ELSEVIER

International Journal of Food Microbiology 42 (1998) 147–158

International Journal of Food Microbiology

Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81

I. Ivanova[a,*], V. Miteva[b], Ts. Stefanova[c], A. Pantev[a], I. Budakov[b], S. Danova[a], P. Moncheva[a], I. Nikolova[b], X. Dousset[d], P. Boyaval[e]

[a]*Department of Microbiology, Faculty of Biology, Sofia University, 8 Dragan Tzankov Boulevard, 1421 Sofia, Bulgaria*
[b]*Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. Bonchev Street, 1113 Sofia, Bulgaria*
[c]*ELBY Bulgaricum PLC, Complex Lagera 44 A, 1612 Sofia, Bulgaria*
[d]*ENITIAA, Laboratoire de Microbiologie, Rue de la Géraudière, 44072 Nantes Cedex, France*
[e]*INRA, Laboratoire de Recherches de Technologie Laitière, 65 Rue de Saint-Brieuc, 35042 Rennes Cedex, France*

Received 7 August 1997; received in revised form 30 January 1998; accepted 21 April 1998

Abstract

A new bacteriocin, produced by *Streptococcus thermophilus* 81 has been isolated, purified and characterized. By its heat sensitivity and broad inhibitory spectrum it does not resemble any other *S. thermophilus* bacteriocin. The mode of action is bacteriostatic. This peptide of 32 amino acids is efficient against several *Bacillus* species, *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. This bacteriocin is heat labile but its activity was not altered by pH variation from 3 to 10. Six months of storage at 40°C did not influence the activity. The inactivation by detergents and the inability to resolve the protein in SDS–PAGE supposes a more complex structure or a possible stabilizing effect of other molecules. The low sensitivity of *Lactobacillus delbrueckii* subsp. *bulgaricus* to the isolated bacteriocin suggests that *S. thermophilus* 81 may be used in yoghurt starters. © 1998 Elsevier Science B.V.

Keywords: Bacteriocin; Lactic acid bacteria; *Streptococcus thermophilus*

1. Introduction

Streptococcus thermophilus is a species with widespread application in milk fermentation processes. As a component of starters for yoghurt and different cheeses *S. thermophilus* is usually cultivated in combination with other lactic acid bacteria (LAB). In mixed cultures it is necessary to use strains which do not negatively influence the growth of each other. In this respect it is important to investigate the inhibitory activity of the starter components.

The strains of *S. thermophilus* are rare producers of bacteriocins and the latter are not well studied. Large amount of research on LAB bacteriocins was

*Corresponding author. Tel.: +359 2 6330341; fax +359 2 656678; e-mail: vitanova@ns.biofac.uni-sofia.bg

0168-1605/98/$19.00
PII: S0168-1605(98)00067-1

COMPARATIVE ANALYSIS OF THE VP1 STRUCTURAL PROTEIN GENE OF THE DISOXARIL MUTANTS OF COXSACKIEVIRUS B1

Ivanka Nikolova[1], Roumena Petkova[2], Stoyan Chakarov[2], Angel S. Galabov[1]

[1]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Scientific Technological Service, Ltd., Sofia, Bulgaria

Abstract

Analysis of the RNA sequence of the disoxaril-resistant and disoxaril-dependent mutants of the Coxsackievirus B1 was carried out. The wild type disoxaril-sensitive strain (Connecticut 5), two disoxaril-resistant mutants, one of them produced in FL cells and the other one isolated from brains of newborn mice, infected with Coxsackievirus B1 and treated with disoxaril, and a disoxaril-dependent mutant obtained from the resistant strain underwent 9 passages in cell culture were included in the present study.

A RT-PCR assay with primer sets selected from a region of the Coxsackievirus B1 genome coding for the capsid protein VP1 was carried out. A parallel comparative analysis of the sequences of resulting fragments from the disoxaril mutants studied and the GenBank sequence of origin of the VP1 gene of Coxsackievirus B1 was performed with the BLAST alignment tool.

The resistant mutant obtained in mice was found to be very similar to the strain, developed in cell cultures (97%). High degree of similarity (97%) between the resistant mutant produced in cell cultures and the dependent strain was observed, as well. The similarity of the resistant and dependent mutants to the wild strain was only 91-92%.

Introduction

The viruses in the family Picornaviridae are clinically important pathogens causing variety of human diseases. The main reason for the failure of antipicornaviral therapy is the quick resistance development. Random genetic mutation can occur at a very high frequency in a number of RNA viruses and especially in picornaviruses (Sierra et al., 2000; Crotty et al., 2001). The high number of mutants in the picornaviral population is a result of point mutations occuring randomly during the viral replication.

A large group of picornavirus inhibitors are compounds (WIN compounds) stabilizing the virion and blocking its uncoating. The target for these compounds

Disoxaril Mutants of Coxsackievirus B1: Phenotypic Characteristics and Analysis of the Target VP1 Gene

Ivanka Nikolova[a], Angel S. Galabov[a,*], Rumena Petkova[b], Stoyan Chakarov[c], and Boris Atanasov[d]

[a] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26, Georgi Bonchev Street, 1113 Sofia, Bulgaria. Fax: +359-2/870 0109. E-mail: galabov@microbio.bas.bg
[b] Scientific Technological Service, Ltd., Sofia, Bulgaria
[c] Department of Biochemistry, Faculty of Biology, Sofia University, Sofia, Bulgaria
[d] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

* Author for correspondence and reprint requests

Z. Naturforsch. **66 c**, 627–636 (2011); received January 7/August 24, 2011

Disoxaril inhibits enterovirus replication by binding to the hydrophobic pocket within the VP1 coat protein, thus stabilizing the virion and blocking its uncoating. Disoxaril-resistant (RES) mutants of the Coxsackievirus B1 (CVB1/RES) were derived from the wild disoxaril-sensitive (SOF) strain (CVB1/SOF) using a selection approach. A disoxaril-dependent (DEP) mutant (CVB1/DEP) was obtained following nine consecutive passages of the disoxaril-resistant mutant in the presence of disoxaril. Phenotypic characteristics of the disoxaril mutants were investigated. A timing-of-addition study of the CVB1/DEP replication demonstrated that in the absence of disoxaril the virus particle assembly stopped. VP1 RNA sequences of disoxaril mutants were compared with the existing Gen Bank CVB1 reference structure. The amino acid sequence of a large VP1 196–258 peptide (disoxaril-binding region) of CVB1/RES was significantly different from that of the CVB1/SOF. Crucially important changes in CVB1/RES were two point mutations, M213H and F237L, both in the ligand-binding pocket. The sequence analysis of the CVB1/DEP showed some reversion to CVB1/SOF. The amino acid sequences of the three VP1 proteins are presented.

Key words: Coxsackievirus B1, Disoxaril Mutants, VP1 Amino Acid Sequence

Introduction

A number of studies have shown that the lack of success in the development of an effective chemotherapy of enteroviral infections is due to the extraordinarily fast development of drug resistance to each of the known specific picornaviral replication inhibitors – protein ligands. This phenomenon is due to the extraordinarily high mutation rate (10^{-3}–10^{-4}) (Richards and Ehrenfeld, 1990) based on the great infidelity of the viral RNA-dependent RNA polymerase thus giving rise to a viral population of billions of quasi-(pseudo-)species. One mutation per each newly synthesized molecule of poliovirus RNA has been established (Agol, 2006). This can explain the vast diversity of clinical manifestations caused by almost every one of the members of the *Enterovirus* genus.

Twenty years ago it was found that WIN compounds (such as arildone, disoxaril, pleconaril) inhibit the virion uncoating process (Fox *et al.*, 1986). The direct crystal X-ray analysis of virus-inhibitor complexes showed that the primary target structure of this action is the hydrophobic pocket beneath the "canyon" on the VP1 protein (Rossmann, 1989). WIN compounds inserted into a cleft formed by a twisted β-sheet increase the structural rigidity of the VP1 subunit thus preventing virus uncoating.

Disoxaril is a typical representative of the WIN compounds – a highly effective inhibitor of a broad spectrum of entero- and rhinovirus serotypes. Enterovirus resistance to disoxaril has been established initially with poliovirus type 3/Sabin (Mosser *et al.*, 1994). Later this resistance was observed in Coxsackievirus B3 (CVB3) to another WIN compound, pleconaril (Groarke and Pevear, 1999).

In our previous research (Nikolova and Galabov, 2003) we have demonstrated the development of resistance to disoxaril during treat-

PERSPECTIVES FOR EFFECTIVE CHEMOTHERAPY OF ENTEROVIRUS INFECTIONS

A. S. Galabov, L. Nikolaeva-Glomb, I. Nikolova, R. Vassileva-Pencheva

Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract

Human enteroviruses distributed worldwide are causative agents of a broad spectrum of diseases with extremely high morbidity, including a series of severe illnesses of the central nervous system, heart, endocrine pancreas, skeleton muscles, etc., as well as the common cold contributing to the development of chronic respiratory diseases, and the chronic obstructive pulmonary disease. The above diseases along with significantly high morbidity and mortality in children, in the high-risk populations (immunodeficiencies, neonates) definitely formulate the chemotherapy as the main tool for the control of enterovirus infections. At present, clinically effective antivirals for use in the treatment of enteroviral infection do not exist, in spite of the lots of work carried out in this field. The main reason for this is the development of drug resistance.

The monotherapy courses were the only approach used till now. For the first time in the research for anti-enterovirus antivirals our team introduced the testing of combination effect of the selective inhibitors of enterovirus replication with different mode of action. We studied alongside the process of development of resistance to the strongest inhibitors of enteroviruses, WIN compounds (VP1 protein hydrophobic pocket blockers), especially in the models *in vivo*, CVB infections in mice. We introduced the tracing of a panel of phenotypic markers (MIC_{50} value, plaque shape and size, stability at 50° C, pathogenicity in mice) for characterization the drug-mutants (resistant and dependent) as a very important stage in the study of enterovirus inhibitors. Moreover, as a result of VP1 RNA sequence analysis performed on the model of disoxaril mutants of CVB1, we determined the molecular basis of the drug-resistance, two point mutations – M213H and F237L, both in the ligand-binding pocket. The sequence analysis of the drug-dependent mutant showed some reversion to the wild drug-sensitive virus strain.

