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Efficacy of novel antibody-based drugs against rhinovirus infection: *In vitro* and *in vivo* results

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ABSTRACT

Rhinoviruses (RVs) cause the common cold and are associated with exacerbations of chronic inflammatory respiratory diseases, especially asthma and chronic obstructive pulmonary disease (COPD). We have assessed the antiviral drugs Anaferon for Children (AC) and Ergoferon (containing AC as one of the active pharmaceutical ingredients) in *in vitro* and *in vivo* experimental models, in order to evaluate their anti-rhinoviral and immunomodulatory potential. HeLa cells were pretreated with AC, and levels of the interferon-stimulated gene (ISG), 2′-5′-oligoadenylate synthetase 1 (OAS1-A) and viral replication were analyzed. In a mouse model of RV-induced exacerbation of allergic airway inflammation we administered Ergoferon and analyzed its effect on type I (IFN-β), type II (IFN-γ) and type III (IFN-λ) IFNs induction, cell counts in bronchoalveolar lavage (BAL), cytokine (interleukin (IL)-4; IL-6) and chemokine (CXCL10/IP-10; CXCL1/KC) levels. It was shown that AC increased OAS1-A production and significantly decreased viral replication *in vitro*. Increased IFNs expression together with reduced neutrophils/lymphocytes recruitment and correlated IL-4/IL-6 declination was demonstrated for Ergoferon *in vivo*. However, there was no effect on examined chemokines. We conclude that AC and Ergoferon possess effects against RV infection and may have potential as novel therapies against RV-induced exacerbations of asthma.

1. Introduction

Asthma is the most prevalent respiratory disease affecting 5–10% of adults and 10–15% of children in European societies (Asher et al., 2006, Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys). Asthma occurrence has increased over recent decades and this trend is likely continued.

The major morbidity and health care costs related to asthma are a result of acute exacerbations (Weiss and Sullivan, 2001) which are generally triggered by viral infections of the lower respiratory tract (respiratory syncytial viruses (RSV), human metapneumoviruses (hMPV), coronaviruses, influenza viruses and the most common—human rhinovirus (RV) (Papadopoulos et al., 2007) (Wood et al., 2011). RV accounts for around 60% of virus induced asthma exacerbations and currently no vaccine or antiviral therapies against them exist. RV-induced asthma exacerbations are therefore a clear unmet medical need.

Inhaled steroids are the mainstay of asthma treatment (Johnston et al., 2005), however, in adults they reduce exacerbation frequency

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by only 30–40% (Pauwels et al., 2003) and if combined with long-acting β_2 agonist therapy – by around 40–50% (Pauwels et al., 1997).

The situation is even worse in children. In pre-school and school age children, prophylactic inhaled steroids were ineffective for reduction of exacerbation frequency, duration or severity (Doull et al., 1997), (Oommen et al., 2003). The great majority of asthma exacerbations continue to occur despite use of current therapies, thus indicating them to be of limited efficacy, and therefore necessitating the development of novel treatments.

In this paper we present the results on protective activity of the antiviral drugs AC and Ergoferon for which antiviral efficacy has been already reported (Tarasov et al., 2012; Kudin et al., 2009; Sergeev et al., 2004; Shishkina et al., 2008; Erman et al., 2009; Rafalsky et al., 2016; Shilovsky I.P. et al., 2012), in *in vitro* and *in vivo* models of RV infection.

2. Materials and methods

2.1. Compounds

Ergoferon, AC and diluent controls were blinded and supplied coded by OOO "NPF "MATERIA MEDICA HOLDING" (Russia, Moscow) as ready-to-use aqueous solutions. Active pharmaceutical ingredients (APIs) of the drugs were manufactured based on a novel patented biotechnological platform (US Patent 8535664, 2013) using the following affinity purified rabbit polyclonal antibodies (Abs) as the starting substances: Abs to interferon (IFN)-γ, Abs to CD4-receptor and Abs to histamine for Ergoferon, and Abs to IFN-γ for AC formulation. Therefore, technologically-treated forms of Abs to IFN-γ are an API of both AC and Ergoferon.

The substances were manufactured in accordance with the current EU requirements for GMP starting materials (EU Directive 2001/83/EC as amended by Directive 2004/27/EC) by Angel Biotechnology Holdings plc (UK, Edinburgh) (Abs to IFN-γ) or by AB Biotechnology (Edinburgh, UK) (Abs to CD4 receptor and Abs to histamine). Diluent, namely purified water was used for test drugs manufacturing and served as a control for all examined procedures. Commercially available supplier provided IFN-β (R&D Systems) as a sterile solution (3.69×10^7 IU/ml) which was used for *in vitro* experiments as a reference product. IFN-γ (an aqueous solution, OOO "NPF "MATERIA MEDICA HOLDING") was used as an inducer for cell culture experiments. An anti-huICAM 14C11 was purchased from R&D Systems (UK 2) at concentration of 8 μg/ml and used in *in vivo* research.

