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Original Article

Anti-Human Rhinovirus 1B Activity of Dexamethasone via GCR-Dependent Autophagy Activation

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ABSTRACT

Objectives: Human rhinoviruses (HRVs) are the major cause of the common cold. Currently there is no registered, clinically effective, antiviral chemotherapeutic agent to treat diseases caused by HRVs. In this study, the antiviral activity of dexamethasone (DEX) against HRV1B was examined.
Methods: The anti–HRV1B activity of DEX was assessed by sulforhodamine B assay in HeLa cells, and by RT-PCR in the lungs of HRV1B-infected mice. Histological evaluation of HRV1B-infected lungs was performed and a histological score was given. Anti-HRV1B activity of DEX via the glucocorticoid receptor (GCR)-dependent autophagy activation was assessed by blocking with chloroquine diphosphate salt or bafilomycin A1 treatment.
Results: In HRV1B-infected HeLa cells, treatment with DEX in a dose-dependent manner, resulted in a cell viability of > 70% indicating that HRV1B viral replication was reduced by DEX treatment. HRV1B infected mice treated with DEX, had evidence of reduced inflammation and a moderate histological score. DEX treatment showed antiviral activity against HRV1B via GCR-dependent autophagy activation.
Conclusion: This study demonstrated that DEX treatment showed anti-HRV1B activity via GCR-dependent autophagy activation in HeLa cells and HRV1B infected mice. Further investigation assessing the development of topical formulations may enable the development of improved DEX effectiveness.

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Introduction

Human Rhinoviruses (HRVs) belong to the genus Enterovirus of the Picornaviridae family. There are over 160 serotypes of HRVs that are responsible for the common cold, which is a mild illness of the upper respiratory tract [1]. HRVs may be associated with more severe diseases such as acute otitis media in children and sinusitis in adults [2]. HRV infection has been also associated with different respiratory tract complications such as bronchiolitis, pneumonia, sinusitis and acute otitis media, and has been implicated in exacerbations of chronic respiratory disorders, such as asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD) [3]. Recently, experimental HRV infection in COPD patients established a causative relationship between virus infection and exacerbations, moreover these exacerbations were predicted by the World Health Organization to become one of the major causes of worldwide death in the next few decades [4].

Due to the large number of serotypes, vaccination against HRVs is not feasible. Despite intensive efforts in antiviral research and development, no effective antiviral therapies have been approved for either the prevention or treatment of

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diseases caused by HRV [5]. In this regard, many trials have been conducted to find antiviral components. For example, Pirodavir lowered HRV replication, whilst oral administration of Pleconaril led to a reduction in duration and severity of the illness [6]. Treatment of HRV-induced exacerbations of asthma is still under clinical evaluation [7]. Therefore, to date, the search for antiviral compounds against HRV is ongoing.

Dexamethasone (DEX) has become one of the most commonly, and intensively used therapeutic agents in pediatric oncology [8]. However, to date, no detailed study has been carried out on DEX's antiviral activity against human rhinovirus 1B (HRV1B) in Hela cells.

In this study the anti–HRV1B activity of DEX in HeLa cells, and in the lungs of HRV1B-infected mice using PCR after DEX treatment was studied. Furthermore, anti-HRV1B activity of DEX correlation with induction of autophagy activation via glucocorticoid receptor (GCR) was investigated.

Materials and Methods

1. Cell culture, viruses, and reagents

Human rhinovirus 1B (HRV1B) was obtained from ATCC (Manassas, VA, USA) and propagated by infection at 33°C in HeLa cells. HeLa cells were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (Invitrogen, Carlsbad, CA, USA). DEX, rupintrivir (Rup), chloroquine diphosphate salt (CQ) and bafilomycin A1 (BAF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Doxycycline (DOX) was purchased from Clontech (Mountain View, CA, USA). For Tet-on system, the GCR shRNA-transfected HeLa cells were treated with doxycycline (2 mg/mL) in culture media every 2 days for 7 days.

