gene without activating the expression of the GR target transactivated gene. Results of molecular docking suggest that PPD(*R*) yields more hydrogen bond interactions and a lower binding energy than its counterparts, resulting in tighter binding between PPD(*R*) and GR. In addition, PPD(*R*) achieves stability in the pocket after 2 ns, thereby facilitating exerting its regulatory role of GR target genes. By contrast, other ginsenosides fluctuate drastically during the simulations. In conclusion, PPD(*R*) may serve as a potential selective GR modulator (SEGRM).

1. Introduction

Endogenous glucocorticoids (GCs) such as cortisol are steroid hormones that function in development, metabolism, and immune responses. Since 1948, numerous synthetic glucocorticoids such as dexamethasone (DEX) have been developed to be used in the treatment of a range of immune-related disorders (Cain and Cidlowski, 2017). Both natural and synthetic glucocorticoids exert their regulatory roles in physiological processes by binding to glucocorticoid receptor (GR), which is expressed in virtually every human cell type (Gulliver, 2017). GR is a ligand-activated transcription factor that belongs to nuclear receptor superfamily. The unliganded glucocorticoid receptor is located in the cytosol as part of a multi-protein heterocomplex that contains various heat shock proteins (HSPs), including HSP90, HSP70, and HSP56, as well as a small acidic protein p23. These chaperone proteins maintain GR in an inactive conformation that allows it to bind ligands with high affinity (Ng et al., 2017; Ranhotra and Sharma, 2001). Upon ligand binding, GR dissociates from chaperone proteins, dimerizes and translocates into the nucleus, where it binds glucocorticoid-responsive element (GRE) in the promoter to induce transcription (Aoyagi and Archer, 2005).

Although glucocorticoids are widely used as anti-inflammatory drugs in clinical practice, they are often accompanied by a series of adverse effects, such as osteoporosis, muscle atrophy, and type 2 diabetes (Potamitis et al., 2019). Recent researches suggest that the adverse effects caused by GCs are mainly due to the transactivation of GR target genes, whereas the clinical efficacy of GCs is predominantly mediated by the transrepression of pro-inflammatory genes (Sundahl et al., 2015). In order to amplify beneficial anti-inflammatory actions and to reduce adverse side effects, there is an urgent need for screening of selective glucocorticoid receptor modulators (SEGRMs). As dissociated ligands for GR, these compounds are capable of separating transrepression from transactivation and may serve as effective and safe candidates for the therapy of the inflammatory diseases.

During the past decades, steroidal ligands (such as RU 24782 and RU 24858) as well as non-steroidal ligands (such as ZK 216348 and ZK

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245186) have been characterized as dissociated GR agonists, with the aim to alter the balance between transrepression and transactivation activities (De Bosscher, 2010). As a phenyl aziridine precursor from the Namibian shrub *Salsola tuberculatiformis* Botschantzev, Compound A (CpdA) is one of the first plant-derived SEGRMs with dissociated characteristics (De Bosscher et al., 2005). Although some of the SEGRMs have exhibited improved therapeutic potential, dissociating transrepression from transactivation completely is so far not possible due to the interdependent nature of these two regulatory pathways (Chen, 2008). Recently, it has been reported that GR-dependent transactivation of anti-inflammatory genes probably involves in the beneficial anti-inflammatory actions of GCs (Potamitis et al., 2019). Hence, the concept of dissociating transrepression from transactivation may be too simplistic to reveal the underlying molecular mechanism of selective actions of SEGRMs.