2.2. Cell and virus cultures

Human rhinovirus serotype 1B (RV1B) and human rhinovirus serotype 16 (RV16) originally obtained from the ATCC (Cat.No VR-1645 and Cat.No VR-283) were titrated and propagated in HeLa cells to ascertain their 50% tissue culture infective dose ($TCID_{50}$). Then, cell lysates were kept at −80 °C at approximately 4×10^7 $TCID_{50}$/ml for *in vitro* and 4×10^8 $TCID_{50}$/ml for *in vivo* experiments, respectively. Both viruses were negative for *Mycoplasma* infection.

Ohio HeLa cells (ATCC, Cat.No CCL-2) were cultured under sterile conditions, according standard protocol (see Supplementary material) and utilized for *in vitro* studies.

HeLa H1 cells (ATCC, Cat.No CRL 1958) were used for viral propagation in *in vivo* experiments.

All procedures with cell cultures and viruses were carried out in class II facilities equipped with high efficiency filters (99.9%) in accordance with the highest environment protection of EEC standards.

2.3. Mice

6-8 week old human ICAM-1 (huICAM-1) transgenic Balb/c mice (Tg+) were generated in house as described elsewhere (Bartlett et al., 2008) and were housed in individually ventilated cages in a specific pathogen free animal facility, with free access to food and water. Mice were acclimatized for 5 days before treatment. The animals were screened for huICAM transgene expression by PCR. 132 huICAM (Tg+) mice (DOB 23/4/12–16/5/12) were identified and 112 were selected for the present study.

All animal procedures were completed in accordance with UK Home Office guidelines for the ethical approval process (UK project license PPL 70/7234 valid 03/03/2011 to 03/03/2016).

2.4. In vitro experiments

2.4.1. Infecting HeLa cells with RV infection

In order to evaluate the antiviral activity of AC *in vitro*, Ohio HeLa cells were inoculated with RV1B. Following infection, cells were cultured either in the presence of AC, diluent or pure DMEM medium as a negative control (see Supplementary material). AC, reference product (IFN-β at 5 different doses: 1, 10, 100, 1000, 10000 UI/ml) and diluent were added daily for the duration of the study. Cell supernatants from all experimental groups were harvested 48 h after the addition of samples or medium distribution to the plate wells. Results were presented as a pool of 5 experiments performed in duplicates. Data are presented as a percentage (%) of medium-treated cell.

2.4.2. qRT-PCR

Quantitative PCR was carried out using specific primers and probes for each gene (see Supplementary material).

2.4.3. Treatment with IFN-γ

Expression of the antiviral ISG OAS1A was measured in HeLa cells treated with IFN-γ alone and in combination with AC following cell stimulation with IFN-γ at a dose range of 3.9–250 UI/ml. For more details see Supplementary material.

Cells were exposed to AC or mixture DMEM medium/IFN-γ for 24 h. After that, cell lysates were harvested and analyzed for ISG presence. The procedures were performed as 3 independent experiments (in duplicates). Results are presented as % relative to medium-treated cells.

2.5. In vivo experiments

2.5.1. Virus-induced asthma exacerbation model

Transgenic huICAM mice (Tg+) were used in the experiments with RV16, as described in (Bartlett et al., 2008). General information about breeding procedures is detailed in Supplementary material. Four mice from each group remained non-infected and were considered as the baseline (0 h). Treatment group ($n = 40$) mice received Ergoferon twice daily for 5 days before and 4 days after inoculation with the virus. Negative control group ($n = 20$) was given diluent in a comparable dosing regimen as for Ergoferon group. The group receiving reference product ($n = 40$) was dosed with anti-huICAM 14C11 intranasally 2 h prior to inoculation (Traub et al., 2013).

The base scheme of experiment presented on Fig. 1.

2.5.2. qRT-PCR

Total RNA was extracted from the upper left lobe of the mouse lung, and placed in RNA later (Qiagen), prior to RNA extraction and cDNA synthesis. To conduct qPCR experiments specific primers and probes for each gene were used as described in manufactures