2. Mice and virus infection

Four-week-old, female BALB/c mice were purchased from SPL animal company (Orient Bio Inc, Sungnam, Korea). Mice were maintained in accordance with the guidelines, and stabilized for 7 days at Kangwon National University. Mice were intranasally infected with HRV1B (1.8×10^7 pfu/mouse) 3 times with 10 µL/PBS at intervals of 10 minutes. DEX was intranasally administered at 0.1 mg/kg after HRV1B infection.

3. SRB assay

The SRB assay was used to measure antiviral activity as previously reported [9]. The day before the experiment, HeLa cells (3×10^4 cells/well) were seeded in a 96-well culture plate. HeLa cells were used for HRV1B infection. After 24 hours, the HRV1B infection medium was replaced with 30 mM $MgCl_2$, 1% FBS in MEM media. The HRV1B-infected HeLa cells were incubated at 33°C and 5% CO2 resulting in CPE. After 48 hours, the 96-well culture plate was washed with PBS, and fixed in 70% acetone (100 µL/well) for 30 minutes, followed by staining with 0.4% SRB (Sigma-Aldrich, St, Louis, MO, USA) in 1% acetic acid. The precipitated SRB crystals were solubilized with 10 mM unbuffered tris-based solution 100 µL/well. The absorbance was read on a SpectraMax i3 microplate reader (Molecular Devices, Palo Alto, CA, USA) at 562nm.

4. Real-time PCR

Total RNA was extracted from HeLa cells and lysate of mice lungs with a QIAamp viral RNA mini kit (Qiagen, Hilden, Germany). Reverse transcription was performed with RNase inhibitor, oligo (dT) 15 primers, dNTP mixture, and Moloney murine leukemia virus reverse transcriptase with 5 X buffer, according to an established protocol (Promega, Madison, WI, USA). Quantitative real- time PCR (qPCR) analysis was performed to amplify complementary deoxyribonucleic acid (cDNA), using the THUNDERBIRD® SYBR® qPCR mix (Toyobo, Osaka, Japan), and CFX96 optics module real-time PCR system (Bio-Rad, Hercules, CA, USA). The following primers: HRV 5'-NCR sense, 5'-TCC TCC GGC CCC TGA ATG-3' and HRV 5'-NCR-antisense, 5'-GAA ACA CGG ACA CCC AAA G-3'; and human β-actin-sense, 5'-CCA TCA TGA AGT GTG ACG TGG-3' and human β-actin-antisense, 5'-GTC CGC CTA GAA GCA TTT GCG-3' were used. The PCR conditions were as follows: 95°C for 3 minutes for 1 cycle, and 95°C, 30 seconds; 60°C, 30 seconds; 72°C, 30 seconds; for 35 cycles.

5. Histological analysis

The HRV1B-infected lungs of mice were washed in PBS, and fixed with 4% (w/v) formaldehyde overnight. The HRV1B-infected lungs were dehydrated in serial gradients of ethanol and xylene, and embedded in paraffin. The tissues were sliced into 5-mm-thick sections and stained with hematoxylin and eosin. After HRV1B infection, the lungs with severe inflammation (score: 0-4) were graded for inflammation, edema, and cellular infiltration, according to the guidelines. A pathologist evaluated the degree of inflammation using a light microscope (200 X) and was blinded whilst scoring each slide. The means of the lung inflammatory score were graded for severity (absent, minimal, mild, moderate, marked).

6. Plasmid cloning and lentivirus production

Plasmid cloning and lentivirus production were carried out as described previously [10]. To establish the Tet-inducible lentiviral shRNA expression system, the shRNA oligo was annealed and inserted into the Tet-pLKO-puro vector, which was gifted by Dmitri Wiederschain (plasmid #21915, Addgene, Cambridge, MA, USA). The Tet-pLKOblast vector was constructed with the blasticidin resistance gene. For shRNA sequences, TRCN0000245004 for GCRsh-1 was used. For lentivirus production in 293T cells, each lentiviral vector was co-transfected with pMD2.G and psPAX2 (gifted from Didler Trono: Addgene plasmid #12259, #12260), using Lipofectamine 2000.