As the main active constituent of the root of *Panax ginseng*, ginsenosides exhibit various biological activities including anti-inflammatory property (Baek et al., 2015, 2016; Kim et al., 2017, 2018; Yu et al., 2017). It has been identified that ginsenoside Re (Leung et al., 2007), Rg1 (Lee et al., 1997), and compound K (Yang et al., 2008) can bind to and activate glucocorticoid receptor. As the metabolites of ginsenosides, 20(*R*, *S*)-protopanaxadiol [PPD(*R*, *S*)] and 20(*R*, *S*)-protopanaxatriol [PPT(*R*, *S*)] are generally regarded as the structural basis for biological activities of ginsenosides. Based on the similar structures of dexamethasone (a typical GR agonist) and ginsenosides (Fig. 1), we postulate that PPD(*R*, *S*) and PPT(*R*, *S*) may also serve as functional ligands for the glucocorticoid receptor. Hence, this work investigated whether these ginsenosides exhibit the potential for selective modulation of GR. A luciferase reporter plasmid that contains three copies of glucocorticoid-responsive element (GRE) was constructed to assess the ginsenoside-induced GR activation in dual-luciferase reporter assay. The cytotoxicity assay was performed and then the transactivation and transrepression activities of ginsenosides were determined. In addition, the structural mechanism of ginsenoside-GR interactions was elucidated by combining molecular docking with molecular dynamics (MD) simulations.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum was purchased from Hyclone (Logan, Utah, USA). Lipofectamine 2000 transfection reagent was purchased from Thermo Fisher Scientific (San Jose, CA, USA). Dexamethasone (DEX) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ginsenosides 20(*R*)-protopanaxadiol [PPD(*R*)], 20(*S*)-protopanaxadiol [PPD(*S*)], 20(*R*)-protopanaxatriol [PPT(*R*)], and 20(*S*)-protopanaxatriol [PPT(*S*)] were purchased from Yuanye Biotech Co., Ltd. (Shanghai, China). Trizol reagent, reverse transcriptional kit (TransScript First-Strand cDNA Synthesis SuperMix), and quantitative PCR kit (TransStart Top Green qPCR SuperMix) were purchased from Transgen Biotech Ltd. (Beijing, China). All other reagents used were of analytical grade.

2.2. Construction of the plasmids

The control plasmid pRL-TK, containing Renilla luciferase, was purchased from Promega (Madison, WI, USA). The full-length coding region of the human glucocorticoid receptor (hGR) was *de novo* assembled by PCR-based accurate synthesis (PAS), then inserted into the pcDNA3.1(+) vector at *Bam*H I-*Xho* I site, producing an expression plasmid pcDNA3.1(+)-hGR. The reporter plasmid, designated as GRE-Luc, was constructed by ligating a synthetic double-stranded oligonucleotide containing three tandem repeats of the consensus GRE (GGT ACATTTTGTTCT) into the modified pGL6-CMV-TA-Luc vector at restriction sites *Bmt* I and *Nde* I (Novotna et al., 2012).

2.3. Cell culture and MTT assay

The human cervical cancer (HeLa) cell line and human hepatoma (HepG2) cell line were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in 10 mL of DMEM containing 10% fetal bovine serum (FBS) and 100 IU L^{-1} of penicillin-streptomycin in 10 cm discs at 37 °C under 5% CO_2 in a humidified incubator, approximately 2.5×10^6 cells per disc. Cells were transferred every 4 d with two medium renewals every day after the transfer.

Ten thousand HepG2 cells per well were cultured in 100 μL DMEM on 96-well plate for approximately 16 h (to the logarithmic phase), then new medium (100 μL) was replaced and cultured for 1 h. One microliter solution of ginsenoside with different concentrations (10, 25, 50, 75 and 100 μM) was supplemented respectively. Three duplicates per each concentration were treated and the plate was gently shaken when the reagents were supplemented to mix the system and dissolve the compounds. DMSO was applied as blank control and its final concentration was kept constant at 1% (v/v) in both control and treated cell cultures. The cells were cultured for 36 h then the medium was removed, and 90 μL new DMEM plus 10 μL MTT solution were applied to each well. The plate was incubated in the incubator for 4 h. The supernate was disposed after the incubation and 150 μL of DMSO was added to dissolve formazan. Absorption at 570 nm was tested with a microplate reader. All data was then normalized to the DMSO treatment.

2.4. Luciferase reporter assay

The GR-driven luciferase reporter plasmid GRE-Luc, the expression plasmid pcDNA3.1(+)-hGR and the control plasmid pRL-TK were co-transfected into the HeLa cells using Lipofectamine 2000 transfection reagent (Zhang et al., 2019). After 24 h of transfection, cells were treated with DMSO, dexamethasone (100 nM), or ginsenosides (100 nM) for another 24 h. The resultant firefly luciferase activity was