A large-scale study of the combined effect of a series of anti-enteroviral agents ith different modes of action resulted in the selection of a number of very effective *in ro* double combinations revealing synergistic character of the combination effect and road spectrum of sensitive enteroviruses. Two antivirals developed in our previous dies were involved in these studies, PTU-23 and oxoglaucine. The most prospective ainment in our study in this field was the development of a novel scheme for the mbined application of anti-enteroviral substances in coxsackievirus B1 neuroinfection newborn mice. It consisted of a consecutive, alternating and non-simultaneous, ministration of the substances in the combination. A triple combination - disoxaril-anidine.HCl-oxoglaucine (DGO), showing good efficacy was selected. Its effectiveness s expressed in a marked reduction of mortality rate in infected mice as compared both the placebo group, and to the partner compounds used alone every day, and to the same mbination applied simultaneously every day. Studies of the drug sensitivity of viral brain lates from mice, treated with DGO combination showed not only preserved, but even reased sensitivity to the drugs included in the combination. Obviously, the consecutive rnating administration of anti-enteroviral substances hinders the occurrence of drug-istance in the course of the experimental enteroviral infections in mice.

Key Words: enteroviruses, antivirals, drug-resistance, combination effects

Резюме

Разпространени повсеместно, ентеровирусите са причинители на широк спек- от заболявания, включващ тежки състояния с патология на ЦНС, сърцето, ске-тните мускули и др., а също така и т. нар. настинки, които биха могли да доведат развитието на хронични респираторни заболявания и хронична обструктивна одробна болест. Много високата заболеваемост и смъртност при децата, както ри високо-рисковите групи (имунодефицитни пациенти и новородени) катего-но определят химиотерапията като основно оръжие за контрол на ентеровирус-е инфекции. Независимо от интензивната работа в тази област, на този етап а антивирусни препарати, които да са клинично ефективни за лечение на тези екции. Основната причина за това положение е развитието на лекарствена ре-тентност.

Изпитването ефекта на монотерапевтични курсове с ентеровирусни инхибито-ри бе единственият подход използван досега. Нашият екип за първи път въведе из-следвания върху комбинираните ефекти на селективни инхибитори на ентеровирус-ната репликация с различен механизъм на действие. Наред с това, ние изследвахме процеса на развитие на резистентност към най-ефективните инхибитори на ентеро-вирусите – WIN съединенията (блокиращи хидрофобния джоб в белтъка VP1). Тези изследвания бяха проведени основно *in vivo,* на модели на коксаки В вирусни инфекции в мишки. С цел характеризиране на лекарствените мутанти (резистентни и зависими), което е много важна фаза в изучаването на ентеровирусните ихиби-тори, ние представихме проследяването на панел от фенотипни маркери ($МИК_{50}$, форма и големина на плаките, стабилност при 50° С, патогенност за мишки). Освен това, като резултат от секвенционен анализ на участъка на РНК, кодираща белтъка VP1, извършен на модел на дизоксариловите мутанти на CVB1, ние установихме молекулната основа на лекарствената резистентност – две точкови мутации (M213H и F237L), намиращи се в лиганд-свързващия джоб. Секвенционният анализ на ле-карствено-зависимия мутант показа една реверсия към дивия лекарствено-чувстви-телен вирусен щам.

Широкомащабно проучване на комбинирания ефект на серия от анти-ентеро-вирусни агенти с различен механизъм на действие доведе до подбора на редица много ефективни *in vitro* двойни комбинациии, които показаха синергичен характер на комбинирания ефект при широк спектър от чувствителни ентеровируси. В тези експерименти бяха включени и два ентеровирусни инхибитора, чийто ефект бе до-казан за първи път при наши предишни изследвания - PTU-23 и оксоглауцин. Най-перспективното постижение на нашите проучвания бе прилагането на нова схема за комбинирано прилагане на анти-ентеровирусни вещества при коксаки В невро-инфекция в новородени мишки. Тази схема се състои в последователно редуващо се, а не едновременно, прилагане на веществата в комбинацията. Бе подбрана една тройна комбинация – оксоглауцин-гванидин HCl-оксоглауцин (DGO), показваща добра антивирусна активност. Нейната ефективност се изразява в отчетлива нама-ляване на смъртността на заразените животни, при сравнение с плацебо групата, с групите третирани ежедневно с монотерапевтичен курс от партниращите си в комбинацията вещества, както и с комбинацията DGO при ежедневно едновремен-но прилагане на трите съединения. Изследването на лекарствената чувствителност на вирусни мозъчни изолати, взети от мишки, третирани с тази комбинация показа

RESEARCH ARTICLE

Petar Grozdanov
Ivanka Nikolova
Angel Galabov

Detection of cytomegalovirus (CMV) DNA by PCR in patients with unknown inflammatory eye diseases

Authors' address:
The Stephan Angeloff Institute of Microbiology,
Bulgarian Academy of Sciences,
Sofia, Bulgaria.

Correspondence:
Petar Grozdanov
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia.
Tel: +359-2-979-6355
e-mail: grozdanov@microbio.bas.bg

ABSTRACT

Cytomegalovirus (CMV) is well known as pathogenic agent of intraocular inflammatory diseases. Light microscopy and immunohistochemical studies have limitations in the identification of this virus, but detection and typing viral DNA with Polymerase Chain Reaction (PCR) offers a rapid, highly specific, and easily interpretable means of identifying CMV in patients with ophthalmic lesions. Two patients (34-year-old male and 48-year-old male) who developed retinitis with unknown causative agent were studied for presence of CMV DNA. We used PCR kit for the qualitative detection of Cytomegalovirus (CMV 500/800 IC) provided for us by "Sacace Biotechnologies". The target of the PCR reaction was the "Major Immediate-Early" (MIE) gene. Positive and negative controls were used to avoid false results. Our PCR analysis showed the presence of CMV-DNA within the samples.

Key words: Cytomegalovirus, CMV, DNA, PCR, inflammatory eye disease

Introduction

Intraocular inflammation caused by viral infection is one of the major known types of endogenous inflammatory eye diseases (Koizumi et al., 2008). Herpesviruses are common pathogens of retinitis but uncertain identification of herpesvirus primary infection and reactivation symptoms can make diagnosis difficult (van Boxtel et al., 2007). CMV rarely causes retinitis in non-immunocompromised adults but is typical in congenitally infected neonates (Usui et al., 1993). It is well known that a number of ocular surface diseases such as papillary conjunctivitis, chronic blepharitis, or dry eye are associated with infections by this family of viruses due to their ubiquitous nature (Ergazaki et al., 1994; Karavellas et al., 2001; Martin et al., 2002; Eid et al., 2008).

In the present study, we employed PCR method to detect viral DNA in aqueous humour from two patients with clinically diagnosed retinitis.

Materials and Methods

DNA extraction

DNA-Sorb-B Kit (Sacace Biotechnologies) was used for total DNA isolation. We used the protocol supplied by the manufacturer. 100 µl of each sample, 10 µl if Internal Control and 300 µl of Lysis Solution were mixed together in appropriate tubes. The tubes were incubated for 5 min at 65°C then centrifuged briefly for 7-10 sec. 20 µl of DNA Sorbent was added to each tube followed by incubation for 6 min at room temperature. Then all tubes were centrifuged for 30 sec at 5000g. The supernatant from each tube was removed by a micropipette without disturbing the pellet. 300 µl of Washing Solution 1 were added to each tube followed by centrifuging for 30 sec at 8000g. The supernatant was removed and discarded. 500 µl of Washing Solution 2 were added to each tube this time. The supernatant was removed and discarded again. This step was repeated and then the tubes were incubated with open cap for 5 min at 65°C. The pellet was resuspended in 50 µl of DNA-eluent and incubated for 5 min at 65°C. The tubes were centrifuged for 1 min at 12000g and the DNA in supernatant was ready to be used for PCR amplification.

Control DNA

The positive and negative controls used in this study were supplied in the CMV 500/800 IC Kit.

ACTA MICROBIOLOGICA BULGARICA

Investigation of Antioxidant and Antiviral Properties of Geraniol

Milka Mileva[1*], Ivanka Nikolova[1], Nadya Nikolova[1], Luchia Mukova[1], Almira Georgieva[2], Anna Dobreva[3], and Angel S. Galabov[1]

[1] *Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences*
[2] *Department of Biological Effects of Natural and Synthetic Substances, Institute of Neurobiology, Bulgarian Academy of Sciences*
[3] *Institute for Rose and Aromatic Plants, Kazanlak, Bulgaria*

Abstract

Geraniol is an acyclic monoterpene alcohol with characteristic rose-like odour. It is an important constituent of Bulgarian *Rosa alba* L. and *Rosa damascena* Mill. essential oils. The purpose of the present study was to investigate antioxidant ability as well to reveal the potential for antiviral activity of geraniol against the replication of viruses belonging to different taxonomic groups and representing important human pathogens. Geraniol significantly depressed the effect of oxidation - it showed good ability to capture 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and to inhibit lipid peroxidation in a egg liposomal suspension. Geraniol showed low cytotoxicity toward HEp-2 cells. It was tested *in vitro* for its activity against viruses representing important human pathogens assigned to different taxonomic groups: coxsackievirus B1 (CV-B1) from the *Picornaviridae* family, respiratory syncytial virus (RSV) from the *Paramyxoviridae* family, and influenza virus A/Aichi/68/H3N2 from the *Orthomyxoviridae* family. *In vitro* antiviral effect was examined by the virus cytopathic effect inhibition assay. Geraniol showed antiviral activity only against CVB1 - the ratio of selective index is 3.9. The investigated biological properties of geraniol, including good antioxidant and antiviral activities against some virus families, together with negligible toxicity, warrant further studies to explore the feasibility of formulating geraniol-containing consumer products with health promoting properties.

