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Antiviral activity of an aqueous extract derived from *Aloe arborescens* Mill. against a broad panel of viruses causing infections of the upper respiratory tract

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ABSTRACT

Background: A number of antiviral therapies have evolved that may be effectively administered to treat respiratory viral diseases. But these therapies are very often of limited efficacy or have severe side effect. Therefore there is great interest in developing new efficacious and safe antiviral compounds e.g. based on the identification of compounds of herbal origin.
Hypothesis: Since an aqueous extract of *Aloe arborescens* Mill. shows antiviral activity against viruses causing infections of the upper respiratory tract *in vitro* we hypothesised that a product containing it such as Biaron C® could have an antiviral activity too.
Study design: Antiviral activity of Bioaron C® an herbal medicinal product consisting of an aqueous extract of *Aloe arborescens* Mill., Vitamin C, and *Aronia melanocarpa* Elliot. succus, added as an excipient, was tested *in vitro* against a broad panel of viruses involved in upper respiratory tract infections.
Methods: These studies included human adenovirus and several RNA viruses and were performed either with plaque reduction assays or with tests for the detection of a virus-caused cytopathic effect.
Results: Our studies demonstrated an impressive activity of Bioaron C® against members of the orthomyxoviridae – influenza A and influenza B viruses. Replication of both analysed influenza A virus strains – H1N1 and H3N2 – as well as replication of two analysed influenza B viruses – strains Yamagata1 and Beiying – was significantly reduced after addition of Bioaron C® to the infected cell cultures. In contrast antiviral activity of Bioaron C® against other RNA viruses showed a heterogeneous pattern. Bioaron C® inhibited the replication of human rhinovirus and coxsackievirus, both viruses belonging to the family of picornaviridae and both representing non-enveloped RNA viruses. *In vitro* infections with respiratory syncytial virus and parainfluenza virus, both belonging to the paramyxoviridae, were only poorly blocked by the test substance. No antiviral activity of Bioaron C® was detected against adenovirus – a non-enveloped DNA virus.
Conclusions: These results represent the first proof of a selective antiviral activity of Bioaron C® against influenza viruses and create basis for further analyses of type and molecular mechanisms of the antiviral activity of this herbal medicine.

Introduction

A number of antiviral therapies are effectively administered to treat respiratory viral diseases, thus providing the physician with a range of compounds including amantadine (Hay et al. 1985), neuraminidase inhibitors (Calfee and Hayden 1998) and nucleoside analogues (Fyfe et al. 1978; Hruska et al. 1990). Therapies with these compounds are very often of limited efficacy and on the other hand, side-effects and systemic toxicity may limit their application,

Abbreviations: Adeno 5, adenovirus C subtype 5; BGM, buffalo-green-monkey cells; CA9, coxsackievirus subtype 9; CPE, cytopathogenic effect; EC50, effective concentration 50; FluA, influenza A virus; FluB, influenza B virus; HEp-2, human epithelial cells; HRV14, human rhinovirus B subtype 14; IC50, inhibitory concentration 50; MDCK, Madin–Darby–Canine–Kidney cells; M.O.I., multiplicity of infection; Para 3, parainfluenza virus type 3; PFU, plaque-forming units; RSV, respiratory syncytial virus.

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particularly in paediatric, geriatric and immunocompromised patients (Bacon et al. 2003, Cassady and Whitley 1997, Englund et al. 1990; Hayden et al. 1983; Janai et al. 1990; Reusser 1996). As a result, there is great interest in developing new efficacious and safe antiviral agents. Plant extracts have been widely used in traditional medicine (DeClercq 2004) due to their antimicrobial and antiviral activity.

Interestingly enough, about 10% of more than 4000 species studied showed a significant antiviral efficacy *in vitro* (Che 1991), although in most studies a systematic investigation of their activity against a broad panel of viruses still remains to be done.

Summerfield et al. described the activity of *Acanthospermum hispidum* DC. (Asteraceae) (Summerfield et al. 1997) against animal pathogenic herpes viruses, pseudorabiesvirus (PRV) and bovine herpesvirus 1 (BHV-1). In 2001 we showed (Glatthaar-Saalmüller et al. 2001) the antiviral activity of an extract from *Eleutherococcus senticosus* Maxim. (Araliaceae), against human rhinovirus (HRV), human respiratory syncytial virus (RSV) and influenza A virus, which was discussed as RNA-virus specific reactivity. Michaelis et al. (2011) investigated the influence of a standardised extract of *Pelargonium sidoides* DC. (Geraniaceae), for the treatment of acute bronchitis, on replication of a panel of respiratory viruses. The authors were able to show that concentrations up to 100 μg/ml interfered with replication of seasonal influenza A virus strains (H1N1, H3N2), RSV, human coronavirus, parainfluenza virus, and coxsackie virus but did not affect replication of highly pathogenic avian influenza A virus (H5N1), adenovirus, or HRV.

Antiviral potential has also been demonstrated for a combination of Gentian root, Primula flower, Elder flower, Sorrel herb, and Verbena herb, which reduced *in vitro* the spreading of influenza A, RSV and parainfluenza type 1 virus (Para 1) (Glatthaar et al. 2012).