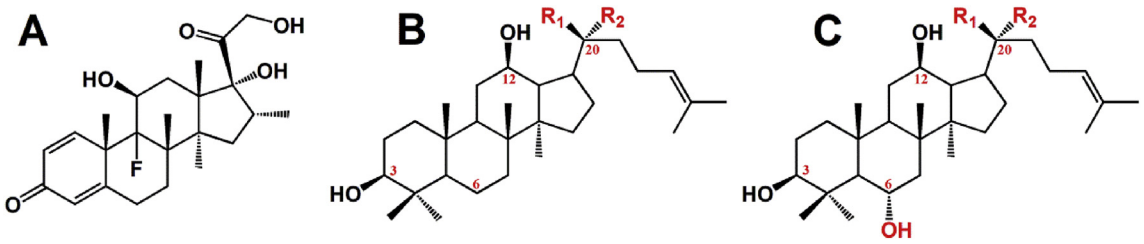

Fig. 1. Structures of dexamethasone (A), 20(*R*, *S*)-protopanaxadiol (B), and 20(*R*, *S*)-protopanaxatriol (C).
B1. 20(*R*)-protopanaxadiol [PPD(*R*)] R_1 = OH, R_2 = CH_3 C1. 20(*R*)-protopanaxatriol [PPT(*R*)] R_1 = OH, R_2 = CH_3
B2. 20(*S*)-protopanaxadiol [PPD(*S*)] R_1 = CH_3, R_2 = OH C2. 20(*S*)-protopanaxatriol [PPT(*S*)] R_1 = CH_3, R_2 = OH.

measured and normalized against that of Renilla by a Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA), according to the manufacturer protocol. The determination was performed in three independent experiments on a microplate reader SpectraMax i3x (Molecular Devices, Sunnyvale, CA, USA).

2.5. Determination of transactivation and transrepression activities

Cells were cultured in 2 mL of DMEM (2.5×10^5 cells per well) for approximately 15 h in 6-well plate, then the medium was discarded and 2 mL of new DMEM was added to each well and cultured for 1 h to make cells adaptive to the medium. Cells were harvested for total RNAs extraction after the treatments with 20 μL of PPD(*S*, *R*) (50 μM), PPT(*S*) (75 μM) and PPT(*R*) (50 μM) respectively for 1, 2, 3, and 4 h. Three duplicates were performed for each treatment time and DMSO was applied as vehicle control (1%, v/v). Total RNAs were extracted using the Trizol reagent according to the user's manual and 500 ng total RNAs from each sample was used for reverse transcription according to the instruction of the kit. Ten times dilution of cDNAs was prepared and 1 μL of each sample was used as templates for the quantitative PCR as instructed in the product manual. Three duplicates were displayed for each sample. Tyrosine aminotransferase (*TAT*) encoding gene was tested using the following primers TATF (5′-CTGAAGTTACCCAGGCA ATGAAAG-3′) and TATR (5′-TAATAAGAAGCAATC-TCCTCCCGAC-3′) (Ishii et al., 2008). Corticosteroid-binding globulin (*CBG*) encoding gene was tested by primers CBGF (5′-CACCAACCAGGCAAATTTCT-3′) and CBGR (5′-GGACGTCAGGTTTAGGGTGA-3′) (Nader et al., 2006). The hypoxanthine phosphoribosyltransferase gene (HPRT) was adopted as reference and was amplified by primers HPRTF (5′-GAAGAGCTATT GTAATGACC-3′) and reverse HPRTR (5′-GCGACCTTGACCATCT TTG-3′) (Qiu et al., 2007). The PCRs were run with LightCycler 96 thermocycler (Roche, Basel, Switzerland) using the following parameters: 94 °C for 10 min; 40 circles of 94 °C for 5 s, 60 °C for 30 s; 94 °C for 10 s, 65 °C for 60 s, 97 °C for 10 s; cooling to 37 °C for 30 s. The expression level was calculated by the $^{\Delta\Delta}$Ct methods. The results were normalized to the DMSO treatment.

2.6. Molecular docking

The X-ray crystal structure of the ligand binding domain of GR (GR-LBD) in complex with dexamethasone (PDB: 4UDC) was obtained from the Protein Data Bank (Edman et al., 2015). The initial structures of dexamethasone, PPD(*R*, *S*) and PPT(*R*, *S*) were constructed manually by GaussView and then optimized by Gaussian 09 W. Firstly, the typical agonist dexamethasone was docked into the binding pocket of GR-LBD by AutoDockTools. The grid box was generated around the co-crystallized ligand with a dimension of 40 × 40 × 40 points and the grid spacing was set at 0.375 Å. After the validation of docking protocol, ginsenosides were docked into the receptor. Ten independent docking runs were carried out for each ginsenoside and the docking pose with the lowest scoring function was selected for subsequent molecular dynamics analysis.