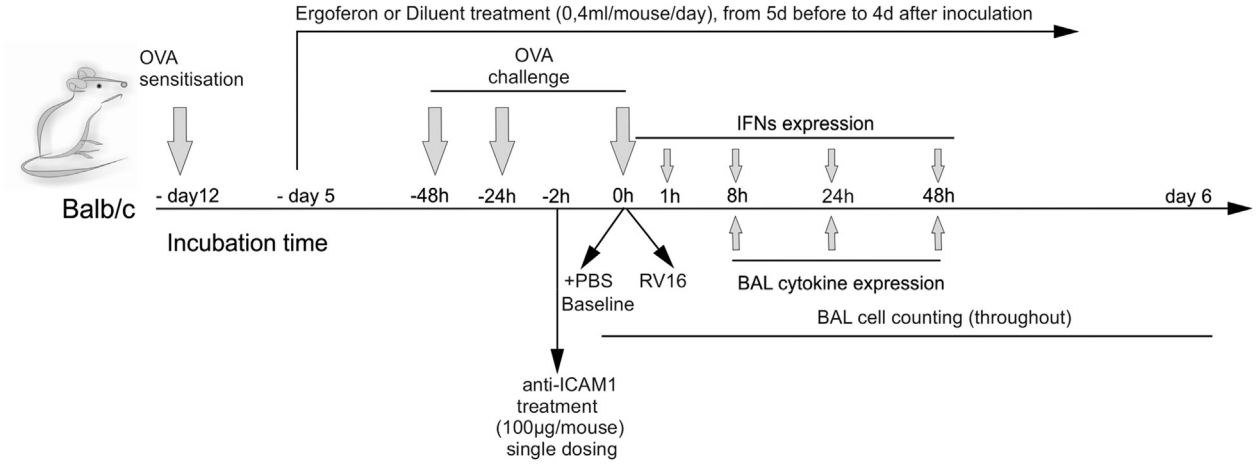

Fig. 1. Timeline of *in vivo* experiment.

procedures. IFN gene expression was measured at − 0, 1, 8, 24, 48 h.

2.5.3. BAL analyses

Lungs of mice were cannulated via the trachea and lavaged with 1.5 ml of BAL buffer (RPMI, 50 mM EDTA). Total BAL was separated into cell and supernatant fractions. Cell counting were performed in trypan blue (Sigma-Aldrich, UK) using a hemocytometer. Changes in BAL level were examined immediately (0 h) and 8, 24, 48 h and on day 6 after viral inoculation.

Cytokine (IL-6, IL-4) and chemokine (IFN-γ-induced protein 10 (IP10/CXCL10), CXCL1/KC) analysis was determined in cell-free BAL fluid by ELISA (RnD Systems, USA) according to the manufacturer's instructions 8, 24 and 48 h post infection (p.i.).

2.6. Statistical analysis

In vitro and *in vivo* data were distributed normally and are presented as means and standard error of the mean (SEM). Depending on experiments, results were presented as % normalized to medium-treated cells. Comparisons of different groups were analyzed by ANOVA (R 3.2.1 software R Foundation for Statistical Computing, Vienna, Austria) with Post-hoc Dunnett's test. For *in vivo* experiments Kruskal-Wallis test with Post-hoc Nemenyi analysis were carried out.

P-values below 0.05 were considered significant.

3. Results

3.1. Influence of AC on RV replication in vitro

In an Ohio HeLa cell model of RV infection we assessed the effect of AC on viral RNA (vRNA) abundance. RV1B was titrated down to 0.025 MOI and used *in vitro,* as it was shown that reference product (IFN-β) could inhibit viral replication in dose-dependent manner. This reduction was statistically significant ($p < 0.001$) for IFN-β at 10000, 1000 and 100 UI/ml doses (Fig. 2).

AC at dilutions of ½ and ¼ were tested alongside IFN-β. Working dilutions were defined based on previously conducted cytotoxicity studies. 48 h p. i., RV1B vRNA was significantly reduced by AC versus DMEM medium alone (Fig. 3, $p < 0.001$). AC reduction of vRNA was observed in a dose dependent manner, with ½ dilution showing greater reduction than ¼ dilution (2-fold decrease).

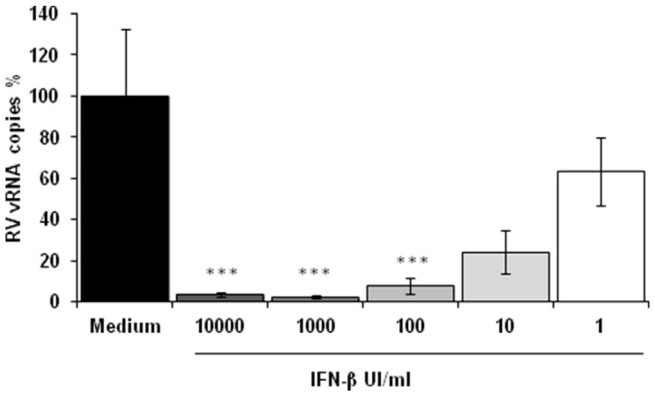

Fig. 2. Effects of IFN-β on mRNA abundance 48 h p.i. HeLa cells were seeded and treated with IFN-β diluted in DMEM medium and were infected with RV1B (0.025 MOI) 24 h later. Freshly prepared IFN-β or medium were replaced every 24 h. At 48 h p.i., cell supernatants were harvested and titrated on HeLa cells to calculate the $TCID_{50}$ ($n = 8$ per IFN-β groups; $n = 6$ per medium group). ***: $p < 0.001$, versus medium treated cells. Results were analyzed with one-way ANOVA with Post-hoc Dunnett's test and expressed as % relative to medium-treated cells.

