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ORIGINAL ARTICLE

A molecular simulation analysis of vitamin D targets interleukin 13 (IL13) as an alternative to mometasone in asthma

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Abstract

Asthma, a chronic lung disease characterized by obstruction of airway passage is characterized by inflammation and hyperresponsiveness with increase in the number of eosinophils. Interleukin-13, plays a significant role in causing inflammation during an asthmatic attack by bronchial constriction. Mometasone, a glucocorticoid has been used as the first line of administration for people affected with asthma for almost a decade. However, in several cases, people treated with mometasone have faced systemic and local side effects. To reduce these side effects, we hypothesized vitamin D that can be used as a substitute to mometasone. For this purpose, we employed the use of molecular docking and simulation studies for comparative study. The docking studies revealed the binding residues of interleukin-13 which are bound to the active site. Among all, we noticed three binding residue Leu83, His84 and Arg86 common for both mometasone and vitamin D. Also, the binding energies share a significant similarity between them. The docked complexes of mometasone and vitamin D with interleukin-13 were evaluated with molecular dynamics simulation. Consistently, the MD analysis uncovered the interesting note on conformational adaptation between the complexes as well as that vitamin D has the complementary binding efficiency to interleukin-13 as compared to mometasone. The substitution of vitamin D might provide a promising gateway to reduce the side effects caused by mometasone and also reduce the cost for treatment of asthma patients.

Keywords Asthma · Vitamin D · Mometasone · IL13 · Docking · Molecular dynamics

Introduction

Allergic asthma is the hyperreactivity characterized by a chronic, reversible airway of the lung, airway remodeling and inflammation and it has been on the rise for past decade. The patients affected with asthma showed characteristic features such as airway blockage with hyperresponsiveness, inflammation due to an increase in the number of eosinophils, elevated levels of serum IgE and hypersecretion of mucus. But, the mechanism involved in the development of asthma is still not well-understood. There have been several hypotheses regarding the causes such as $CD4^+$ T cells producing a Th2 pattern of cytokines which includes interleukins IL-4, IL-5, IL-13 and IL-9 playing a pivotal role in the pathogenesis of the disease. Among the interleukins, IL-13 is known to act as a potent activator in the inflammatory process associated with asthma. Many studies reported IL-13 signaling results in hyper-regulation of mucin, fibrosis and up-regulation of chitinase. IL-13 and IL-4 together share common biological functions in both signalings as well as the complex receptor network (Wynn 2003; Elias et al. 2003). Interleukin 13 is also known to play a significant role in the recruitment regulation, homing and activation of inflammatory responsive cells. IL-13 is known to induce the production of IL-5 in the lung airway smooth muscles thereby leading to regulating the conscription of eosinophils into the airway spaces (Wills-Karp 2004). An illustrative pathway depicted the vital role in mentioning the progenitor role of IL13 in the asthmatic pathway (Fig. 1).

The treatment regimen for asthma is under intensive research. For the past few decades, inhaled corticosteroids (ICS) are popularly known for the effective treatment of asthma. Among ICS, mometasone furoate (MF),