2.7. Molecular dynamics simulations

In order to elucidate the dynamic interactions of dexamethasone and ginsenosides with GR-LBD, molecular dynamics simulations of ligand-GR-LBD complexes were performed by GROMACS 2019 package using the CHARMM36 all-atom force field (Vanommeslaeghe et al., 2010). The topology of dexamethasone and ginsenosides were generated via the CGenFF server (Vanommeslaeghe and MacKerell, 2012; Vanommeslaeghe et al., 2012). The ligand-GR-LBD complex was solvated with water in a cubic box with buffering distance of 2 nm between the receptor fragment and the edges of the box. The simple point charge (SPC) model was chosen to simulate water molecules and three sodium ions were added to neutralize the system. After energy minimization, the system was equilibrated with position restraints on both the receptor and ligand at constant temperature and pressure. The position restraints were released during subsequent 10-ns molecular dynamics simulation. The root mean squared deviation (RMSD) values of the backbone of GR-LBD with respect to the original crystal structure (PDB: 4UDC) were calculated to assess the stability of this simulation system (Edman et al., 2015). Furthermore, the RMSD values of ligands in corresponding systems were also calculated to compare their binding stabilities during the simulations.

3. Results and discussion

3.1. Evaluation of the GR transcriptional activity by ginsenosides in HeLa cells

In order to evaluate the activation effect of ginsenosides on the GR pathway, HeLa cells were transiently transfected with reporter plasmid GRE-Luc and expression plasmid pcDNA3.1(+)-hGR. As a control, cells were also co-transfected with pRL-TK to correct for differences in transfection efficiency. After transfection, cells were treated with different concentrations of ginsenosides. The luciferase activity was measured following incubation for 24 h. The responses were expressed as the ratio of firefly activity to Renilla activity. As shown in Fig. 2, the transcriptional activity was increased approximately 30-fold by the agonist dexamethasone at a concentration of 100 nM, indicating that the GR reporter gene system was well established. Compared with the DMSO control, the tested ginsenosides did not increase luciferase expression in the glucocorticoid-responsive HeLa cell line (Fig. 2). These results suggest that PPD(*R*, *S*) and PPT(*R*, *S*) cannot induce transcriptional activation of glucocorticoid receptor.

3.2. Cytotoxicity of ginsenosides in HepG2 cells

HepG2 cells were treated with various concentrations of ginsenosides. The results suggest that PPT(*S*), PPT(*R*), and PPD(*S*) are able to inhibit the proliferation of the cell. The inhibition is dose-dependent on the condition that cells are cultured for 16 h and then treated for 36 h in 100 μL of DMEM with 10% FBS. As shown in Fig. 3A, B, and C, the IC_{50} values of PPT(*S*), PPT(*R*), and PPD(*S*) are 75, 50, and 50 μM respectively. The inhibition of PPD(*R*) is not as acute as that of other ginsenosides, which may be due to its low solubility in the medium. Actually, PPD(*R*) is separated out when the concentration is higher than 50 μM at the cell culture condition. As shown in Fig. 3D, the cell proliferation is much inhibited at the concentration of 50 μM, and thus we suppose the IC_{50} value could be achieved around 100–150 μM, if the solubility issue is addressed. According to these results, the IC_{50} values of PPT(*S*), PPT(*R*), and PPD(*S*), together with 50 μM of PPD(*R*) were adopted for

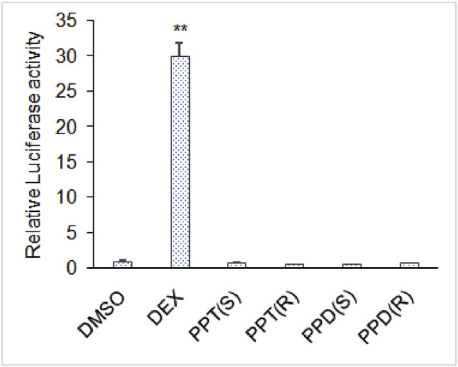

Fig. 2. The GR activation potency of dexamethasone (DEX) and ginsenosides tested using GRE-luciferase reporter gene assay in HeLa cells. Results are given as means ± SD of three independent experiments. **, extremely significantly different from DMSO control group ($p < 0.01$).