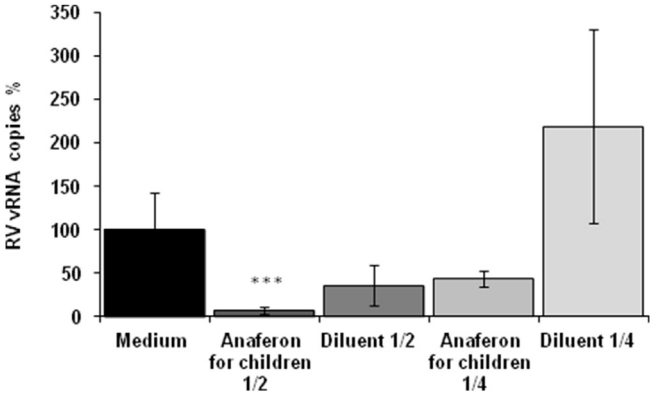

Fig. 3. Rhinovirus vRNA decrease in the presence of AC. HeLa cells infected with RV1 (MOI 0.025) test sample, diluent or medium were added 24 h prior to infection. RV1 induced mRNA levels of RV replication. Results were measured 48 h p.i. by real-time qPCR ($n = 5$, in duplicates). ***: $p < 0.001$, versus medium treated cells. Data were analyzed by one-way ANOVA with Post-hoc Dunnett's test and presented as % relative to medium-treated cells.

3.2. IFN-γ enhancement of OAS1-A expression under AC treatment in vitro

In order to assess the influence of AC treatment on ISG

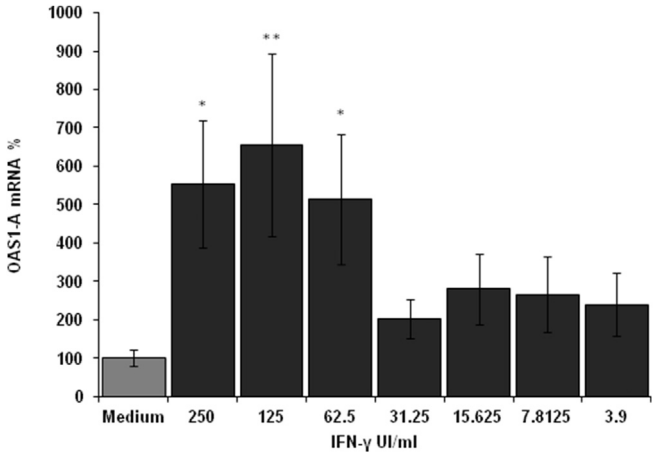

Fig. 4. The effect of a dose range of IFN-γ (250–3.9 IU/ml) on the number of OAS1-A copies. HeLa cells were seeded and treated with IFN-γ at 250–3.9 IU/ml in DMEM medium. After 24 h, cell lysates were harvested. The number of OAS1-A copies was measured by qPCR normalized to 18S rRNA and was expressed as normalized copy number per μl cDNA (n = 6). **: $p < 0.01$; *: $p < 0.05$ as compared to medium treated cells. Results were analyzed by one-way ANOVA with Post-hoc Dunnett's test and presented as % relative to medium-treated cells.

expression, AC at ¼ dilution and IFN-γ in a range of doses (3.9–250 UI/ml) were added to HeLa cells. OAS1-A gene was analysed as an important antiviral gene involved in innate immune responses and as an ISG capable of regulating RNA-based virus replication.

As expected, OAS1-A was barely detectable in the absence of IFN-γ, but was strongly stimulated at 250 IU/ml dose of IFN-γ, showing significant level of ISG induction (Fig. 4). OAS1-A gene level increased in dose-dependent manner and exceeded that of control cells after stimulation with IFN-γ at 250, 125 and 62.5 IU/ml doses, at least 5-times (Fig. 4). In view of this, the dose range of 250–3.9 IU/ml was considered ideal to assess the effect of AC.

In contrast to diluent, in case of which the level of OAS1-A was decreased; AC had a promoting effect. The trend has been particularly pronounced at doses of 250, 31.5 and 15.625 IU/ml of IFN-γ. However, observed increases were not statistically significant (Fig. 5).

3.3. Ergoferon and RV infection in vivo

3.3.1. RV16 induced IFN gene expression in vivo

Using a mouse model of major group RV16-induced exacerbation of allergic airway inflammation, we tested the efficacy of oral treatment with Ergoferon on induction of an antiviral environment as well as reduction in airways inflammation. Ergoferon significantly increase the IFN-β ($p < 0.05$) gene expression 48 h after infection in comparison with reference (Fig. 6). The amount of IFN-γ was gradually elevated during the studies of experiment, but results were insignificant ($p > 0.05$). An augmentation trend in IFN-λ gene production was observed only in the presence of Ergoferon, at 24 h p.i., for other groups the quantity of gene was almost at the initial level with no differences between the groups (Fig. 6C).