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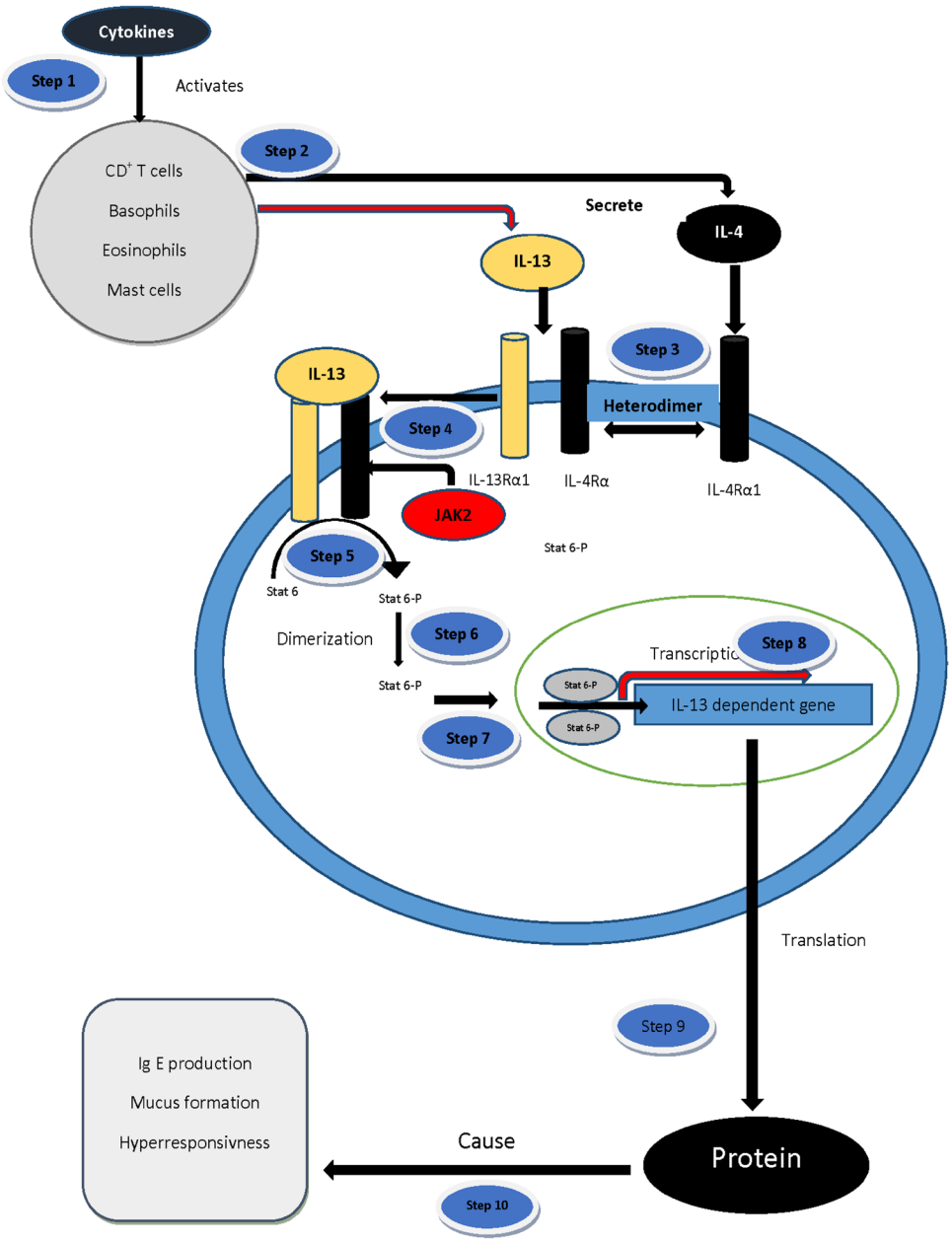

Fig. 1 Illustrative pathway depicting the role of interleukin-13 in the pathogenesis of asthma

a glucocorticoid which is commonly known as mometasone has been used for the treatment of asthma patients. It has been under implementation from the year 2005 for the patients above 12 years of age and children between 4 and 11 years in the year 2008. The dosage was dependent on the age of the patients. The usage of mometasone is applied in the first line of administration for the patients with mild persistent asthma (Dahl 2006). Mometasone is known to have practical impact against IL-13 signaling. Thereby, by reducing the Broncho constriction in the asthmatic patients it provides relief as it decreases the blockage in the airway passage. The efficacy of ICS in reduced inflammation and hyperresponsiveness might be the prime factors behind the extensive usage in the moderate to stern asthmatic attack (Suissa et al. 2000; Hanania et al. 1995). However, the usage of this drug is associated with some systemic and local side effects. Systemic side effects include glaucoma, osteoporosis, hindrance in the normal growth of children and thinning of skin (Hanania et al. 1995; Cave et al. 1999; Kelly and Nelson 2003). Local side effects include dysphonia, constant cough, bronchospasm and oropharyngeal candidiasis (Hanania et al. 1995; Kelly and Nelson 2003). We hypothesized that vitamin D could have a beneficial effect based on some of the research carried on asthmatic patients. Gale et al. reported that vitamin D has a beneficiary effect on pregnant women (Gale et al. 2008). Later, in recent times,

i.e., in the year 2017, Mohamed et al., depicted that there is a gradual decrease in vitamin D levels in bronchial asthma and supplementation which showed a prominent effect on bronchial relaxation (Shahin et al. 2017). In our present study, the docked complexes of mometasone and vitamin D with interleukin-13 and their binding complementarities, conformational adaptation were evaluated with auto dock and molecular dynamics simulation (Raghuraman et al. 2017). The obtained results were promising, so vitamin D could be used as a potential replacement for mometasone against IL-13 in asthma reducing the side effects known to be caused by ICS in standard drug treatment.