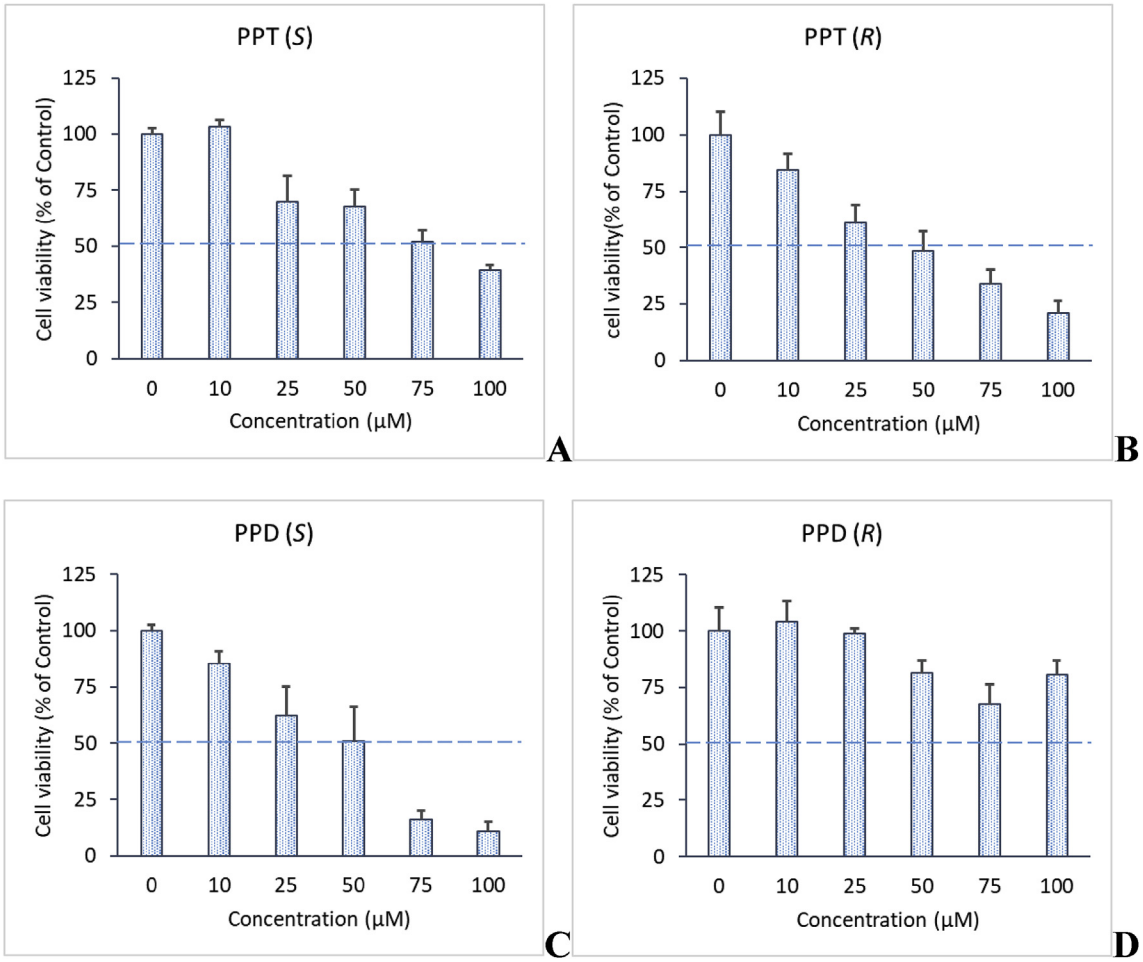

Fig. 3. Cytotoxicity of PPT(*S*) (A), PPT(*R*) (B), PPD(*S*) (C), and PPD(*R*) (D) in HepG2 cells. Results are given as means ± SD of three independent experiments and normalized to DMSO control group. Imaginary lines indicate the 50% cell viability.

the experiments afterwards in this work.