Key words: geraniol, antioxidant activity, antiviral properties

Резюме

Гераниол е ацикличен монотерпенов алкохол с характерен мирис на роза. Той е важна съставна част от етеричните масла на българската *Rosa alba* L. и *Rosa damascena* Mill. Целта на настоящото проучване е да се изследва антиоксидантната способност, както и да се разкрие потенциала за антивирусна активност на гераниол срещу репликацията на вируси, принадлежащи към различни таксономични групи, които са важни човешки патогени. Гераниол показа добра способност да улавя 2,2-дифенил-1-пикрилхидразил (DPPH) радикали и да инхибира липидната пероксидация в моделна система от яйчени липозоми. Гераниол демонстрира ниска цитотоксичност към HEp-2 клетки. *In vitro* беше тествана неговата активност срещу вируси, които са важни човешки патогени, принадлежащи към различни таксономични групи: Коксаки B1 вирус (CV-B1) от семейство *Picornaviridae*, респираторен синцитиален вирус (RSV) от семейство *Paramyxoviridae* и грипен вирус A/Aichi/68/H3N2 от семейство *Orthomyxoviridae*. Антивирусният ефект беше изследван *in vitro* в постановка на многоциклов ЦПЕ (цитопатичен ефект)-инхибиращ тест. Гераниол показа антивирусно действие само срещу CVB1 - селективният индекс е 3.9. Изследваните биологични свойства на гераниол, сред които са добрата антиоксидантна и антивирусна активност срещу някои вирусни семейства, заедно с незначителната токсичност, налагат провеждането на допълнителни изследвания, за да се проучи приложимостта на гераниол-съдържащите продукти с добри здравословни показатели.

*Correspondence to: Milka Mileva
E-mail: milkamileva@gmail.com

Original Article

Antiviral Chemistry and Chemotherapy
2015, Vol. 24(5–6) 136–147

DOI: 10.1177/2040206616671571
avc.sagepub.com
SAGE

Anti-enteroviral triple combination of viral replication inhibitors: activity against coxsackievirus B1 neuroinfection in mice

Adelina Stoyanova[1], Ivanka Nikolova[1], Gerhard Pürstinger[2], Georgi Dobrikov[3], Vladimir Dimitrov[3], Stefan Philipov[3] and Angel S Galabov[1]

Abstract

Background: Chemotherapy is an important tool for controlling enterovirus infections, but clinically effective anti-enterovirus drugs do not currently exist, mainly due to the development of drug resistance. We investigated the combination effects of enterovirus replication inhibitors in order to limit this process. In previous studies, we showed the efficacy of consecutive alternating administration of the triple combinations disoxaril/guanidine/oxoglaucine and pleconaril/guanidine/oxoglaucine against coxsackievirus B1 infection in newborn mice. Drug sensitivity tests of the viral brain isolates showed that these drug combinations prevented the development of drug resistance.

Methods: In the current study, we replaced guanidine-HCl with enteroviral RNA synthesis inhibitor MDL-860 to test the effect of a new triple combination—pleconaril/MDL-860/oxoglaucine—applied via consecutive alternating administration in newborn mice infected subcutaneously with 20 MLD_{50} of coxsackievirus B1.

Results: The pleconaril/MDL-860/oxoglaucine combination via consecutive alternating administration showed high activity at the 75 mg/kg MDL-860 dose: a protective effect of 50% and a pronounced suppression of brain virus titers. Moreover, along with prevention of drug resistance, a phenomenon of increased drug sensitivity was established. MDL-860 sensitivity in pleconaril/MDL-860/oxoglaucine increased 8.2 times vs. placebo (29 times vs. monotherapy) on day 7 and oxoglaucine sensitivity—4.9 times vs. placebo (by 6.8 times vs. monotherapy) on day 13. As concerns pleconaril, a demonstrable prevention of drug resistance was registered without increase of drug sensitivity. Daily, simultaneous administration of pleconaril/MDL-860/oxoglaucine showed no protective effects and led to a rapid development of drug resistance.

Conclusions: These results add new support for using consecutive alternating administration treatment courses to achieve clinically effective chemotherapy of enterovirus infections.

Keywords

Animal model, compounds, drug combination, drug resistance, picornaviridae

In recent decades, the interest on the role of enteroviruses (EVs) in human infectious pathology has increased.[1–3] This is in part due to the large number of investigations carried out on a series of EV-induced infections manifested for the first time by epidemic spread in several regions of the globe, for example, the enterovirus 71 (EV 71) epidemic in Southeast Asia[4,5] and EV D68 in the USA.[6] Moreover, EVs are causative agents of an unusual phenomenon for the infectious pathology: one virus, in one region, during one period of time (the summer season), to cause more than 10 different clinical pictures affecting different

[1]Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria
[3]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Corresponding author:
Angel S Galabov, The Stephan Angeloff Institute of Microbiology, 26 Academician Georgi Bonchev St., Sofia, Bulgaria.
Email: galabov@microbio.bas.bg.

Antiviral Research 121 (2015) 138–144

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Effect of consecutive alternating administration (CAA) of a triple anti-enteroviral combination on Coxsackievirus B1 neuroinfection in mice

Adelina Stoyanova, Ivanka Nikolova, Angel S. Galabov *

Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. G. Bonchev Str., 1113 Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 26 March 2015
Revised 15 July 2015
Accepted 16 July 2015
Available online 18 July 2015

Keywords:
CVB1
Mice
Pleconaril
Oxoglaucine
Guanidine-HCl
Drug sensitivity

ABSTRACT

Currently, clinically effective antivirals for use in the treatment of enteroviral (EV) infections do not exist. The main reason is the development of drug resistance, the principle obstacle in the development of EV infection chemotherapy, based til now on monotherapy. The most important achievement of our previous studies was the development of a novel scheme for *in vivo* application of a triple combination of EV inhibitors with different modes of action against Coxsackievirus B (CVB) infections in mice. It consists of consecutive alternating administration (CAA) of the substances in the combination. Here, we tested the effect of the triple combination pleconaril, guanidine-HCl, and oxoglaucine (PGO) via CAA in newborn mice infected with a neurotropic strain of CVB1 (20 LD_{50} per mouse). This combination manifested a considerable protective effect with pleconaril doses of 25–200 mg/kg: it decreased mortality rate (protection index, PI, between 31.3% and 67.7%) and increased mean survival time (MST) by 4–6 days. Pleconaril monotherapy demonstrated activity similar to that of PGO via CAA, as measured by PI values, but MST values were slightly lower. However, it also greatly suppressed growth of infected suckling mice, especially at 200 mg/kg. This toxic effect was avoided with CAA of PGO at pleconaril doses of 25–100 mg/kg. Pleconaril monotherapy administered every 3 days was ineffective. The PGO with CAA treatment course decreased infectious virus content, whereas pleconaril monotherapy did not. Analysis of drug-sensitivity in brain samples from CVB1 infected mice, based on IC_{50} (50% inhibitory concentration) values from cell culture experiments, showed that the CAA course counteracted the development of drug resistance to pleconaril and oxoglaucine in the triple PGO combination and increased drug sensitivity. In contrast, pleconaril and oxoglaucine monotherapies resulted in drug resistance. This data clearly proves the effectiveness of the proposed novel approach—the CAA treatment course—for combined application of EV replication inhibitors.

1. Introduction

Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with enormously high morbidity, including a series of severe illnesses that involve pathologies of the CNS, heart, β-cells of pancreas, skeletal muscles, and so on. With the common cold, they contribute to the development of chronic respiratory diseases, including chronic obstructive pulmonary disease. It should be stressed that there is significantly high morbidity and mortality in children and high-risk populations (people with immunodeficiencies, neonates) (Pallansch and Roos, 2006; Tan, 2005; Mallia et al., 2007). The unusually large number of enterovirus serotypes that are causative agents of clinical illness hamper the establishment and introduction of anti-enterovirus vaccines. All these facts point to chemotherapy as the main tool for controlling enterovirus infections. The high prevalence of inapparent cases (more than 85%) (Morens and Pallansch, 1995; Pallansch and Roos, 2006; Strauss and Strauss, 2008) is a strong argument for beginning administration of specific anti-enteroviral chemotherapeutic agents during the disease latency period. Such urgent prophylaxis is especially indicated for all children during enteroviral infection outbreaks in childcare settings (nursery schools, kindergartens, primary schools, pediatric hospital units, and so on) (Morens and Pallansch, 1995). Doing so could result in reduced risk of acquired diabetes and other severe forms of enterovirus-induced infections (Hyöty et al., 1995; Hyöty and Taylor, 2002; Galabov and Angelova, 2006).

* Corresponding author.
E-mail address: galabov@microbio.bas.bg (A.S. Galabov).

http://dx.doi.org/10.1016/j.antiviral.2015.07.004
0166-3542/

DE GRUYTER
OPEN

ПРИЛОЗИ. Одд. за мед. науки, XXXVI 2, 2015 **МАНУ**
CONTRIBUTIONS. Sec. of Med. Sci., XXXVI 2, 2015 **MASA**

10.1515/prilozi-2015-0057

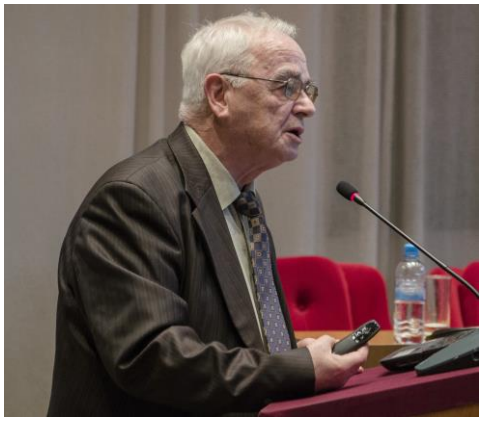
ISSN 1857-9345
UDC: 578.835.1.017.4

ANTIVIRAL COMBINATION APPROACH AS A PERSPECTIVE TO COMBAT ENTEROVIRUS INFECTIONS

Angel S. Galabov, Ivanka Nikolova, Ralitsa Vassileva-Pencheva and Adelina Stoyanova

The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Corresponding Author: Angel S. Galabov, Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 26 Georgi Bonchev str., Sofia 1113, Bulgaria, Tel. +359 2 9 79 31 57; Fax: +359 2 8 70 01 09, E-mail: micb@microbio.bas.bg



Abstract

Human enteroviruses distributed worldwide are causative agents of a broad spectrum of diseases with extremely high morbidity, including a series of severe illnesses of the central nervous system, heart, endocrine pancreas, skeleton muscles, etc., as well as the common cold contributing to the development of chronic respiratory diseases, including the chronic obstructive pulmonary disease. The above mentioned diseases along with the significantly high morbidity and mortality in children, as well as in the high-risk populations (immunodeficiencies, neonates) definitely formulate the chemotherapy as the main tool for the control of enterovirus infections. At present, clinically effective antivirals for use in the treatment of enteroviral infection do not exist, in spite of the large amount of work carried out in this field. The main reason for this is the development of drug resistance. We studied the process of development of resistance to the strongest inhibitors of enteroviruses, WIN compounds (VP1 protein hydrophobic pocket blockers), especially in the models *in vivo*, Coxsackievirus B (CV-B) infections in mice. We introduced the tracing of a panel of phenotypic markers (MIC_{50} value, plaque shape and size, stability at 50ºC, pathogenicity in mice) for characterization of the drug-mutants (resistant and dependent) as a very important stage in the study of enterovirus inhibitors. Moreover, as a result of VP1 RNA sequence analysis performed on the model of disoxaril mutants of CVB1, we determined the molecular basis of the drug-resistance.