Materials and methods

Dataset collection

The protein interleukin-13 information was obtained from the UniProt database (2008) with the accession id P35225. IL-13 consists of 146 amino acid residues and the crystallized X-ray structure of interleukin-13 was retrieved from the PDB ID: 4I77 (Ultsch et al. 2013) from RCSB Protein Data Bank (PDB) (Berman et al. 2000). Followed by, the chemical structures of the ligands mometasone and vitamin D, the active form of vitamin D were obtained from PubChem with the compound id 441335 and 5280453, respectively.

Ligand preparation and active site prediction

The crystal structure of IL-13 (PDB ID: 4I77) complex with the ligand was refined for docking procedures. All the heteroatoms, the non-essential water molecules and the cofactors were removed. The hydrogen atoms were added to the protein structure. The ligand structures of mometasone and vitamin D obtained as sdf format were converted to PDB using PyMol and were further used for docking analysis. The active site of IL-13 protein was predicted using HotSpot Wizard 2.0 (Bendl et al. 2016). The selection criteria of the active site residues were based on their functionality and stabilizing residues.

Molecular docking

The comparative docking analysis for mometasone and vitamin D was carried out using Auto Dock v4.2 (Morris et al. 2009). Docking protocols help in the elucidation of the best ligand binding pose to the receptor using the interactive maps. The protein structure of IL-13 was processed with the addition of all the hydrogen atoms and then merging with the non-polar hydrogen atoms using Auto Dock tools. Later, the Gasteiger charges and Kollman charges were assigned to calibrated structural charge. The torsions were processed for the ligand. The grid for the protein was set around the active site of IL-13 which was predicted using CASTp where the ligand interaction takes place. The dimensions of the grid are $62 \times 62 \times 62$ with 3.75 Å around the protein active site. This rigid grid box was attained using Auto grid followed by Auto dock with a Lamarckian genetic algorithm for the best docking conformation. The docking was performed and the binding energies were calculated. The best conformation was selected and was visualized using PyMol.

Evaluation of protein–ligand complex using dynamic simulation

The docked complexes of mometasone and vitamin D with interleukin-13 were used as the initial point for molecular dynamics simulation. The GROMACS 4.6.5 package is adopting the GROMOS53a6 force field which is optimal for performing ligand protein MD simulation (Kahn and Bruice 2002; Raghuraman et al. 2016). The topology of the ligand was generated using PRODRG server for including the heteroatom file due to its limitation of GROMACS for parameterization of the ligand files consisting of the heteroatom group in the structural PDB file (Schüttelkopf and Aalten 2004). The two protein–ligand complexes were individually introduced and placed in a cubic box of 0.9 nm and solvated by SPC water model consisting of water molecules. Before energy minimization, the total charge of the system was neutralized with six sodium ions (Na^+). Later, the energy minimization was carried out for 50,000 steps using the steepest descent method. Furthermore, the equilibrium of the system was achieved by maintaining a constant volume, the temperature at 300 k and pressure of 500 ps. Finally, the equilibrated structures were finally subjected to the final molecular dynamics simulation of 50 ns with a LINICS algorithm of 2-f time step for each of the protein–ligand complex. The non-bonded list was calculated based on the atom-based cut-off value of 10 Å. The MD trajectory were analyzed through Gromacs utilities for structural analysis for every nanosecond between the protein and ligand in the docked complex using the inbuilt modules of gromacs such as g_rmsd, g_rmsf, g_hbond and g_sas calculating root mean square deviation (rmsd), root mean square fluctuation (rmsf), hydrogen bond formation (hbond) and solvent accessible surface area (sasa), respectively.

Results and discussion

Structure of the target protein and active site prediction

The 3-D structure of IL-13 was constrained to 107 residues after refinement. Later, the residues identified in the

active site from HotSpot Wizard 2.0 were Ser81, Ser82, Leu83, His84, Val85, Arg86, Asp87, Phe107, and Arg108 corresponding to the binding site for docking analysis for mometasone and vitamin D.