3.3. Transactivation and transrepression activities of ginsenosides in HepG2 cells

Real-time quantitive PCR was performed to test three liver specific genes to confirm the transactivation and transrepression effects of ginsenosides. The *TAT* and *CBG* genes were employed as delegates of the target genes transactivated and transrepressed by GR (Duret et al., 2006; Psarra et al., 2005), while the *HPRT* was used as reference. As transcription is considered to happen in short time, thus 1-, 2-, 3- and 4-h treatments were tested. The results of transactivation are shown in Fig. 4A. At the concentration of 50 μM, PPD(*R*) does not exhibit transactivation activity, and it is not significant to the DMSO control at all the test points. However, PPT(*S*) and PPT(*R*) exhibit transactivation activity with time-dependent at dose of IC_{50} in the early treatments for 1, 2, and 3 h, so does PPD(*S*) in the first 2 h, but the activity decreases later on. Both PPT(*R*) and PPD(*S*) are unable to repress the expression of *CBG*, on the contrary, the expression was activated. Although PPT(*S*) activates the expression of *CBG* in 1-h treatment, the activity vanishes in later treatments. Conversely, the expression was statistically significant repressed in 4-h treatment (Fig. 4B). On the other hand, PPD(*R*) represses the expression of *CBG* significantly in a short time and the repression is continuous. Given the above observation, PPD(*R*) does not affect cell proliferation at the concentration available in aqueous solution but does repress the expression of inflammation-associated genes without activating the expression of side effect associated genes. According to the concept that ideal candidates for anti-inflammatory drugs should be with higher transrepression activity dissociated from transactivation activity (De Bosscher et al., 2005), it is supposed that PPD(*R*) may be a potential SEGRM for further investigation.

3.4. Binding modes of ginsenosides with GR

In order to investigate the structure-activity relationship of ginsenoside-GR-LBD binding, their binding modes were explored by using molecular docking. As can be seen in Fig. 5, the docked dexamethasone binds to GR-LBD in a similar orientation to that of co-crystallization dexamethasone, indicating that the docking method is reliable and can be extended to explore the binding poses for ginsenosides. The hydrophobic cavity is large enough to accommodate dexamethasone as well as ginsenosides. All these compounds completely fit into the binding pocket without disrupting the co-activator binding site, which is known as the ligand-dependent transcriptional activation function 2 (AF-2). Interestingly, ginsenosides adopt a binding conformation similar to that of the agonist dexamethasone (Fig. 5).

As shown in Fig. 6, the hydrogen bond networks between ligands and residues within the hydrophobic binding pocket play a significant role in stabling the ginsenoside-GR-LBD binding. Both dexamethasone and ginsenosides form hydrogen bonds with Asn564, Gln570, and Arg611, as listed in Table 1. The C-12 hydroxyl group of ginsenosides also makes a hydrogen bond interaction with Met560, while dexamethasone forms a hydrogen bond with Thr739. It is noteworthy that the C-3 hydroxyl group of protopanaxadiols makes a hydrogen bond interaction with Phe623, however, this hydrogen bond has not been observed in protopanaxatriols. In addition, the C-20 hydroxyl group of