7. Statistical analysis

Multiple groups were compared using a 1-way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA). Values of $p < 0.05$ were considered significant at a 95% confidence interval.

Results

1. Dexamethasone showed antiviral activity against HRV1B in HeLa cells

Treatment of HRV1B-infected HeLa cells with DEX was performed at concentrations of 16, 80, 400 and 2000 nM. and showed cell viability of > 70% in a dose-dependent manner (Figure 1A). Treatment with Rup gave higher cell viability in HRV1B-infected HeLa cells (Figure 1A).

The antiviral activity of DEX in HRV1B-infected HeLa cells assessed by RT-PCR, significantly reduced. HRV1B viral replication (Figure 1B). Treatment with Rup strongly reduced HRV1B viral replication (Figure 1B).

2. Dexamethasone ameliorates pulmonary inflammation and inhibits HRV1B replication in mice

Histological evaluation of HRV1B-infected murine lung tissue showed characteristic HRV1B-infected lesions including necrotizing bronchiolitis, and interstitial pneumonia and scored a high histological score compared with lungs from control BALB/c mice which exhibited typical normal pulmonary tissue with a low histological score (Figures 2A and 2B). HRV1B-infected mice treated with DEX had moderate inflammation including increased necrosis, increased number of inflammatory cells and pulmonary edema and scored a moderate histological score (Figures 2A and 2B).

To determine whether the decreased pulmonary inflammation was associated with decreased viral replication, RT-PCR was performed on lung tissue homogenate to assess HRV1B NCR gene expression. HRV1B-infected mice had increased viral replication, as detected by HRV1B NCR gene expression (Figure 2C). Treatment with DEX in HRV1B-infected

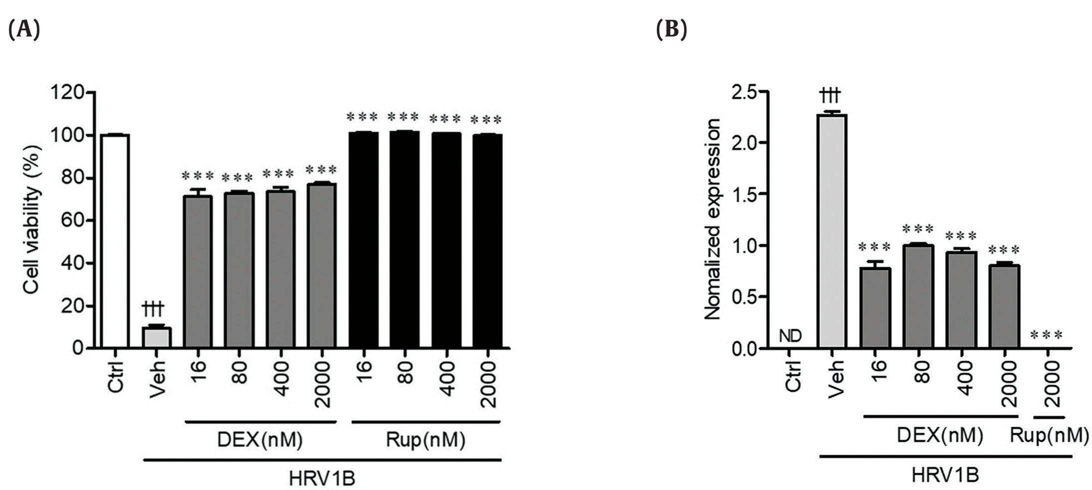

Figure 1. The antiviral activity of dexamethasone against HRV1B in vitro. (A) The antiviral activities of dexamethasone (DEX) against HRV1B were determined by inoculating HeLa cells with HRV1B at an MOI of 10 and treated with DEX. The viability of cells was measured using SRB assay, and the antiviral activity was calculated based on cell viability. Results are shown as means ± SEM. $^{†††}p < 0.001$ for comparison with non-infected control group (Ctrl). $^{***}p < 0.001$ for comparison with HRV1B-infected vehicle group (Veh). (B) Relative HRV1B gene expression in HRV1B-infected HeLa cells was determined by real-time PCR. $^{†††}p < 0.001$ for comparison with non-infected control group (Ctrl). $^{***}p < 0.001$ for comparison with HRV1B-infected vehicle group (Veh).
HRV1B = human rhinovirus 1B; ND = not detected.