Docking analysis

To investigate and analyze the binding efficiency of vitamin D, docking analysis was carried out using standalone Auto Dock software. On docking the mometasone to IL-13, the least binding energy was observed to be − 4.16 kcal/mol with total internal energy of 0.56 kcal/mol. The vitamin D showed the binding energy of − 4.54 kcal/mol with a total internal energy of 1.03 kcal/mol. The binding residues surrounding the ligand were also noted to observe similarity from the interaction plots as shown in Fig. 2. The binding sites for mometasone was found out to be Phe80, Ser81, Leu83, His84 and Arg86, whereas for vitamin D binding sites were found to be Ile37, Asn38, Thr40, Gly42, Ala46, Ser82, Leu83, His84, Val85, Arg86 and 87 as shown in Table 1. The number of hydrogen bonds formed for mometasone and vitamin D were 2 and 3, respectively. Thereby, docking pose analysis showed that vitamin D and mometasone has one of the same binding residues in the binding pocket of IL-13 and the distribution of hydrogen donor and acceptors around the ligands in the binding pocket reveals that both of the lead candidates have the same affinity as shown in Fig. 3.

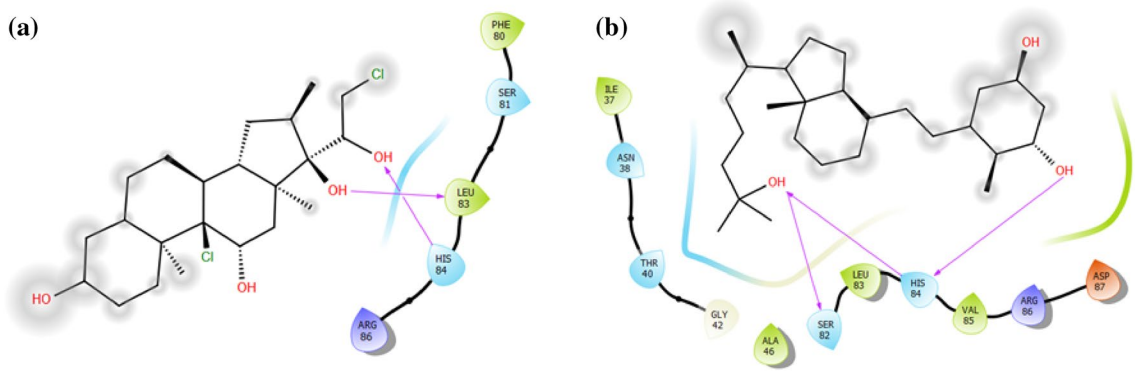

Fig. 2 Interactive plots of interleukin-13. **a** Mometasone. **b** Vitamin D

Table 1 Docking scores and binding residues of the compounds with interleukin-13

Compound	Binding energy (kCal/mol)	Total internal energy (kCal/mol)	Binding residues	H bonds
Mometasone	− 4.16	0.56	Phe80, Ser81, **Leu83,His84, Arg86**	2
Vitamin D	− 4.54	1.03	Ile37, Asn38, Thr40, Gly42, Ala46, Ser82, **Leu83, His84**, Val85, **Arg86**, Asp87	3