The monotherapy courses were the only approach used till now. For the first time in the research for anti-enterovirus antivirals our team introduced the testing of combination effect of the selective inhibitors of enterovirus replication with different mode of action. This study resulted in the selection of a number of very effective *in vitro* double combinations with synergistic effect and a broad spectrum of sensitive enteroviruses. The most prospective attainment in our examinations in this field was the development of a novel scheme for the combined application of anti-enteroviral substances in coxsackievirus B1 neuroinfection in newborn mice. It consisted of a consecutive, alternating and non-

simultaneous administration of the substances in the combination. The triple combination - disoxaril-guanidine. HCl-oxoglaucine (DGO) showed a high effectiveness expressed in the marked reduction of the mortality rate in infected mice as compared both to the placebo group, and to the partner compounds used alone every day, and to the same combination applied simultaneously every day. The studies of the drug sensitivity of viral brain isolates from mice treated with DGO combination showed not only preserved, but even increased sensitivity to the drugs included in the combination. Obviously, the consecutive alternating administration of anti-enteroviral substances hinders the occurrence of drug-resistance in the course of the experimental enteroviral infections in mice.

Key words: enteroviruses, antivirals, drug-resistance, combination effects.

Enteroviruses in human pathology

Human enteroviruses, members of a large genus in the *Picornaviridae* family, are distributed worldwide and represent the most common of human viruses, possibly infecting a billion or more individuals annually and resulting in hundreds of thousands of hospitalizations per year in the developed world only (Pallansch, 2011). Enteroviruses are very efficient infective agents although the majority of infections are asymptomatic or mild in their clinical course (Morens, 1995; Pallansch, 2006; Strauss, 2008). The contagiousness is such that actually, if an individual in a given group developed the symptoms of an enteroviral infection, it would be certain that all members of the group were infected (Pallansch, 2011). Alongside the asymptomatic or mild clinical course of the majority of infections, a wide variety of diverse syndromes and diseases are associated with non-polio enterovirus infections. The most common clinical manifestation is the upper respiratory tract discomfort (Chuang, 2010), fever, and, occasionally, mild gastrointestinal symptoms (Morens, 1995). But non-polio enteroviruses may cause a broad spectrum of diseases due to their ability to affect various organs and systems including the central nervous system, the respiratory system, the skin, the heart, the pancreas, the eyes. The most common tissue specific localization of the enteroviral infection is the central nervous system, resulting in aseptic meningitis, or much more rarely, in encephalitis. Some enteroviruses may manifest tropism to other definite tissues like the cardiotropic coxsackieviruses or those viruses affecting the pancreatic beta cells, but the tropism is neither unique, nor specific (Archard, 1987; Tauriainen, 2011). Disseminated infection can lead to exanthema, non-specific myalgias or severe multiorgan disease in neonates (Morens, 1995). Individual serotypes can be associated with different clinical manifestations and *vice versa*, a particular clinical manifestation can be caused by several different serotypes (Strauss, 2008). Severe and life-threatening conditions, such as meningitis, encephalitis, neonatal sepsis, myocarditis, pancreatitis, as well as further complications and some chronic diseases later in life, i.g. insulin dependent type 1 diabetes mellitus (Hyoty, 2002; Galabov and Angelova, 2006) and dilated cardiomyopathy (Schultheiss, 2006) can be attributed to an enterovirus infection, especially early in life. Symptomatic infections include also the summer cold, herpangina, hand-foot-and-mouth disease, pleurodynia (Bornholm disease), rashes, hemorrhagic conjunctivitis, uveitis, chronic fatigue syndrome. Human rhino viruses, which comprise another numerous group within the enterovirus genus, cause the common cold in individuals of all ages contributing to the possible development or exacerbation of chronic respiratory diseases later in life (Tan, 2005; Mallia, 2007). All enteroviruses due to their high stability in the environment can also be the causative agents of nosocomial outbreaks, particularly severe in neonatal units and nurseries (Aitken, 2001).

Chemotherapy in the control of enterovirus infections

As the number of human enteroviruses is quite big and their mutation rate is rather high, it is considered that efforts for the development of a successful vaccine would not lead to an efficient control. This definitely formulates chemotherapy as the main tool to overcome the infection. What is more, the new strategy for poliomyelitis eradication claims for the development of safe and efficient anti-polio drugs.

VirusDis. (July–September 2016) 27(3):271–276
DOI 10.1007/s13337-016-0338-4

ORIGINAL ARTICLE

Characterization of genomic changes in the cervical pre-cancerous lesions and tumors induced by different types of human papillomaviruses

Petar Grozdanov[1] · Savina Hadjidekova[2] · Ivanka Dimova[2] · Ivanka Nikolova[1] · Draga Toncheva[2] · Gancho Ganchev[3] · Victor Zlatkov[4] · Angel S. Galabov[1]

Received: 2 June 2016 / Accepted: 2 August 2016 / Published online: 9 August 2016

Abstract Cervical carcinoma is the second most common malignancy among women in both incidence and mortality. Although much is known about the etiology and treatment of cervical cancer, the role of genetic alterations in the multistep pathway of cervical tumorigenesis is largely unknown. The aim of this study was to characterize the genomic changes in the cervical pre-cancerous lesions and tumors, induced by different types of human papillomaviruses. In this research was used the BlueGnome CytoChip oligo 2 × 105 K microarray for whole-genome oligo-array CGH. Microarray CGH analysis of 40 specimens was carried out—12 specimens from patients with early-stage squamous cell carcinomas; 19 specimens from patients with mild to moderate dysplasia and 9 with severe dysplasia. First we performed microarray CGH analysis of five DNA pools which contained the DNA from homogeneous groups of patients. The results revealed presence of micro chromosomal aberrations in chromosome region 14q11.2. According to the genome database these aberrations represent polymorphisms. Microarray analysis of DNA from 9 separate carcinoma lesions revealed a total of 26 aberrations in 14 chromosomes of nine patients. Our results showed the advantages of high-resolution chips in the clinical diagnosis of patients with cancerous and pre-cancerous lesions caused by viral infection with HPV, but also highlight the need for extensive population studies revealing the molecular nature and clinical significance of different CNVs and the creation of detailed maps of variations in the Bulgarian population. This would facilitate extremely precise interpretation of specific genomic imbalances in the clinical aspect.

Keywords Human papillomavirus · Microarray CGH analysis · Genomic aberration · CNV · Cervical cancer

Introduction

Cervical cancer (CC) is the second most common cancer in women worldwide, affecting 500,000 individuals each year, and it is the main cause of death of women with cancer in developing countries. Despite the damage caused by the oncoviral proteins, CC is a rare complication of the viral infection because most infections are transient and do not evolve into neoplastic lesions. Although 95 % of the patients with precancerous lesions harbor HPV, only a small fraction of the cases eventually progress to invasive cancer [25]. On average, it takes 12–15 years before a persistent HPV infection may, via the premalignant stages of cervical intraepithelial neoplastic lesions (CIN), lead to CC. These findings suggest HPV infection alone does not cause the disease and other factors, such as abnormal host genes, could be associated with the development of invasive cancer. Further identification of such genetic alterations is critical in our understanding of the molecular basis of CC development [18].

Several genomic regions have been identified with changes in the number of DNA copies copy number-altered

✉ Petar Grozdanov
grozdanov@microbio.bas.bg

[1] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26 Georgi Bonchev, 1113 Sofia, Bulgaria

[2] Department of Medical Genetics, Medical University, Sofia, Bulgaria

[3] Specialized Hospital for Active Treatment in Oncology, Sofia, Bulgaria

[4] The University Obstetrics and Gynecology Hospital "Maichin Dom", Sofia, Bulgaria

DETECTION OF ORAL HUMAN PAPILLOMAVIRUSES (HPV)

Grozdanov P.[1], Simeonova L.[2], Nikolova I.[2], Galabov A.S.[2]

[1]Laboratory Center Pasteur, [2]Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences,

Abstract

Squamous cell carcinomas (SCCs) are the most common malignancy in the head and neck region. Common risk factors in head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol abuse, however, in an increasing proportion of cases, no significant smoking or drinking history has been reported.

Approximately 35 years ago, a role of human papillomavirus (HPV) in cervical cancer was postulated. Today, it is well established how this very heterogeneous virus family represents an important human carcinogen, causing not only the vast majority of cervical and ano-genital tumors, but also a variable number of cancers in other districts of the human body including the head and neck. The fifth leading cause of cancer mortality rate proved oropharyngeal squamous cell carcinoma (OPSCC) – throat cancer associated with positive results for the presence of HPV. HPV oral and oropharygeal cancers are harder to discover than tobacco related cancers because the symptoms are not always obvious to the individual who is developing the disease, or to professionals that are looking for it. They can be very subtle and painless. We present cases of three patients (47-year-old male, 48-year-old male and 55-year-old male) diagnosed with benign warts in the oral cavity were studied for the presence of HPV DNA. We used PCR kit for the qualitative detection of human papillomaviruses (REF V-10/14-50F) provided for us by "Sacace Biotechnologies". The targets of the PCR reaction were E6, E2, E1, L1 genes. Positive and negative controls were used to avoid false results. Our PCR analysis showed the presence of HPV-DNA (HPV 6) within the samples.

Key Words: Human papillomavirus, PCR, oropharyngeal squamous cell carcinoma

Резюме

Плоскоклетъчните карциноми (SCCs) са най-честото злокачествено заболяване в областта на главата и шията на човека. Най-общите рискови фактори за развитие на рак в областта на главата (HNSCC) са тютюнопушенето и злоупотребата с алкохол. През последните години обаче растат случаите на този вид заболяване при хора, които не са изложени на тези рискови фактори.