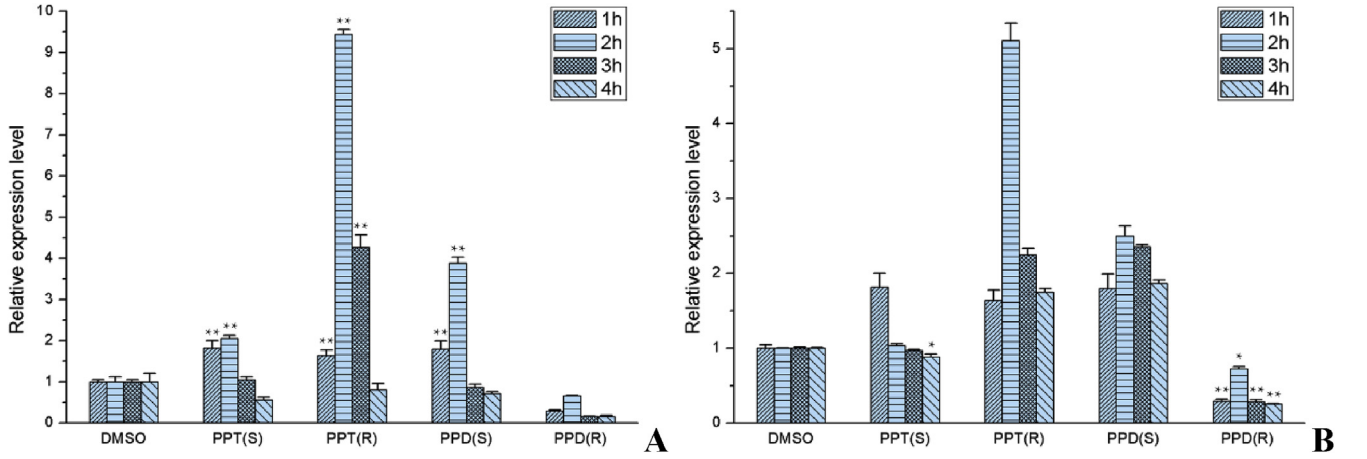

Fig. 4. Transactivation and transrepression activity of ginsenosides. The cells were treated with PPD(*S*, *R*) (50 μM), PPT(*S*) (75 μM) and PPT(*R*) (50 μM) respectively for 1, 2, 3, and 4 h. The DMSO treatment was used as blank control. The transactivation (A) and transrepession (B) analysis were based on the expression level of *TAT* gene and *CBG* gene referenced to that of *HPRTR* gene. Results are given as means ± SD of three independent experiments and normalized to DMSO control group. *, **, statistically significant differences ($p < 0.05$ and $p < 0.01$ respectively).

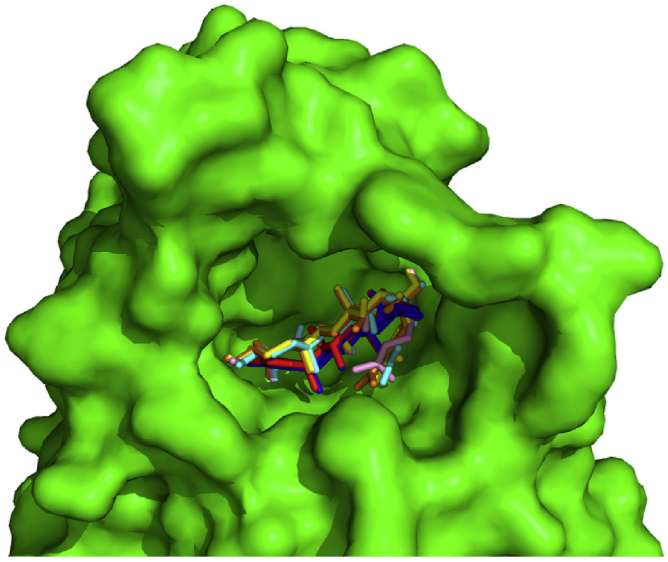

Fig. 5. The original crystallographic pose of dexamethasone (red) and docking poses of dexamethasone (blue), PPD(*R*) (yellow), PPD(*S*) (orange), PPT(*R*) (pink), and PPT(*S*) (cyan). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

20(*R*)-ginsenosides as well as dexamethasone form a hydrogen bond with Gln642, whereas this hydrogen bond is absent in 20(*S*)-ginsenosides. Accordingly, the stereostructure of the C-20 hydroxyl group exhibits remarkable influence on the hydrogen bond networks.

3.5. Dynamic binding process of ginsenosides with GR

Ligand binding mediates conformational changes of nuclear receptors, which is crucial for nuclear translocation and subsequent transcriptional activity (Blanquart et al., 2003). The dynamic binding process of dexamethasone and ginsenosides with GR-LBD was further explored by 10-ns molecular dynamics simulations starting from the binding conformations. As shown in Table 2, the average RMSD values of the backbone of GR-LBD vary from 0.12 to 0.16 nm, suggesting that all these ligands cause little conformational changes essential to stabilize GR-LBD. Clearly, the conformation of dexamethasone exhibits small deviations from its initial docking structure, as represented in Fig. 6. Compared with dexamethasone, ginsenosides undergo much significant