Common interaction residues are highlighted in bold

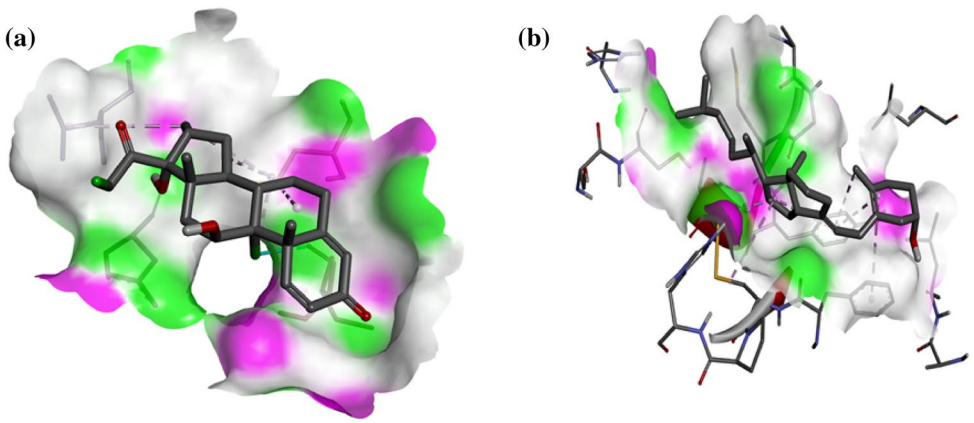

Fig. 3 Binding poses of interleukin-13 active site. **a** Mometasone **b** Vitamin D

Complex dynamics simulation analysis

For comparison of flexibility and structural behavior of IL-13 and mometasone (complex 1), IL-13 and vitamin D (complex 2), we incorporated the use of GROMACS 4.5.5 for a 50 ns simulation for each of the complex. The root mean square deviation (rmsd) of the two complexes was calculated against both the complexes and the graphs were generated for comparison of backbone flexibility implying the stability of the protein upon binding. The mometasone and vitamin D complexes attained a state of convergence of 15 ns which implied that candidates binding at the active site of the protein are stable as well as robust entailing that there is not much disturbance in the backbone of protein structure. The rmsd values of mometasone showed noticeable fluctuations in a window size between ~0.15 and 0.3 nm, whereas vitamin D showed the rmsd values between ~0.1 and 0.35 nm as shown in Fig. 4a. Here, our investigations exposed that both mometasone and vitamin D has equal affinity showing proper, stable and strong binding affinity. The residual mobility of the protein in each of the lead molecule complexes was calculated using root mean square fluctuation (rmsf) plotted against residue number and the time scale of the trajectory of the MD simulation as shown in Fig. 4b. The fluctuations among the complexed protein structures with the lead molecules did not show any noticeable changes at their residual level without any abnormal fluctuation, which shows that the vitamin D also showed a near similar trajectory as that of mometasone at the residual level.

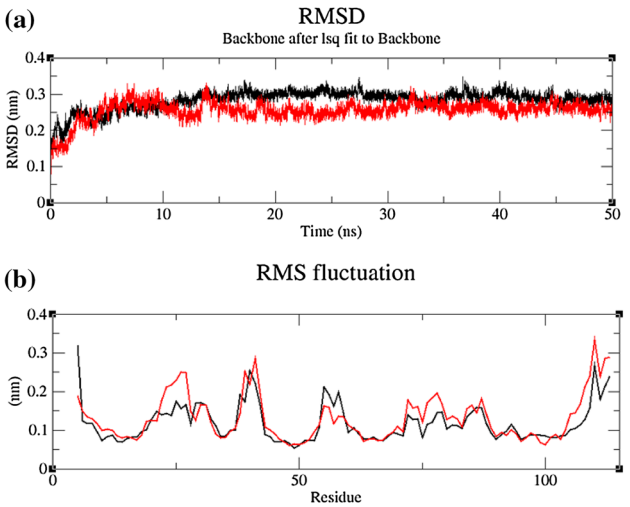

Fig. 4 Time dependence of interleukin-13 complexes of mometasone and vitamin D. **a** Root mean square deviation of backbone structure. **b** Root mean fluctuation of C-alpha of the structure. The black color indicates the complex of IL13-mometasone and the red color indicated the IL13-vitamin D complex

Hydrogen bond analysis

The formation and stability of hydrogen bonds of the drug candidates in binding with interleukin 13 were determined. The g_hbond utility of GROMACS was used to calculate the hydrogen bonding profile using the cut-off of 0.35 nm of donor–acceptor distance among the IL13 complexes (Jeffrey and Takagi 1978). The analysis revealed that the average number of hydrogen bonds formed between mometasone and IL-13 was 2 and 3 H bonds were formed over the time period as shown in Fig. 5a. Whereas, vitamin D comprised higher average hydrogen bonds comprising of 2–3 H bonds at a maximum level and partially 4 H bonds during the trajectory period as shown in Fig. 5b. The average number of hydrogen bond formation throughout the MD simulation signifies their continuous hydrogen bonding interactions with the binding site of the target protein. This characteristic feature suggests that vitamin D has a better binding capacity than mometasone to interleukin-13 for efficient inhibition.