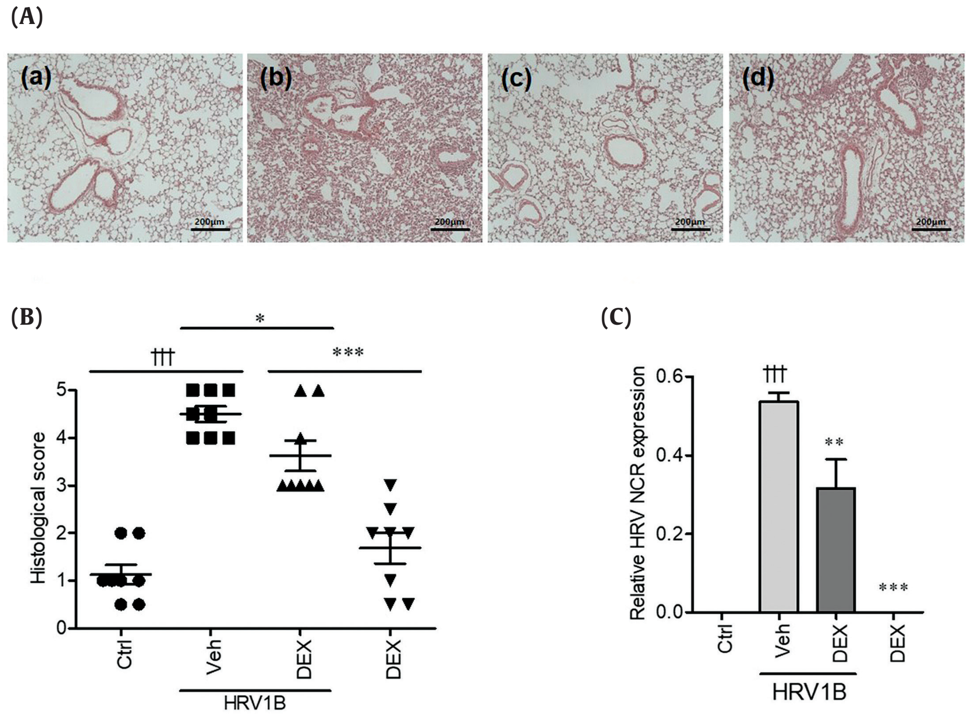

Figure 2. In vivo study for antiviral activity of DEX against HRV1B. BALB/c mice were infected with HRV1B, and 0.1 mg/kg DEX was intranasally administered. (A) Lung pathology was assessed after H&E staining in uninfected mice (a), HRV1B-infected mice (b), uninfected mice treated with DEX (c), and HRV1B-infected mice treated with DEX (d). (B) Pathological scores were determined according to the severity of edema, hemorrhage and immune cell infiltration. $^{†††}p < 0.001$ for comparison between non-infected control group (Ctrl) and HRV1B-infected vehicle group (Veh). $^{*}p < 0.05$ for comparison between HRV1B-infected vehicle group (Veh) and HRV1B-infected treated with DEX group. $^{***}p <$ 0.001 for comparison between HRV1B-infected treated with DEX group and nuinfected treated with DEX group. (C) The relative HRV NCR expression levels were analyzed in lung tissue homogenates. Results are shown as means ± SEM. $^{†††}p < 0.001$ for comparison with non-infected control group (Ctrl). $^{**}p < 0.01$ and $^{***}p < 0.001$ for comparison with HRV1B-infected vehicle group (Veh).
DEX = dexamethasone; HRV1B = human rhinovirus 1B.

mice decreased HRV1B NCR gene expression, suggesting a reduction in viral replication (Figure 2C).