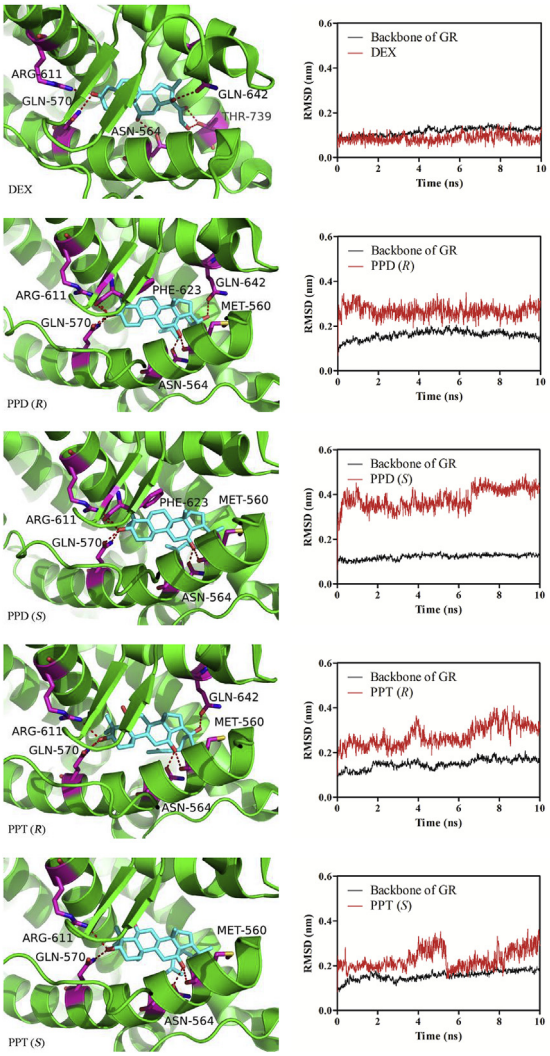

Fig. 6. The hydrogen bond networks in the binding pocket (left) and variations of the root mean square deviation (RMSD) during the molecular dynamics simulations (right).

Table 1
The docking results of dexamethasone (DEX), PPD(*R*, *S*) and PPT(*R*, *S*) with GR-LBD.

Compound	Binding energy (kcal mol^{-1})	Hydrogen bonds
DEX	−12.68	Asn564, Gln570, Arg611, Gln642, Thr739
PPD(*R*)	−10.50	Met560, Asn564, Gln570, Arg611, Phe623, Gln642
PPD(*S*)	−9.63	Met560, Asn564, Gln570, Arg611, Phe623
PPT(*R*)	−9.45	Met560, Asn564, Gln570, Arg611, Gln642
PPT(*S*)	−10.10	Met560, Asn564, Gln570, Arg611

Table 2
The average values and standard deviations of the root mean square deviation (RMSD).

Compound	Backbone of GR-LBD (nm)	ligand (nm)
DEX	0.12 ± 0.02	0.09 ± 0.02
PPD(*R*)	0.16 ± 0.02	0.27 ± 0.03
PPD(*S*)	0.12 ± 0.01	0.38 ± 0.05
PPT(*R*)	0.15 ± 0.02	0.27 ± 0.05
PPT(*S*)	0.16 ± 0.02	0.23 ± 0.04

conformational changes relative to their initial docking poses during the MD simulations. The average RMSD values of ginsenosides are in the range of 0.23–0.38 nm (Table 2), larger than that of dexamethasone (0.09 nm). Interestingly, the calculated binding energies of ginsenosides ($-10.50 \sim -9.45$ kcal mol^{-1}) are also higher than that of dexamethasone (-12.68 kcal mol^{-1}), as represented in Table 1. The results of molecular docking combined with MD simulations show that dexamethasone achieve more strong and stable binding with GR-LBD than ginsenosides. It can be speculated that the initial docking poses of ginsenosides undergo conformational adjustments to be embedded in the cavity of GR-LBD during the simulations. By contrast, the initial docking pose of dexamethasone is close to its crystallographic binding pose, as shown in Fig. 5.

Interestingly, PPD(*R*) achieves stability in the binding pocket after 2 ns, whereas other ginsenosides fluctuate drastically within the simulation period. Furthermore, PPD(*R*) yields more hydrogen bond interactions and a lower binding energy than its counterparts (Table 1), resulting in tighter binding between PPD(*R*) and GR-LBD, thereby facilitating exerting its regulatory role of GR target genes.

4. Conclusion

In this work, PPD(*R*, *S*) and PPT(*R*, *S*) were evaluated for their potential for selective modulation of glucocorticoid receptor. The results of molecular simulations and reporter gene assay show that ginsenosides can bind to GR-LBD but cannot induce transcriptional activation of GR. The GR binding potency of PPD(*R*) is slightly higher than that of other ginsenosides. The cytotoxicity assay shows that PPT(*S*), PPT(*R*), and PPD(*S*) are able to inhibit cell proliferation while PPD(*R*) does not suppress the cell growth at available concentration. Unlike the other tested ginsenosides, PPD(*R*) maintains the transrepression activity but does not exhibit the transactivation activity. The results of molecular simulations may provide a possible mechanism for the transrepression of GR target genes by PPD(*R*). In conclusion, PPD(*R*) may serve as a potential selective GR modulator, capable of dissociating transactivation from transrepression without cell proliferation inhibition.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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