Molecular surface analysis

The solvent accessible surface area (SASA) of a molecule is an area of the exposed molecular surface of the protein which is directly proportional to the free energy of the molecule using the GROMACS analysis utility tool g_sas. The value change of SASA indicates the conformational change whose interactions are associated with the water. Hence, we can calculate the hydrophobic and hydrophilic areas which are freely accessible to water. For the complexes of mometasone and vitamin D with IL-13, we observed the average accessible surface was in the range of ~30–40 nm^2 and ~32–39 nm^2 over a time period (Fig. 6). This shows that vitamin D has close proximity for the binding affinity towards the IL-13 binding site as that of mometasone.

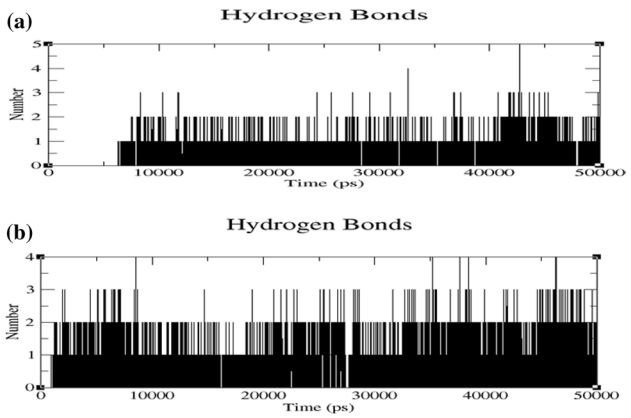

Fig. 5 Estimated hydrogen bond pattern with the cut-off of 0.35 nm of donor–acceptor distance between **a** IL13–mometasone complex. **b** IL13–vitamin D complex

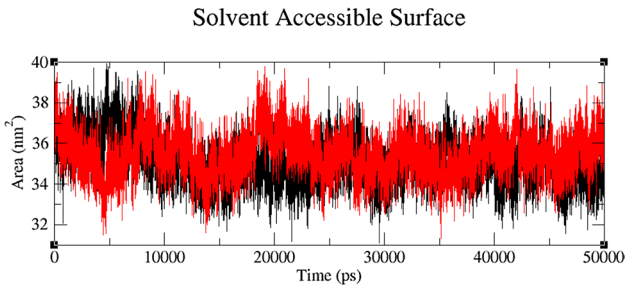

Fig. 6 Solvent accessible surface area (SASA) of interleukin-13 complexes. The black color indicates the complex of IL13-mometasone and the red color indicated the IL13-vitamin D complex

The 50 ns molecular dynamics simulation trajectories revealed that vitamin D is in close proximity with IL13 correlating to that of mometasone. The results obtained from our hypothesis perfectly conceded that vitamin D have a predominant role in an asthmatic patient. A study conducted by Majak et al. showed that the vitamin D supplementation in children tends to prevent the triggering of acute respiratory inflammation (Majak et al. 2011). There have been some clinical reports such as those of Tachimoto et al. that conducted a testological survey with placebo and low dosage of vitamin D supplementation and it has shown to have an improving effect on the children with asthma (Tachimoto et al. 2016).

Conclusion

We performed the molecular docking study for mometasone which is widely known for its use against the temporary relief of asthma patients. However, it is also inflicted with the side effects that come with it. Here, we propose the use of vitamin D as a replacement drug for mometasone. For this purpose, we employed the use of molecular docking and simulation to evaluate our hypothesis of replacement of mometasone with vitamin D. Results showed that docking conformation and binding energies which are in close proximity with each other. Also, we have found three common residues of Leu83, His84 and Arg86 which binds to both mometasone as well as vitamin D. Later, molecular dynamic simulation analysis proved our hypothesis that vitamin D provides a valuable replacement for mometasone as the energy states, i.e., the rmsd and rmsf analysis showed the complex stability of the trajectory. The molecular interactions of formation of hydrogen bonds provide the higher stability in both the trajectories. The substitution of vitamin D might provide a promising gateway to reduce the side effects caused by mometasone and also reduce the cost for the treatment of asthma patients. However, further validation is required through experimental studies to prove the therapeutic potential of vitamin D concerning asthma.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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