Преди 35 години се доказа ролята на човешкия папиломен вирус (HPV) при рака на маточната шийка. Днес е добре известно, че вируси от това хетерогенно семейство представляват важен канцерогенен фактор за човека, причинявайки не само рак на маточната шийка, а и ано-генитални тумори, като вече има данни и за различен брой злокачествени заболявания в други области на човешкото тяло, включително главата и шията. Петата водеща причина за смъртност от ракови заболявания се оказва орофарингеалния карцином (OPSCC) - рак на

гърлото, асоцииран с положителни резултати за наличие на HPV. Раковите заболявания на устата и гърлото, свързани с вирусна етиология, са по-трудни за откриване от тези свързани с тютюнопушенето, тъй като симптомите не винаги са очевидни за пациента и лекуващия го лекар. В настоящата работа представяме трима пациенти, с диагноза доброкачествени брадавици в устната кухина, изследвани за наличие на вирусна ДНК. За целта използвахме полимеразно верижна реакция PCR кит за качествено откриване на човешките папиломни вируси (REF V-10/14-50F, „Sacace Biotechnologies"). Мишената на полимеразно верижната реакция бяха вирусните гени E6, E2, E1 и L1. За валидиране на резултатите бяха използвани положителни и отрицателни контроли. Резултатите ни показаха наличие на вирусна ДНК (HPV 6) във всички изследвани проби.

Introduction

Squamous cell carcinomas (SCCs) are the most common malignancy in the head and neck region. The most common risk factors for cancer in the head and neck (HNSCC) are smoking and alcohol abuse (Ragin and Taioli 2007; NCCN 2016; Soares et al. 2015). Common risk factors in head and neck squamous cell carcinoma (HNSCC) are smoking and alcohol abuse, however, in an increasing proportion of cases, no significant smoking or drinking history has been reported (Kumar et al. 2008; Frakes et al. 2016).

Approximately 35 years ago, a role of human papillomavirus (HPV) in cervical cancer was postulated (Gillison et al. 2012; Ukpo et al. 2013). Today, it is well established how this very heterogeneous virus family represents an important human carcinogen, causing not only the vast majority of cervical and ano-genital tumors, but also a variable number of cancers in other districts of the human body including the head and neck. The fifth leading cause of cancer mortality rate proved oropharyngeal squamous cell carcinoma (OPSCC) – throat cancer associated with positive results for the presence of HPV (O'Rorke et al. 2012; Lohaus et al. 2014; Ward et al. 2014).

Undoubtedly, all problems referring to the prophylaxis of socially significant diseases in Bulgarian population are current. Nowadays, a particular attention is paying to the etiological factors of the treated disease, making the topic of the current study up-to date, as well as the research on the molecular mechanisms underlying in the oropharyngeal carcinoma and in that way the prevention and treatment of this disease will be improved.

Until now, molecular epidemiological survey on virus-throat cancer in Bulgarian patients has not been conducted.

There are over 100 types of human papillomavirus. Many people get infected with it in certain moment of their lives. Two strains of human papilloma virus are associated with the greatest risk of cancer – HPV 16 and HPV 18. It is believed that HPV 16 is responsible for about 60% of cervical cancers, 80% of the diseases of anal cancer and 60% of cancers of the mouth. Treatment and prognostic analysis of the disease depends on demonstrating the link between human papillomavirus and developing oropharyngeal cancer (Hong et al. 2010).

Approximately 8260 cases of throat cancer are registered every year in our country, of which statistically around 4000 are fatal, putting it in fifth place as a leading cause of death from cancer. Some studies have shown that the incidence

Bioorganic & Medicinal Chemistry Letters 27 (2017) 4540–4543

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-enterovirus activity of new analogues of MDL-860

Georgi M. Dobrikov [a,*], Ivaylo Slavchev [a], Ivanka Nikolova [b], Adelina Stoyanova [b], Nadya Nikolova [b], Lucia Mukova [b], Rosica Nikolova [c], Boris Shivachev [c], Angel S. Galabov [b,*]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, bl. 9, Acad. G. Bonchev str., Sofia 1113, Bulgaria
[b] Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, bl. 26, Acad. G. Bonchev str., Sofia 1113, Bulgaria
[c] Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences, bl. 107, Acad. G. Bonchev str., Sofia 1113, Bulgaria

ARTICLE INFO

Article history:
Received 1 July 2017
Revised 24 August 2017
Accepted 27 August 2017
Available online 30 August 2017

Keywords:
Enterovirus
Coxsackieviruses
Polio
Diarylethers
MDL-860

ABSTRACT

A series of twelve novel compounds, analogues of antiviral agent MDL-860 were synthesized and their antiviral activity was evaluated *in vitro* against enteroviruses poliovirus 1 (PV1), Coxsackieviruses B1 (CVB1) and Coxsackieviruses B3 (CVB3). Compounds **14**, **24** and **25** manifested strong antiviral effects against CVB1 and PV1 (SI values of 405 and 118 for CVB1 and PV1 respectively). In contrast to the wide anti-enteroviral activity of MDL-860, these three compounds were inactive against CVB3. Compounds **14**, **24** and **25** along with MDL-860 were tested *in vivo* in mice infected with CVB1. Marked protective effects of compounds **14** and **24** were established, PI values of 50% and 33.3%, respectively. In addition, almost all of the tested compounds manifested very low toxicity.

Enteroviruses (EV) are non-enveloped, single-stranded (+) RNA viruses belonging to the *Picornaviridae* family. This large family includes several pathogens that are implicated in a wide range of clinical manifestations, affecting humans as well as animals. Indeed, enteroviruses are responsible for at least 10–15 million symptomatic infections yearly. EV may also be linked to even more serious illnesses, which can subsequently be life-threatening. Such threads includes meningitis, encephalitis, myocarditis and insulin dependent diabetes etc.[1] Coxsackieviruses, and in particular Coxsackie B group, have often been associated with the development of myocarditis, which may lead to sudden death in young adults or progress to dilated cardiomyopathy if untreated.[2–4] Over the past decades, several classes of non-peptidic compounds have been reported to be selective inhibitors of enterovirus replication after *in vitro* testing (cell culture experiments). However, a sharp discrepancy exists between the antiviral activity established *in vitro* and *in vivo* (experiments involving laboratory animals). In contrast with hundreds virus replication inhibitors showing *in vitro* effects less than twenty manifested some *in vivo* activity. Unfortunately, the efficient anti-enteroviral chemotherapy for clinical use is still not established. The development of drug-resistance is the main reason for the lack of antivirals in clinical use for enteroviral infections.[5] Nevertheless, some anti-enteroviral compounds have entered clinical trials – isoxazoles ("WIN compounds" – disoxaril, pleconaryl), pirodavir and its analogues, imidazolidinones, chalcones, flavanes, diarylethers etc. (Fig. 1).[6]

Diarylether derived compound MDL-860 (2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile, also known as DNB) was first reported in 1980's. Indeed MDL-860 possesses a broad-spectrum of *in vitro* activity against picornaviruses, by inhibiting an early event in virus replication, after initial uncoating.[7,8] MDL-860 mechanism of action was elucidated in our recent publication, the identified target being the host phosphatidylinositol-4 kinase III beta (PI4KB).[9] MDL-860 also elicited *in vivo* efficacy in a model of coxsackievirus B3 (CVB3) induced myocarditis.[10] The promising results inspired the development of many analogues of MDL-860 over the past decades. For example, Markley et al.[11] synthesized and tested over than 70 diarylethers (and their isosteric analogues) against several piconaviruses. The latest work of Pürstinger et al. reports on the synthesis of 60 new diarylethers and their activity against CVB3 replication.[12] All obtained results clearly showed that the 2-cyano-4-nitrophenoxy group is an essential building block for the existence of antiviral activity of this class of compounds. However, varying the substituents in the other aromatic ring can have a significant impact on both the antiviral activity and cytotoxicity. Identified hit compounds, usually contain two to three halogen atoms in the second aromatic ring.

Despite the reported promising results, recently this class of compounds has been neglected in terms of further investigation with respect to their antiviral activity. Thus, the current study

* Corresponding authors.
E-mail addresses: gmdob@orgchm.bas.bg (G.M. Dobrikov), galabov@microbio.bas.bg (A.S. Galabov).

http://dx.doi.org/10.1016/j.bmcl.2017.08.056
0960-894X/

BO4. TESTING OF MEROCYANINES ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I IN CELL CULTURES

Ivanka Nikolova[1], Neli Vilhelmova-Ilieva[1], Tsonko Kolev[2], Petar Grozdanov[1],
[1] *Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 26, 1113 Sofia, Bulgaria*
[2] *Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgari*

Corresponding author e-mail: vanianik@mail.bg

Abstract

The cytotoxicity of four merocyanines (L-6, L-8, L-9 and L-10) on monolayer cell culture MDBK was determined. The lowest toxicity of all compounds showed L-9 (CC_{50} = 843.5μM), followed by L-10 (CC_{50} = 603.5μM). Highest toxicity showed L-8 (CC_{50} = 11μM).

The antiviral activity of the substances on the replication of herpes simplex virus type 1 (HSV-1) was determined. The significant effect on the replication of intracellular HSV-1 showed substances L-10 with a selective index (SI) = 20.8.

The effect of the test substances on the activity against the virulence of the extracellular HSV–1 virions was also investigated. Activity was determined in five time intervals: 15, 30, 60, 90and 120 minutes. The strongest activity showed L-8 which decreases the virus titer with Δlg = 2, followed by L-6, L-9 and L-10 with Δlg = 1.75.

Keywords: Herpes simplex virus type I, viral replication, antiviral drugs, therapy, synthesis, acyclovir (ACV)

Introduction

Herpes simplex virus (HSV) exists as two types: HSV-1 and HSV-2, of which HSV-1 primarily causes infections of the mouth, throat, face, eyes and central nervous system but can also cause genital infection. HSV can cause various diseases but is most often characterized by the formation of lesions on the skin and mucous membranes of the infected area. After the primary infection, the virus always forms latent lifetime infection. Especially severe herpes infections are those in the eye [17] and herpes encephalitis [23]. HSV-1 may lead to partial damage to the nervous system and increase the risk of developing Alzheimer's disease [8, 9, 6]. One of the diseases ending often fatal is Neonatal herpes simplex caused by vertical transmission of HSV (type 1 or 2) from the mother to the newborn [1, 20].