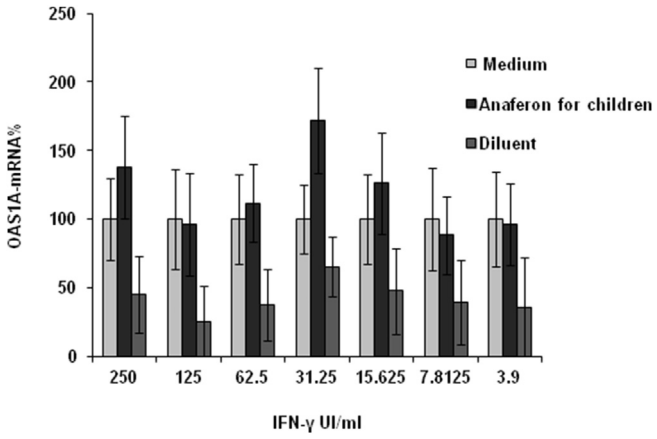

Fig. 5. Effect of AC at ¼ dilution combined with an IFN-γ dose range. HeLa cells were seeded and treated with the mixture of IFN-γ at 250–3.9 IU/ml AC, diluent or pure DMEM medium. After 24 h of IFN-γ stimulation, mRNA was extracted, the results were normalized to 18S rRNA and were expressed as normalized copy number per μl cDNA (n = 6). Data were presented as % relative to medium-treated cells.

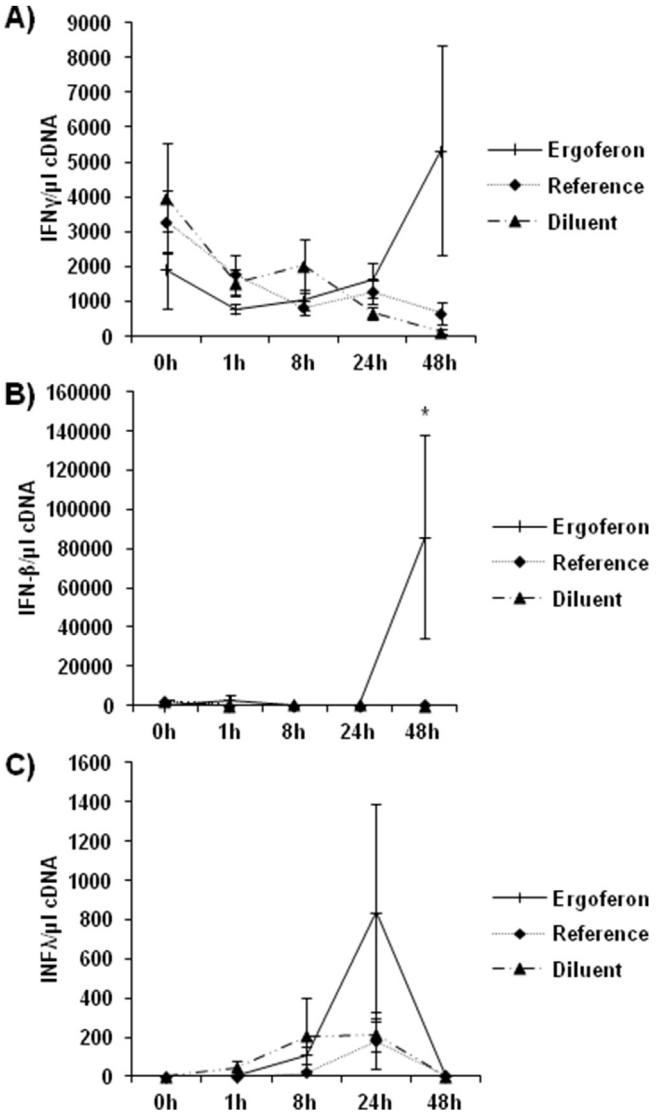

Fig. 6. RV-16 induction of antiviral IFNs in transgenic Balb/c mice (Tg+). Animals were infected with RV16 (2.5×10^6 $TCID_{50}$) and were given a test sample or diluent twice daily for 5 days before and 4 days after viral inoculation, reference product (100 μg per mouse) was administered intranasally 2 h prior to infection. PBS treated mice were used as the baseline; results were measured at 0 h time point. RV16-induced mRNA levels of A) IFN-γ, B) IFN-β and C) IFN-λ were obtained at 5 time points - 0, 1, 8, 24, 48 h p.i. (n = 8 for Ergoferon and reference groups; n = 4 mice per diluent group). *: $p < 0.05$ difference between Ergoferon and reference group. Data were assessed by Kruskal-Wallis test with Post-hoc Nemenyi analysis and presented as mean ± SEM.

3.3.2. Results of BAL cell counting

We next sought to determine the effect of Ergoferon treatment on accumulation of inflammatory cells within BAL following RV