3. Dexamethasone showed anti-HRV1B activity via GCR receptor

To assess whether the anti-HRV1B activity of DEX was mediated via the GCR in HeLa cells, the cells were conditionally depleted using the Tet-inducible knock out system. Treatment of GCR shRNA-transfected HeLa cells with DOX abolished the expression of GCR (Figure 3). The antiviral activity of DEX against HRV1B was significantly reduced in GCR knock-down HeLa cells (Figure 3), suggesting that the antiviral activity of DEX was mediated by GCR.

4. Dexamethasone inhibits HRV1B infection via activation of autophagy

To investigate whether autophagy activation by DEX correlated with the antiviral activity of DEX against HRV1B infection, autophagy was blocked with inhibitors including CQ or BAF. The results demonstrated that only treatment of DEX decreased cell viability in HRV1B-infected cells in a dose-dependent manner (Figure 4). Both CQ and BAF significantly attenuated the protective activity of DEX against HRV1B-infected cell viability (Figure 4).

Discussion

HRVs are involved in a range of clinical manifestations [11], but up to now viable options for the prevention or treatment of HRV infections remain limited. DEX has become one of

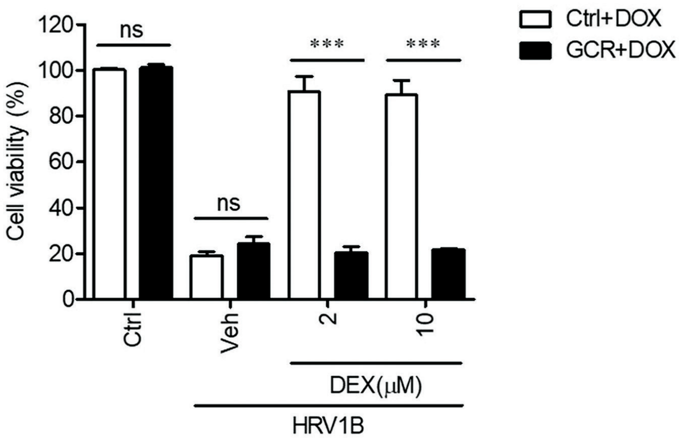

Figure 3. Antiviral activity of DEX against HRV1B in GCR knockoutHeLa cells. To determine whether the DEX antiviral activity was mediated via the glucocorticoid receptor (GCR), HeLa cells transfected with GCR shRNA were treated for 7 days with 2 mg/mL doxycycline. The HeLa cells were then infected with HRV1B at an MOI of 10 in the presence of 2 and 10 mM DEX. Results are shown as means ± SEM. ns, not significant and $^{***}p < 0.001$.
DEX = dexamethasone; HRV1B = human rhinovirus 1B.

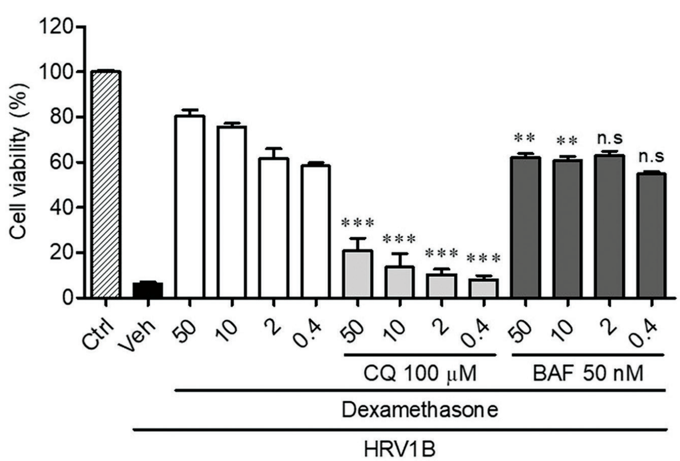

Figure 4. Blockade of autophagy reduced antiviral activity of DEX. HeLa cells were infected with HRV1B at an MOI of 10 and treated with DEX in the presence of 100 mM chloroquine or 0.025 mM bafilomycin A1. Cells were incubated for 48 hours, and cell viability was measured by SRB assay.
Data are presented as means ± SEM. $^{**}p < 0.05$ and $^{***}p < 0.001$ for comparisons between chloroquine and bafilomycin A1 treatment at the same dose of DEX alone.
DEX = dexamethasone; HRV1B = human rhinovirus 1B.