Acyclovir (ACV) as well as other nucleoside analogues are effective in the treatment of HSV infections, but in a number of cases therapy fails due to the occurrence of ACV-resistant mutants [4, 14, 22]. Therefore, it is necessary to find new therapies whose mechanism of action is different from that of acyclovir.

For the past decades, many studies were conducted in the field of photodynamic inactivation of viruses. Numerous efforts have been made to seek new methods of virus inactivation because of their ability to develop resistance to antiviral agents. Particular attention is paid to photosensitizers and their antiviral activity in the treatment of contaminated blood as no side effects in cells or plasma proteins are seen in this process [3, 7, 11,13].

Volume 8, Issue 1., 2018, 3053 - 3059 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article **Open Access Journal**

Received: 12.01.2018 / Revised: 10.02.2018 / Accepted: 12.02.2018 / Published on-line: 15.02.2018

Evaluation of antimicrobial, biofilm inhibitory and cytotoxic activities of a new hiperbranched polymer modified with 1,8-naphthalimide units

Evgenia Vasileva-Tonkova [1,*], Peter Grozdanov [1], Ivanka Nikolova [1], Desislava Staneva [2], Paula Bosch [3], Sandra Medel [3], Ivo Grabchev [4,*]

[1] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] University of Chemical Technology and Metallurgy, Sofia, Bulgaria
[3] Institute of Science and Technology of Polymers, Madrid, Spain
[4] Sofia University "St. Kliment Ohridski", Faculty of Medicine, Sofia, Bulgaria

*Corresponding authors e-mail addresses: ***i.grabchev@chem.uni-sofia.bg; evaston @yahoo.com***

ABSTRACT

New hyperbranched polymer modified with six 1,8-naphthalimide units (P1000-Napht) was tested *in vitro* for antimicrobial activity against Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*, Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter johnsonii*, and the yeasts *Candida lipolytica*. The new modified polymer displayed moderate to good antimicrobial potential against the tested strains. The effect of the P1000-Napht on membrane permeability of bacterial cells was evaluated, and cytotoxicity assay was performed against HEp-2 cell line. The antibacterial finishing of the treated cotton fabric and polylactic acid film was assessed toward Gram-positive and Gram-negative bacteria. The obtained results suggest that the new modified dendritic polymer may be developed as promising antimicrobial alternative for biomedical application.

Keywords: *antimicrobial activity, antibacterial cotton fabrics, cytotoxic activity, hiperbranched polymer, 1,8-naphthalimide, polylactic acid films.*

1. INTRODUCTION

The emergence of microbial resistance to conventional antibiotics is a serious threat to the effectiveness of current antimicrobial therapy [1]. Therefore, the discovery of novel antimicrobial agents to which the pathogenic microbes cannot develop resistance easily is one of the major medical concerns of the 21st century [2]. Nanomaterials could serve as a long-term solution to the growing problem of antimicrobial resistance because they have shown antimicrobial effect against a wide range of drug-resistant pathogens. Dendrimers are a special class of nano-sized, polymeric macromolecules with highly branched three-dimensional architecture consisting of an initiator core, a radiating interior structural layer composed of repeating generations (G0-G10), and end-groups attached on an outer layer of repeat units [3, 4]. These end-groups can be functionalized, thus modifying physicochemical or biological properties of dendrimers [5]. The low polydispersity and the ability to precisely control their surface chemistry make dendrimers especially useful for pharmaceutical and biomedical applications [6-9]. Many researchers are focused on dendrimers as potential antimicrobial compounds or agents improving antibacterial or antifungal activity of existing chemotherapeutics [10]. As the mechanism by which dendrimers kill or inhibit the growth of bacteria depends in particular on the type of dendrimer peripheral groups, special design is required for the synthesis of dendrimers for biomedical applications [11]. It has been found that dendrimer biocides are more potent than both polymeric biocides and small molecule biocides. The significant improvements of biocide action of dendrimers are attributed to the high number of functional groups in a compact space and their polycationic structure [12].

Hyperbranched polymers (HBP) are a class of synthetic tree-like macromolecules called dendritic polymers with densely branched structure and a large number of end functional groups [13]. Recently, a new fluorescent HBP containing 1,8-naphthalimide units in the side-chain of a commercial HBP, designated as P1000-Napht, has been synthesized for the first time through click chemistry, and its photophysical properties have been investigated [14]. In the present study, the antimicrobial, biofilm inhibitory and cytotoxic activities of the newly synthesized P1000-Napht are investigated and some possible applications are discussed.

2. EXPERIMENTAL SECTION

The syntesis and characterization of HBP P1000-Napht (Scheme 1) have been described recently [14].

Microorganisms. The antimicrobial activity of the new HBP P1000-Napht was tested against the following model pathogenic strains (Collection of the Institute of Microbiology, Bulgarian Academy of Sciences): Gram-positive bacteria *Bacillus subtilis* and *Bacillus cereus*, Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter johnsonii*, and the yeasts *Candida lipolytica*. The cultures were maintained at 4°C on Mueller-Hinton agar (MHA) slants and transferred monthly.

Agar diffusion assay. The antimicrobial activity of the P1000-Napht was firstly tested by the agar well diffusion assay. MHA plates of 3-4 mm thickness were seeded with aliquots of overnight grown test cultures. Stock solution of the investigated HBP in DMSO (0.5%) was prepared and equal amounts (30 µl) were added into wells (8 mm in diameter) punched into MHA.

BO5. TWO SALTS OF VIOLURIC ACID INHIBITORS OF THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I

Neli Vilhelmova-Ilieva[1], Petar Grozdanov[1] , Tsonko Kolev[2], Ivanka Nikolova[1*]
[1]*Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 26, 1113 Sofia, Bulgaria*
[2]*Institute of Molecular Biology "Roumen Tsanev", BulgarianAcademy of Sciences, Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgari*

Corresponding author e-mail: nelivili@gmail.com

Abstract

The cytotoxicity of two salts of violuric acid (K-12 and K-98) on monolayer cell culture MDBK was determined. Both substances showed close toxicity values K-12 (CC_{50} = 640μM) and K-98 (CC_{50} = 630 μM).

The antiviral activity of the substances on the replication of herpes simplex virus type 1 (HSV-1) was determined. Stronger effect on the replication of intracellular HSV-1 showed substances K-98 with a selective index (SI) = 74. Significant activity also demonstrates and the other substance K-12 (SI = 64).

The effect of the test substances on the activity against the virulence of the extracellular HSV–1 virions was also investigated. Activity was determined in five time intervals: 15, 30, 60, 90 and 120 minutes. A low virucidal effect is marked at 30 minutes with Δlg = 1.5 for both compounds. K-12 showed a higher inhibition value of Δlg = 1.75 when measured at 90 min which has time-dependent properties.

Keywords: Herpes simplex virus type I, viral replication, salts of violuric acid, therapy, synthesis, acyclovir (ACV)

Introduction

According to the World Health Organization data, more than 3.7 billion people aged up to 50 years (67%) of the world's population have HSV-1 infection. Primary infection with the virus is most common in infancy through oral-to-oral contact. More than 417 million people aged between 15-49 years (11%) worldwide have an HSV-2 infection that is defined as sexually transmitted. The results are even more disturbing, considering that more than 140 million people aged between 15-49 years have an already established genital HSV-1 infection, also taking into account the fact that both HSV-1 and HSV-2 are lifetime infections (8).

Therapy against the replication of herpes simplex viruses based on the use of nucleoside analogues, the largest application of which received acyclovir (ACV), has been developed. The disadvantage of this therapy is the relatively rapid formation of resistant mutants, leading to failure of the treatment (3).

Therefore, numerous studies have been carried out in different directions, both with natural and synthetic products for the detection of new therapies against herpes infection (3, 7).

ACTA MICROBIOLOGICA BULGARICA Volume 34 / 3 (2018)

Testing of Silanes for Antiviral Activity

Angel S. Galabov[1*], Lyubomira Nikolaeva-Glomb[1], Adelina Stoyanova[1], Ivanka Nikolova[1], Nikolay Petrov[2], Neli Vilhelmova-Ilieva[1], Luchia Mukova[1], RalitzaVassileva-Pencheva[1], Zoser B. Salama[3]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]New Bulgarian University ; [3]Siogen Biotech SDN BHD, Berlin, Germany

Abstract

Forty-three organosilicon class of compounds were tested for antiviral activity using a wide scope screening program *in vitro* (in cell cultures) including eight model strains of viruses belonging to seven taxonomic groups including causative agents of infections in which applications of chemotherapy is indicated. The results obtained demonstrated a marked activity of di(hexadecanoyloxy)diphenylsilan (compound 27) only against human cytomegalovirus (SI = 30.9). A marked activity toward this virus at a low m.o.i. was recorded also by 1-o-dimethyl(octadecyl)silyl-(2,3,4,6-o-tetraacetyl-β-D-glucopyranosid) (compound 20). Small to borderline effect against this virus was found by silanes 2, 7, 15, 19 and 34, at silanes 2, 3 and 25 toward influenza virus A(H3N2) and at silane 9 versus vaccinia virus. As concerns the cytotoxicity it was established a strong variation towards different cell cultures used, the higher susceptibility of the HEp-2 cells been recorded.

Keywords: silanes, antiviral testing, *in vitro*, cytotoxicity

Резюме

Четиридесет и три съединения от класа органосиликони бяха изпитани за антивирусна активност чрез широко-спектърна скринингова програма *in vitro*, състояща се от осем моделни щамове на вируси, принадлежащи към седем таксономични групи, включващи причинители на инфекции, при които е показано приложение на химиотерапия. Получените резултати показаха отчетлива активност на ди(хексаноилокси)дифенилсилан (съединение 27) само срещу човешкия цитомегаловирус (SI = 30.9). Отчетлива активност спрямо този вирус при ниска множественост на инфекцията бе отчетена също от 1-о-диметил(октадецил)силил-(2,3,4,6-о-β-D-глюкопиранозид) (съединение 20). Слаб до граничен ефект спрямо този вирус бе намерена при силани 2, 7, 15, 19 и 34, при силани 2, 3 и 25 срещу грипен вирус A(H3N2) и при силан 9 срещу вирус вакциние. Що се касае до цитотоксичността, установено бе силно вариране спрямо различните използвани клетъчни култури, като най-висока чувствителност бе отчетена при клетки HEp-2.