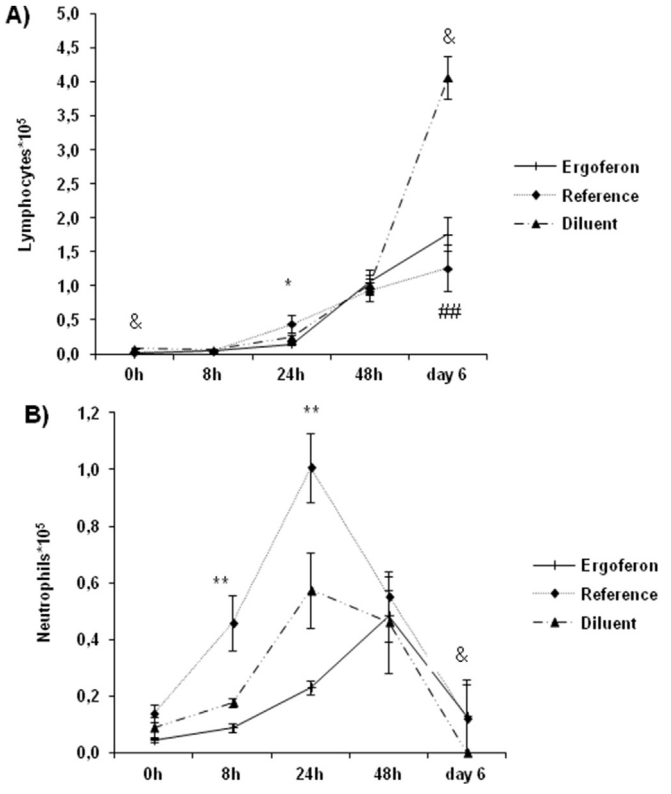

Fig. 7. Bronchoalveolar cell profiles in transgenic Balb/c mice (Tg+) in time. Animals were infected with RV16 (2.5×10^6 $TCID_{50}$) and were given the test sample or diluent twice daily for 5 days before and 4 days after viral inoculation, reference product (100 μg per mouse) was administered intranasally 2 h prior to infection. PBS treated mice were used as the baseline; results were measured at 0 h time point. A) Lymphocyte profiles *: $p < 0.05$ (24 h) difference between Ergoferon and reference; &: $p < 0.05$ (0 h; day 6) difference between Ergoferon and diluent; ##: $p < 0.01$ statistical difference, reference product versus diluents B) Neutrophil **: $p < 0.01$ (8 and 24 h) difference between Ergoferon and reference product; &: $p < 0.05$ difference between Ergoferon and diluent. Total count of BAL cells were assessed by cytospin at 5 time points - 0, 8, 24, 48 and 6 days p.i. ($n = 8$ for Ergoferon and reference groups; $n = 4$ mice per diluent group). Data were assessed by Kruskal-Wallis test with Post-hoc Nemenyi analysis and presented as mean ± SEM.

infection. Ergoferon showed significant efficacy in altering cell trafficking. The number of lymphocytes was decreased ($p < 0.01$) at 24 h p.i. as compared to reference product, and on day 6 ($p < 0.05$) versus diluent (Fig. 7). A difference between reference - anti-huICAM Abs and diluent on day 6 p.i. ($p < 0.01$) was also shown. The number of neutrophils in mice treated with Ergoferon was significantly reduced at 8 h ($p < 0.01$) and 24 h p.i. ($p < 0.001$) than those in the reference group. Neutrophil counts in the presence of reference were higher than that in diluent-treated mice, but these differences were not statistically significant. On day 6, neutrophils returned to the baseline values, however, cell numbers in diluent treated mice remained lower than those of Ergoferon ($p < 0.05$) and reference groups.

There were no statistically significant changes in macrophage and eosinophil counts for each of examined groups (data are not shown).

3.3.3. BAL cytokines

Level of cytokines and chemokines within the airways were determined via ELISA of BAL. Results of the experiments on BAL cytokine levels indicated that Ergoferon increased IL-6 levels at 24 h p.i relative to reference product ($p < 0.05$) and then had a sharp and significant decline compared to diluent at 48 h ($p < 0.05$ versus diluent). The reference group had higher level of BAL cytokines as compared to Ergoferon ($p < 0.05$) and diluent at 8 h p.i.

The dynamic of IL-4 expression was similar to that of IL-6. Statistically significant reduction between the groups was observed only at 48 h p.i ($p < 0.05$ Ergoferon vs diluent).

Chemokine levels in BAL were analyzed in terms of CXCL10/IP-10 and CXCL1/KC expression. Results were similar for both analytes. Data was statistically insignificant through the whole timeline between the groups. The data obtained for the reference group corresponded to those for Ergoferon treated mice (Fig. 8C–D).

4. Discussion

A vital need exists for new treatment options against RV infection (Song, 2016; Hyden, 2013; Weinberger, 2004; Zhu et al., 2014). It is well known that RV entering the host cells leads to direct and rapid activation of PI-3 kinase, downstream activation of protein kinase B (Akt) and nuclear factor kappa B (NF-κB) (Christman et al., 2000). Infection results in IFN responses, which are deficient in asthma (Contoli et al., 2006), generation of pro-inflammatory cytokines (IL-1, IL-6, IL-8), neutrophil and lymphocyte infiltration (Zhu et al., 2014)-(Papi et al., 2006). Therefore, one of the potential approaches in therapy of RV infection is to augment host IFN responses and to induce the production of antiviral ISG expression (Dhariwal et al., 2013).

Here, we were aimed to assess the expression of antiviral gene mRNA, viral replication, BAL cells and cytokines in *in vitro* and *in vivo* models. *In vitro* experiments were carried out using HeLa cells as a standard model to analyze the effects of interventions on RV coping (Hofer et al.,1994; Shafren et al., 1997). OAS1-A, being an important agent involved in one of the most established antiviral systems – the coupled 2′-5′-oligoadenylate synthetase (OAS)/RNase L pathway (Liang et al., 2006; Solinas et al., 1993; Shulman and Revel, 1980; Castelli et al., 1998) was chosen among the list of IFN- γ induced genes. Here we demonstrated that the ISG response in the presence of AC had an upward dynamic: % of OAS1A expression for the whole IFN-γ dose range exceeded the diluent levels at least two-fold. Moreover, an enhanced OAS1A production compared to medium-treated cells was detected at 250, 62.5, 31.25 and 15.625 UI/ml IFN-γ.