In the present study the antiviral effect of an herbal medicine used for the prevention and treatment of upper respiratory tract infections consisting of *Aloe arborescens* Mill. (Xanthorrhoeaceae), Vitamin C and as excipient *Aronia melanocarpa* Elliot. (Rosaceae) succus, named Bioaron C® has been investigated.

A. arborescens has been used in the treatment of upper respiratory tract infections in Central and Eastern European countries for many decades. Recent pre-clinical studies with Bioaron C® showed *in vitro* a clear dose-dependent antiviral activity against human rhinovirus 14 (HRV 14) (Glatthaar-Saalmüller et al. 2012). In addition clinical studies showed that anti-inflammatory and antiviral activities contribute to its therapeutic efficacy against viral infections of the upper respiratory tract (Bastian et al. 2013). Last published data about Bioaron C® refer to observational studies involving children characterised by susceptibility to upper respiratory tract infections. The results of this study suggest that Bioaron C® can be successfully used in the treatment of viral infections and as an adjuvant during antibiotic therapy because of shortening duration of infection and causing milder course of the infection (Fal and Michalak 2013).

The following study focuses on the antiviral activity of Bioaron C® against some viruses causing infections of the upper respiratory tract to get more detailed information about the specificity of the plant extract and to get some hints for a possible mode of action. Therefore the antiviral activity of Bioaron C® was tested on a DNA virus (adenovirus 5, Adeno 5) as well as on a broad panel of enveloped or non-enveloped RNA viruses. For non-enveloped viruses HRV14 (responsible for the majority of acute respiratory infections in both children and adults) and coxsackievirus type 9 (CA9 – tend to infect the skin and mucous membranes, causing herpangina, acute haemorrhagic conjunctivitis, and hand-foot-and-mouth (HFM) disease) were included in the analyses. Among the enveloped RNA viruses we chose parainfluenzavirus 3 (Para 3) and RSV – both associated with bronchiolitis and pneumonia. But the main focus of these studies was set on the reactivity of the test substance against different strains of influenza viruses belonging either to Type A influenza viruses (influenza H1N1 and H3N2) or to type B influenza viruses (influenza B Yamagatal and influenza B Beiying).

Material and methods

Test substance

Bioaron C® syrup is a commercial available herbal medicinal product of an aqueous extract of *A. arborescens* plus Vitamin C from Phytopharm Klęka S.A., Klęka 1, 63-040 Nowe Miasto nad Wartą, Poland. As excipients sucrose, concentrated chokeberry juice (*A. melanocarpa*), sodium benzoate, and purified water are used. The aloe extract of *A. arborescens* leaves is the key ingredient of the syrup which contains 1920 mg of the extract and 51 mg Vitamin C per 5 ml. In the present investigation the original product Bioaron C® has been investigated without sugar.

A preparation with all ingredients with the exception of *A. arborescens* and sugar served in all assays as a negative (solvent) control.

Medicinal products based on *A. arborescens* aqueous extracts are widely used in Poland, Russia, and Ukraine with a focus on upper respiratory tract infections in children and lack of appetite during or after long-lasting illness (Bastian et al. 2013).

Aloe arborescens *Mill.*

In the European Pharmacopoeia and HMPC monographs different Aloe species, especially *Aloe ferox* Mill. and *Aloe barbadensis* Mill., are described for their laxative effects based on their high content in hydroxyanthraquinones. However, *A. arborescens* is characterised by a very low anthranoid content and therefore without laxative effects (Phytopharm Klęka S.A., Klęka, personal communication). The natural habitats of *A. arborescens* are mountain regions of Southern Africa. *A. arborescens* is also grown as a source material for medicinal, cosmetic, and food uses in various countries (China, Israel, Italy, Japan, Poland (green houses), and in Ukraine (Crimea peninsula)) (Jambor 2012). Plants used for this study were cultivated and harvested in a greenhouse plantation by Phytopharm Klęka S.A., in Poland.

Chromatograms of Bioaron C®

Aloenin A is an analytical marker specified for Bioaron C® and *Aloe* extractum fluidum as an active substance of this medicinal product. Reproducibility of the manufacturing process was confirmed by checking of Aolenin A level in every batch of the active substance and the medicinal product (Phytopharm Klęka S.A., Klęka) Both analytical procedures were validated according to ICH guidance (CPMP/ICH/381/95 and CPMP/ICH/281/95). The range derived from linearity studies of the assay method is covering concentration from 2 to 12 mg of Aloenin A per 100ml of *Aloe* extractum fluidum, whereas the average content of the marker is 9 mg/100 ml. Aloenin A content in Bioaron C® varied between 2.2 and 3.8 mg/100 ml, the average value was 3.0 mg/100 ml. The identity of Bioaron C® has been confirmed by the detection of Aloenin A. The chromatogram profile of Bioaron C® together with Aloenin A is presented in Fig. 1 (a: Aloenin A and b: Bioaron C®).

Determination of Vitamin C in Bioaron C® was conducted by a direct redox titration method in acidic condition with an iodine solution ($c = 0.05$ mol/l). One millilitre of 0.05 mol/l iodine solution corresponded to 8.81 mg of Vitamin C. Molality of the titrant was determined by direct titration by means of a 0.1 mol/l sodium thiosulphate solution.

For the *in vitro* assays the test substance was diluted as described with the respective cell culture media. As control served a "solvent control" without the active component *A. arborescens* prepared under the same conditions.

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