Introduction

The presented organosilicon class of compounds comprises di(acyloxy)dialkyl silanes, di(acyloxy)diaryl silanes, di(acyloxy)dialkoxysilanes, tetra(acyloxy)silanes and sugar-organosilicon compounds – mono-, di- or trialkyl, alkoxysilyl sugar (saccharide(s)) as pyranosid.

By appropriate composition of the four substituents at the central silicon atom, the properties and uses of both the non-aggregated organo- and sugar-organosilicon compounds as well as the vesicles formed by these compounds (Siosomes®) can be varied on a broad basis, thus enabling adaptation to a particular problem. A large number of the silanes and sugar silanes such as alkylsilylsilanes, alkoxysilylsilanes, alkylsilylphosphonate, alkylsilylphosphites, hydroxyl and methoxy derivatives of the sugar silanes and diemethylalkylsilylethyl saccharides have been prepared and physicochemically characterised These compounds can be used: (i) in non-aggregated form, as organosilicon and sugar-organosilicon molecules; (ii) in aggregated

* Corresponding author: 26, Academician Georgi Bonchev Str., BG-1113, Sofia; galabov@microbio.bas.bg; phone: +359 2 870 0108; fax: +359 2 870 0109

Bioorganic Chemistry 85 (2019) 487–497

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Anti-enteroviral activity of new MDL-860 analogues: Synthesis, *in vitro/in vivo* studies and QSAR analysis

Ivanka Nikolova[b], Ivaylo Slavchev[a], Martin Ravutsov[a], Miroslav Dangalov[a], Yana Nikolova[a], Irena Zagranyarska[a], Adelina Stoyanova[b], Nadya Nikolova[b], Lucia Mukova[b], Petar Grozdanov[b], Rosica Nikolova[c], Boris Shivachev[c], Victor E. Kuz'min[d,e], Liudmila N. Ognichenko[d,e], Angel S. Galabov[b,*], Georgi M. Dobrikov[a,*]

[a] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, bl. 9, Acad. G. Bonchev Str., Sofia 1113, Bulgaria*
[b] *Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, bl. 26, Acad. G. Bonchev Str., Sofia 1113, Bulgaria*
[c] *Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences, bl. 107, Acad. G. Bonchev Str., Sofia 1113, Bulgaria*
[d] *A.V. Bogatsky Physical-Chemical Institute NAS of Ukraine, Department of Molecular Structure and Chemoinformatics, Odessa, Ukraine*
[e] *Odessa National Polytechnic University, Department of Theoretical Foundation of Chemistry, Odessa, Ukraine*

ARTICLE INFO

Keywords:
Coxsackieviruses
QSAR
Synthesis
Nitrobenzonitriles
MDL-860
Anti-enteroviral

ABSTRACT

A series of 60 nitrobenzonitrile analogues of the anti-viral agent MDL-860 were synthesized (50 of which are new) and evaluated for their activity against three types of enteroviruses (coxsackievirus B1, coxsackievirus B3 and poliovirus 1). Among them, six diaryl ethers (**20e**, **27e**, **28e**, **29e**, **33e** and **35e**) demonstrated high *in vitro* activity (SI > 50) towards at least one of the tested viruses and very low cytotoxicity against human cells. Compound **27e** possesses the broadest spectrum of activity towards all tested viruses in the same way as MDL-860 does. The most active derivatives (**27e**, **29e** and **35e**) against coxsackievirus B1 were tested *in vivo* in newborn mice experimentally infected with 20 MLD_{50} of coxsackievirus B1. Compound **29e** showed promising *in vivo* activity (protection index 26% and 4 days lengthening of mean survival time). QSAR analysis of the substituent effects on the *in vitro* cytotoxicity (CC_{50}) and anti-viral activity of the nitrobenzonitrile derivatives was carried out and adequate QSAR models for the anti-viral activity of the compounds against poliovirus 1 and coxsackievirus B1 were constructed.

1. Introduction

Enteroviruses are members of the *Picornaviridae* family, comprising small non-enveloped viruses with single stranded positive sense RNA genome. They are usually agents of mild infections but also cause encephalitis, myocarditis, poliomyelitis, acute heart failure, and diabetes mellitus. Enteroviruses are subject to significant changes over time because of errors introduced during genome replication. Intraspecies recombination between enteroviruses is also common, further promoting genetic diversity. This genetic plasticity allows for widespread epidemics and sporadic outbreaks to occur. Enteroviruses are now classified into 15 distinct species. Among them are polioviruses (causal agents of poliomyelitis in humans and nonhuman primates), coxsackie A viruses (associated with herpangina, human central nervous system disease, and flaccid paralysis in suckling mice), coxsackie B viruses (human central nervous system and cardiac disease, diabetes, spastic paralysis in mice), and the echoviruses (nonpathogenic in mice, and not initially linked to human disease). New strains of coxsackievirus B1 (CVB1), enterovirus-A71 (EV-A71), and enterovirus-D68 (EV-D68) have emerged as causes of recent outbreaks in the United States, South-Eastern Asia, and other countries, including more severe disease manifestations than previously described. A recent outbreak of CVB1 has once again demonstrated the epidemic potential of enteroviruses. In mid-2007, cases of severe neonatal disease due to CVB1 were recognized nearly simultaneously in several USA cities. In general, this virus was recognized as one of the most commonly circulating enteroviruses in USA between 2009 and 2013 [1].

Significant progress has been made in the global effort to interrupt poliovirus transmission and eradicate polio. However, attempts to eliminate poliovirus 1 (PV1) circulation are still running in countries like Afghanistan, Pakistan and Tadjikistan, but progress has been delayed by factors that have made vaccination unavailable for approximately 5–25% of children in the region [1]. In addition, to the best of our knowledge, an efficient and approved chemotherapy against

* Corresponding authors.
E-mail addresses: galabov@microbio.bas.bg (A.S. Galabov), gmdob@orgchm.bas.bg (G.M. Dobrikov).

https://doi.org/10.1016/j.bioorg.2019.02.020
Received 22 November 2018; Received in revised form 3 February 2019; Accepted 6 February 2019
Available online 12 February 2019
0045-2068/

Fibers and Polymers 2019, Vol.20, No.1, 19-24
DOI 10.1007/s12221-019-8687-4

ISSN 1229-9197 (print version)
ISSN 1875-0052 (electronic version)

Antimicrobial, Antibiofilm and Cytotoxicity Activity of a New Acridine Hyperbranched Polymer in Solution and on Cotton Fabric

Evgenia Vasileva-Tonkova[1]*, Desislava Staneva[2], Sandra Medel[3], Paula Bosch[3], Petar Grozdanov[1], Ivanka Nikolova[1], and Ivo Grabchev[4]*

[1]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*
[2]*University of Chemical Technology and Metallurgy, Sofia 1756, Bulgaria*
[3]*Institute of Science and Technology of Polymers, ICTR-CSIC, Madrid 28006, Spain*
[4]*Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia 1407, Bulgaria*
(Received July 27, 2018; Revised October 9, 2018; Accepted October 18, 2018)

Abstract: For the first time a fluorescent hyperbranched polymer modified with acridine (P1000-Acrid) was tested *in vitro* for antimicrobial activity against different model pathogens. The results showed strong activity of the compound against the used Gram-positive and Gram negative bacteria and yeasts. Cytotoxicity effect of P1000-Acrid has been studied towards HEp-2 cell line. The antibacterial finishing of cotton fabric treated with the P1000-Acrid was evaluated towards Gram-positive and Gram-negative bacteria. It has been shown that the studied P1000-Acrid polymer reduces bacterial growth and prevents the formation of biofilm. The obtained results indicate that the studied P1000-Acrid hyperbranched polymer possess good antimicrobial potential with the greatest effectiveness against the used Gram-positive strains.

Keywords: Hyperbranched polymer, Acridine, Antimicrobial activity, Cytotoxicity, Antibacterial cotton fabric

Introduction

The rapidly increasing emergence of antibiotic resistant pathogenic bacteria creates an urgent need for alternative antibiotics with new mechanisms of action [1]. Acridine derivatives are very promising organic fluorophores which can emit fluorescence with different color and intensity depending of their molecule polarisation. Acridine is a heteroaromatic polycyclic molecule that is well known for its DNA intercalating abilities and pharmacological properties. To date, many acridine derivatives have been synthesized and exhibited a broad spectrum of biological activities such as anticancer, antimicrobial, antitubercular, antimalarial, antiviral and antifungal activities [2,3].

Hyperbranched polymers (HBP) are highly branched three-dimensional macromolecules with unique structures and properties such as abundant functional groups, intramolecular cavities, low viscosity, and high solubility [4]. Their pharmaceutical and biomedical applications are of great interest and have been extensively investigated. Recently, the synthesis and spectroscopic properties of a new fluorescent acridine-modified hyperbranched polymer (P1000-Acrid) has been described [5]. It has been shown that the polymer emits yellow-green fluoresence and has pH sensor properties. The preliminary test for screening its microbiological activity against different Gram-positive and Gram-negative bacteria and yeasts has shown promising results.

In the recent years, due to the resistance of pathogenic microorganisms to a number of products widely used in medical practice, antimicrobial properties of textile materials have been intensively investigated. Antibacterial textile materials have find different practical applications as hospital bed linen, wound dressing, surgical implant tissues, surgical sutures, towels, filters, work clothes, etc. due to their potential for reducing the transmission of infections in medical and healthcare environments [6]. Different synthetic organic compounds, quaternary ammonium salts, metal ions and nanoparticles with antimicrobial properties are used as biologically active compounds [7]. In this regard in our group, we conduct systematic investigations in search of new effective compounds with antimicrobial activity. An important part among them is the modified with fluorophores dendrimers and hyperbranched polymers that exhibit biological activity at low concentrations [8,9]. The use of such structures in the preparation of antibacterial textile materials is a new direction in our investigations [10-13].

In the present study, more detailled studies of antimicrobial, biofilm inhibitory and cytotoxic activities of the new P1000-Acrid have been performed. The first investigation of antimicrobial activity of cotton fabric treated with the new acridine derivative has also been assessed.