RV replication data corresponded to ISG mRNA research. Indeed, vRNA abundance was statistically lower for the ½ dose of AC versus medium treated cells. Results were comparable to that of IFN-β (100 UI/ml). This behavior was also true for ¼ dose of AC, however, there was no statistical significance between analyzed groups. The observed effect could be potentially explained by low sample concentration which was not sufficient to inhibit RV replication with equal respect to IFN-β (100 UI/ml), but still enough to stimulate OAS1A production.

It was previously shown that a key mechanism of AC action is the ability to improve ligand-receptor interactions of IFN-γ and IFN-γ receptor via conformational changes of the IFN-γ molecule (Zhavbert et al., 2013; Epstein, 2013). These changes lead to functional activity regulation, production of endogenous IFNs, hence affecting the antiviral defense. Consequently, OAS1A induction demonstrated in the experiments corresponds to the AC mechanism of action and coincides with the literature, where an increased production of ISGs is connected to the augmentation of IFN induction. Most likely that AC augments other than OAS1A genes that are also important in the anti-viral response. In fact, there are over 380 characterized ISGs (Der et al., 1998), some of which are also induced by IFN- γ. Unfortunately, it was beyond the scope of the present study to measure other ISGs, stressing in this respect the necessity for conducting additional studies to address the mechanism of the observed anti-RV effect.

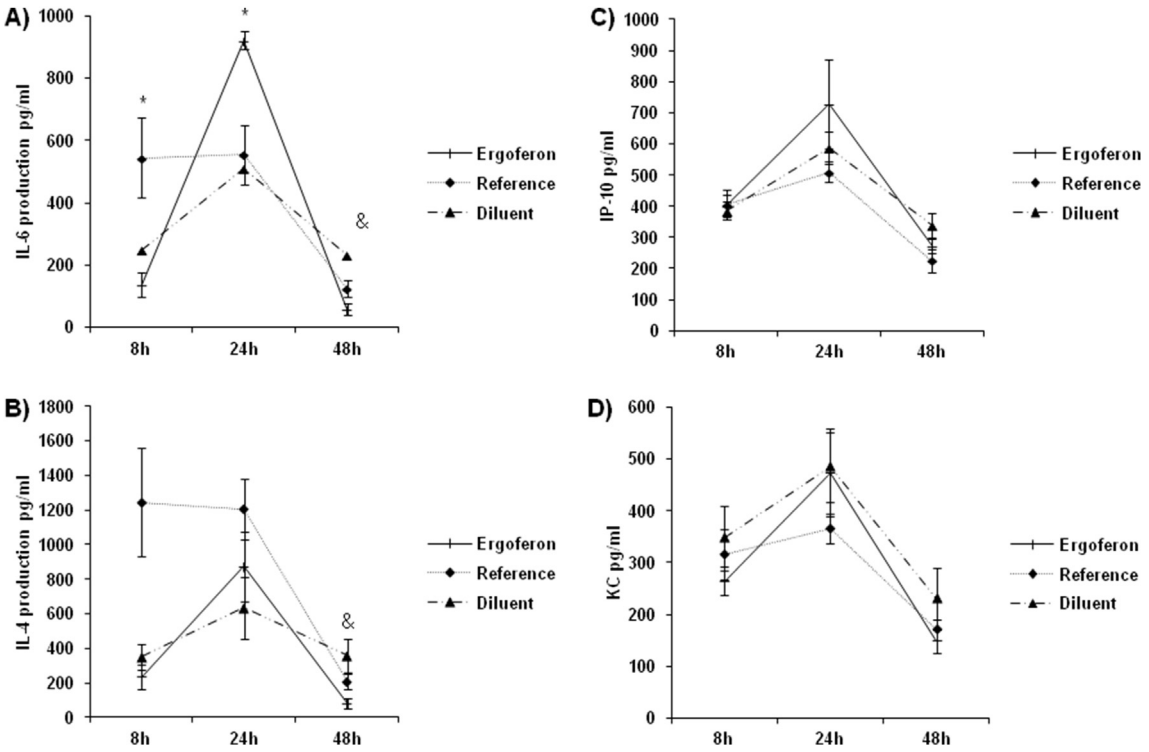

Fig. 8. Pro-inflammatory cytokine and chemokine levels of bronchoalveolar lavage supernatants in transgenic Balb/c mice (Tg+). Animals were infected with RV16 (2.5×10^6 $TCID_{50}$) and were given the test sample or diluent twice daily within 5 days before and 4 days after viral inoculation, 100 μg reference product per mouse was administered intranasally, 2 h prior to infection. A) IL-6; B) IL-4; C) CXCL10/IP-10; D) CXCL1/KC chemokine levels *: $p < 0.05$ difference between Ergoferon and reference groups; &: $p < 0.05$ significant difference between Ergoferon and diluent. Results determined by ELISA 8 h, 24 h and 48 h p.i. ($n = 8$ for Ergoferon and reference groups; $n = 4$ mice per diluent group). Data were assessed by Kruskal-Wallis test with Post-hoc Nemenyi analysis and are presented as mean ± SEM.