the most commonly and intensively used therapeutic agents in pediatric oncology [8]. Currently, no effective antiviral therapeutic effect of DEX has been reported for either the prevention or treatment of diseases caused by HRV infection. In the present study, the anti-HRV1B activity was evaluated in vitro. DEX significantly improved cell viability to > 70% in a dose-dependent manner in HRV1B-infected HeLa cells (Figure 1A). Furthermore, the antiviral activity of DEX was assessed by RT-PCR in HRV1B-infected HeLa cells. HRV1B viral replication was significantly reduced by DEX treatment (Figure 1B). Our results indicate that DEX exhibits anti–HRV1B virus activity in vitro.

HRV predominantly infects cells of the upper respiratory tract, although HRV RNA has also been found in lower airways in experimental models of HRV infection [12]. HRV is the etiological agent of approximately 50% to 70% of common colds [13]. HRV infection is associated with bronchiolitis in human, pneumonia in the immunosuppressed and exacerbations of pre-existing pulmonary conditions such as asthma or chronic obstructive pulmonary disease [14,15]. In this study, only HRV1B-infected mice exhibited characteristic HRV1B-infected lesions including necrotizing bronchiolitis and interstitial pneumonia (Figure 2A), showing increment viral replication reflected by HRV1B NCR gene expression (Figure 2C). Following treatment with DEX, there was moderate inflammation, with increased necrosis and numbers of inflammatory cells and pulmonary edema in the lungs of HRV1B-infected mice with decreasing viral replication reflected by reduced HRV1B NCR gene expression (Figure 2C). Therefore, DEX greatly decreases HRV1B-associated inflammation and viral replication reflected by reduced HRV1B NCR gene expression in HRV1B-infected mice.

Chronic inflammatory respiratory diseases are characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person [16]. Glucocorticoids (GCs) are among the mainstays of treatment in asthmatics and exert their effects mainly via binding to their intracellular receptors (GCRs) [17]. In this study, to assess whether the anti-HRV1B activity of DEX was mediated via the GCR, GCR in HeLa cells were conditionally depleted using the Tet-inducible knockout system. Treatment of DOX in GCR shRNA-transfected HeLa cells abolished the expression of GCR (Figure 3). DEX showed anti-HRV1B activity via mediation by the GCR (Figure 3).

In our recent study, we reported that the glucocorticoid budesonide, showed anti-rhinoviral activity by inducing GCR-dependent autophagy activation [18]. Another study reported that dexamethasone produced reactive oxygen species to activate autophagy [19]. Autophagy is 1 of the 2 main cellular catabolic pathways, which is finely regulated to maintain cellular homeostasis [17]. Moreover, autophagy strongly contributes to the innate and adaptive immune response [20]. Autophagy plays an essential role in antiviral immune responses. Epstein-Barr virus (EBV), like other viruses that persist in the infected host, must interfere with this process to avoid its own elimination [21]. The majority of herpesviruses have developed strategies to inhibit autophagy, especially in immune cells [22]. In this current study, DEX treatment induced antiviral activity against HRV1B via autophagy activation (Figure 4).

Collectively, DEX has demonstrated antiviral activity against HRV1B via GCR-dependent autophagy activation. Therefore, DEX is an attractive anti-HRV1B candidate with the potential to successfully prevent or treat HRV1B-associated malignancies.

Conflicts of Interest

There are no potential conflicts of interest relevant to this article to report.

Acknowledgments

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References

[1] Conti C, Proietti Monaco L, Desideri N. 3-Phenylalkyl-2H-chromenes and -chromans as novel rhinovirus infection inhibitors. Bioorganic Med Chem 2017;25(7):2074-83.

[2] Park SW, Kwon MJ, Yoo JY, Choi HJ, Ahn YJ. Antiviral activity and possible mode of action of ellagic acid identified in Lagerstroemia speciosa leaves toward human rhinoviruses. BMC Comp Alt Med 2014;14:171.

[3] Bochkov YA, Gern JE. Rhinoviruses and Their Receptors: Implications for Allergic Disease. Curr Allergy Asthma Rep 2016;16(4):30.

[4] Gunawardana N, Finney L, Johnston SL, Mallia P. Experimental rhinovirus infection in COPD: implications for antiviral therapies. Antiviral Res 2014;102:95-105.

[5] Choi HJ, Bae EY, Song JH, Baek SH, Kwon DH. Inhibitory effects of orobol 7-O-D-glucoside from banaba (Lagerstroemia speciosa L.) on human rhinoviruses replication. Letters Appl Microbiol 2010;51(1):1-5.

[6] Pevear DC, Hayden FG, Demenczuk TM, Barone LR, McKinlay MA, Collett MS. Relationship of pleconaril susceptibility and clinical outcomes in treatment of common colds caused by rhinoviruses. Antimicrob Agents Chemother 2005;49(11):4492-9.

[7] Lacroix C, Laconi S, Angius F, et al. In vitro characterisation of a pleconaril/pirodavir-like compound with potent activity against rhinoviruses. Virol J 2015;12:106.

[8] Crofton PM, Ahmed SF, Wade JC, et al. Bone turnover and growth during and after continuing chemotherapy in children with acute lymphoblastic leukemia. Pediatric Res 2000;48(4):490-6.

[9] Song JH, Shim A, Kim YJ, et al. Antiviral and Anti-Inflammatory Activities of Pochonin D, a Heat Shock Protein 90 Inhibitor, against Rhinovirus Infection. Biomol Ther (Seoul) 2018;26(6):576-83.

[10] Choi EJ, Jung BJ, Lee SH, et al. A clinical drug library screen identifies clobetasol propionate as an NRF2 inhibitor with potential therapeutic efficacy in KEAP1 mutant lung cancer. Oncogene 2017;36(37):5285-95.

[11] Rotbart HA. Treatment of picornavirus infections. Antiviral Res 2002;53(2):83-98.

[12] Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. Am J Resp Crit Care Med 1997;155(3):1159-61.

[13] Arruda E, Boyle TR, Winther B, Pevear DC, Gwaltney JM, Jr., Hayden FG. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. J Infect Dis 1995;171(5):1329-33.

[14] Jartti T, Gern JE. Rhinovirus-associated wheeze during infancy and asthma development. Curr Respir Med Rev 2011;7(3):160-6.

[15] Kaiser L, Aubert JD, Pache JC, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Resp Crit Care Med 2006;174(12):1392-9.

[16] Saeedfar K, Behmanesh M, Mortaz E, Masjedi MR. Different Gene Expressions of Alpha and Beta Glucocorticoid Receptors in Asthmatics. Iran J Pharm Res 2018;17(2):790-800.

[17] Fader CM, Colombo MI. Autophagy and multivesicular bodies: two closely related partners. Cell Death Diff 2009;16(1):70-8.

[18] Kim SR, Song JH, Ahn JH, et al. Antiviral and anti-inflammatory activity of budesonide against human rhinovirus infection mediated via autophagy activation. Antiviral Res 2018;151:87-96.

[19] Shen C, Cai GQ, Peng JP, Chen XD. Autophagy protects chondrocytes from glucocorticoids-induced apoptosis via ROS/Akt/FOXO3 signaling. Osteoarthritis Cartilage 2015;23(12):2279-87.

[20] Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol 2013;13(10):722-37.

[21] Cirone M. EBV and KSHV Infection Dysregulates Autophagy to Optimize Viral Replication, Prevent Immune Recognition and Promote Tumorigenesis. Viruses 2018;10(11):599.

[22] Shoji-Kawata S, Levine B. Autophagy, antiviral immunity, and viral countermeasures. Biochim Biophys Acta 2009;1793(9):1478-84.