AC, being a monocomponent drug (contains as API only technologically-treated Abs to IFN-γ) was used in the initial experiments, to see whether we could demonstrate antiviral activity against RV *in vitro*, where only innate responses can be induced. Having observed encouraging results we then opted to use Eroferon, which is in addition to technologically-treated Abs to IFN-γ, also contains Abs to CD4-receptor and Abs to histamine, in order to show whether we could in addition observe immunomodulatory/anti-inflammatory effects in a more complex *in vivo* environment where both innate and acquired immune responses, as well as inflammatory and Th2 responses can be studied. It was previously shown that Ergoferon could influence on various elements of host antiviral protection systems, inducing endogenous IFNs, CD4 (Emelyanova et al., 2016) and histamine receptors (Zhavbert et al., 2014), generally expressed by macrophages, dendritic cells, and T-cells (both Th1 and Th2).

In this study we report that treatment with Ergoferon improves virus-induced exacerbation of ovalbumin-induced allergic airway inflammation. Ergoferon resulted in an increase of IFN-mRNA expression (mainly IFN-β) with the greatest degree detected at 48 h p.i. The IFN gene expression levels were assessed in RNA extracted from the upper left lobe of the mouse lung, we therefore cannot say which cell type they were induced in.

Additionally, decreases in lymphocytic and neutrophilic airway inflammation were also observed after Ergoferon exposure. It is known that, Th2 cytokines play an important role in the pathophysiology of allergic diseases such as asthma, and could be used as potential therapeutic targets for their future management (Beale et al., 2014) We have previously shown in this model of RV-induced exacerbation of allergic airway inflammation that on day 7 p.i. large numbers of lymphocytes in the lungs and airways of exacerbated mice have Th2 characteristics. It is therefore very likely that Th2 cell recruitment has been suppressed by Ergoferon given the magnitude of the reduction in total lymphocytes reported herein on day 6. A suppressed Th2 response is supported by the IL-4, IL-6 suppression (Steinke and Borish, 2001) observed in Ergoferon treated mice as compared to the diluent group at 48 h p.i. (Fig. 8A and B).

Neutrophils are also an important component of RV-induced inflammation and may contribute to both upper and lower airway responses in the process of RV infection (Turner, 1990; Jarjour et al., 2000). Temporal progression in responses to RV eventually increases airway neutrophilic inflammation and could, contribute to exacerbations of asthma (Gern et al., 2000). Thus, neutrophilic reduction in the Ergoferon-treated group observed at 8 h and 24 h p.i. could be connected to a reduction in inflammatory processes.

Surprisingly for us, BAL cytokine profiles caused a sudden increase in IL-6 and IL-4 production 24 h p.i. with subsequent and significant reduction at 48 h as compared to diluent treated group. The biological meaning of observed IL-6 increase at 24 h at this point is not clear. We cannot differentiate whether the increase in pro-inflammatory cytokines was due to IFN activation (started at 8 h p i), but still it did not result in increased lymphocytic or neutrophilic inflammation in the lungs. To sum it up, the decrease in both cell attraction and cytokine expression noticed for Ergoferon at 48 h p.i., could be an advantageous mechanism which is lead to attenuation of airway inflammation (Zhu et al., 1996). However, further studies will be needed to clarify the effect of Ergoferon on these and other cytokine and cellular responses to RV infection in asthma.

Interestingly, the level of BAL cell counts and cytokines

(especially the level of neutrophils and IL-4) for the huICAM 14C11 group was higher as compared to Ergoferon-treated mice and was equal to the diluent groups. Probably, huICAM 14C11 reduced RV-induced allergic airway inflammation after 24 h p.i. For instance, Traub and colleagues showed significant reduction in BAL cell profiles with huICAM 14C11 at the same doses (100 μg/mice) only at 48 h p.i., while there were no measurements at the earlier time-points (Traub et al., 2013).

Taking together, observed results of the study demonstrate an antiviral effect for AC *in vitro* and beneficial responses with Ergoferon *in vivo*. AC treatment showed an evident induction in OAS1A expression together with reduction in viral replication. Ergoferon in its turn present an increase in IFN production and reduction in cell and cytokine profiles *in vivo*.

To sum it up, proposed drugs could therefore be candidates for development of novel treatments, for illnesses, including induced by rhinoviruses.

Conflict of interest

MRE, RPW, NWB, JA, EB, LG, MRK and SLJ received consultancy payments from OOO "NPF "MATERIA MEDICA HOLDING" for performing these studies. EAG and SAT are employees and EOI is a founder of OOO "NPF "MATERIA MEDICA HOLDING".

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2017.03.017.

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