Experimental

The synthesis and characterisation of hyperbranched polymer P1000-Acrid (Scheme 1) has been described recently [5]. All organic solvents and chemicals used in this study have been used as obtained from Sigma-Aldrich whitout any adistional purifications.

Treatment of Cotton Fabric with P1000-Acrid

0.005 g of P1000-Acrid was dissolved in 5 m*l* ethanol-water 1:4 (v/v) solution. Cotton fabric sample (1 g) (weight

*Corresponding author: evaston@yahoo.com
*Corresponding author: i.grabchev@chem.uni-sofia.bg

Thieme

Anti-Herpes Simplex Virus Type 1 Activity of Specially Selected Groups of Tannins

Authors
Neli Vilhelmova-Ilieva[1], Rémi Jacquet[2], Denis Deffieux[2], Laurent Pouységu[2], Tahiri Sylla[2], Stefan Chassaing[2], Ivanka Nikolova[1], Stéphane Quideau[2], Angel S. Galabov[1]

Affiliations
1 Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
2 Univ. Bordeaux, ISM (CNRS-UMR 5255), Cedex, France

Key words
ellagitannins, gallotannins, herpesvirus, antiviral screening in vitro

received 23.03.2018
accepted 01.06.2018

Bibliography
DOI https://doi.org/10.1055/a-0640-2557
Published online: 2018
Published online: 2018 Drug Res

ISSN 2194-9379

Correspondence
Stéphane Quideau
University of Bordeaux
ISM (CNRS-UMR 5255)
351 cours de la Libération
Talence
F-33405 Cedex
France
Tel.: +33/5/40 00 62 82, Fax: +33/5/40 00 69 94
stephane.quideau@u-bordeaux.fr

Angel S. Galabov
The Stephan Angeloff Institute of Microbiology
Bulgarian Academy of Sciences
26 Georgi Bonchev Street
BG-1113, Sofia
Bulgaria
Tel.: +359/2/870 0108, Fax: +359/8700109
galabov@microbio.bas.bg

ABSTRACT

Anti-herpes simplex virus (HSV-1) activity of 9 ellagitannins, including 6 natural compounds (castalin, vescalin, acutissimin A, epiacutissimins A and B, mongolicain) and 3 vescalagin synthetic derivatives (VgSBuSH, VgSOctSH, VgOMe), and 13 gallotannin-type compounds [Gal-01A, Gal-01B, Gal-02A, Gal-02B, Gal-03M, Gal-04A, Gal-04B, Gal-05M, Gal-07, Gal-08, Gal-09, Gal-11M (tannic acid), as well as Gal-12 (gallic acid), Gal-13 and Gal-14 (ellagic acid)] were examined in MDBK monolayer cell culture. Their antiviral activity was determined by the cytopathic effect (CPE) inhibition test and their cytotoxicity was evaluated through the neutral red uptake assay. In general, the series of ellagitannins showed a significantly stronger activity against HSV-1 replication than that of the gallotannins. Six of the tested ellagitannins manifested a well-pronounced activity: epiacutissimin B (selectivity index, SI > 60.6), epiacutissimin A (SI > 55.5), acutissimin A (SI > 34.8), mongolicain (SI > 32.5), VgSBuSH (SI > 24.6) and VgOMe (SI > 22.0). Four gallotannin-type compounds inhibited the replication of HSV-1 at a lower but still significant extent: Gal-04B (SI > 35.7), Gal-04A (SI > 28.5), Gal-11M (tannic acid) (SI > 25) and Gal-05M (SI = 15.6).

Introduction

Infection with HSV-1 is highly contagious and ubiquitous worldwide. In most cases, infection with the virus occurs during childhood and is lifelong. HSV-1 infection usually manifests itself as oral herpes (infection around or inside the mouth called oral-labial or oral-facial herpes), and HSV-2 is the main causative agent of genital herpes (infection in the genital or rectal area). In many cases, HSV infections can lead to clinically expressed diseases of liver, lungs, eyes, and central nervous system. The primary infection is usually asymptomatic, but it can lead to gingivostomatitis, sometimes associated with pharyngitis or with small localized lesions. Then, the virus is transported via peripheral sensory nerves to the trigeminal sensory ganglion, which subsequently led to the establishment of a lifelong latent infection. Upon external stimulations (overexposure to wind, cold, wet, heat, sun, and other triggers), latent virus reactivates and causes the recurrence of the disease [1].

The clinically established chemotherapy against HSV infections is routinely based on the utilization of anomalous nucleosidic analogs that reduce the duration of symptoms of HSV invasion and lead to more rapid healing of the lesions. The nucleosidic analogs used have structural similarities with the natural nucleosides, and they are capable of blocking the elongating growth of the newly biosyn-

materials

MDPI

Article

New Poly(Propylene Imine) Dendrimer Modified with Acridine and Its Cu(II) Complex: Synthesis, Characterization and Antimicrobial Activity

Paula Bosch [1,*], Desislava Staneva [2], Evgenia Vasileva-Tonkova [3], Petar Grozdanov [3], Ivanka Nikolova [3], Rositsa Kukeva [4], Radostina Stoyanova [4] and Ivo Grabchev [5,*]

1 Institute of Science and Technology of Polymers, Institute of Science and Technology of Polymers-Spanish National Research Council (ICTP-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain
2 Department of Chemical Technology, University of Chemical Technology and Metallurgy, 1756 Sofia, Bulgaria
3 The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
4 Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
5 Faculty of Medicine, Sofia University "St. Kliment Ohridski", 1407 Sofia, Bulgaria
* Correspondence: pbosch@ictp.csic.es (P.B.); i.grabchev@chem.uni-sofia.bg (I.G.); Tel.: +359-28161319

Received: 21 July 2019; Accepted: 16 September 2019; Published: 18 September 2019

Abstract: A second-generation poly(propylene imine) dendrimer modified with acridine and its Cu(II) complex have been synthesized for the first time. It has been found that two copper ions form complexes with the nitrogen atoms of the dendrimeric core by coordinate bonds. The new compounds have been characterized by nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), fourier-transform infrared spectroscopy (FTIR) and fluorescence spectroscopy. The spectral characteristics of the modified dendrimer have been measured in different organic solvents, and a negative fluorescence solvatochromism has been observed. The antimicrobial activity of the dendrimers has been tested against model pathogenic microorganisms in agar and by broth dilution method. The cotton fabric treated with both dendrimers has been evaluated towards pathogenic microorganisms. The obtained modified cotton fabrics have been shown to hamper bacterial growth and to prevent biofilm formation. Dendrimer cytotoxicity has been investigated in vitro in the model HEp-2 cell line.

Keywords: dendrimer; metallodendrimer; acridine; antimicrobial activity; antibacterial cotton

1. Introduction

Antibacterial surfaces are very important with regard to minimizing infectious diseases which are one of the main causes of mortality worldwide [1]. This problem is mainly due to the increasing resistance of pathogenic microorganisms to antibiotics applied in clinical practice [2] Heterocyclic compounds have major role in the design and investigations of new bioactive drugs [3,4] Therefore acridine derivatives are one of the intensively exploited organic fluorophores in which fluorescence color intensity depends strongly on the polarization of their chromophoric system [5,6]. Possessing a heteroaromatic polycyclic molecule acridine derivatives are well known for their DNA intercalating abilities and pharmacological activity. That has led to the design and preparation of acridine compounds with anticancer, antimalarial, antiviral and antifungal activities [5–10].

Dendrimers are macromolecules with well-defined molecular weight and a high degree of branching units containing different reactive functional groups to which substances with biological activity may be attached by a chemical bond or by weak intermolecular interactions [11]. Compared to the low molecular weight bioactive compounds, dendrimers have the potential to deliver a large dose

Antiviral, Cytotoxic and Antioxidant Effects of *Tanacetum vulgare* L. Crude Extract *in vitro*

Neli Vilhelmova[1], Lora Simeonova[1], Nadya Nikolova[1], Elitsa L. Pavlova[2], Zlatina Gospodinova[3], Georgi Antov[3], Angel S. Galabov[1], Ivanka Nikolova[1*]

[1]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26 Georgi Bonchev, 1113 Sofia, Bulgaria

[2]Sofia University "St. Kliment Ohridski", Faculty of Physics, 5 James Boucher Blvd., 1164 Sofia, Bulgaria

[3] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 21 Georgi Bonchev, 1113 Sofia, Bulgaria

Corresponding author: Ivanka Nikolova vanianik@mail.bg

ABSTRACT

Introduction: Due to the high prevalence of viral infections having no specific treatment and the constant development of resistant viral mutants the search of effective antiviral compounds is essential. The present work explores in vitro antiviral activity of ethanolic extract from aerial parts of *Tanacetum vulgare* L. against viral strains of three taxonomic groups, including agents that cause socially significant diseases in humans for which antiviral chemotherapy is indicated, namely Coxsackievirus B1 (family *Picornaviridae*), Herpes simplex virus type1 (family *Herpesviridae*) and Influenza A virus (family *Orthomyxoviridae*).

Aim: The aim of the current study was to evaluate antiviral activity of ethanolic extract from herbaceous plant *Tanacetum vulgare* L. against some important human viruses for which antiviral chemotherapy is needed and to characterize extract for its antioxidant activity *in vitro*.

Materials and methods: The crude aqueous ethanolic extract from aerial parts of *Tanacetum vulgare* L. contained: flavonoids, determined as apigenin; coumarins, determined as aesculintannic compounds, determined as tannin and others. Antiviral activity of ethanolic extract from herbaceous plant *Tanacetum vulgare* L. against Coxsackieviris B1, Influenza A and Herpes simplex virus type 1 was evaluated by viral yield reduction technique. The total antioxidant activity was determined by measuring the capacity of the sample to inhibit the generation of thiobarbituric acid reactive substances (TBARS).

Results: The results show the lowest toxicity of the extract on the MDBK cell line and close cytotoxicity in Hep-2, whereas in the MDCK cells shows more than twice highest toxicity. Testing the antiviral activity of *Tanacetum vulgare* L. extract revealed a slight inhibition of replication of HSV-1 with a selective index 7.07 and IAV/H3N2 (SI = 3.69) but no specific antiviral effect against CVB1 replication was reported. The evaluation of the antioxidant activity showed great antioxidant activity of the ethanolic extract from *T. vulgare* – 26 mmol/l for the applied 20 mg/ml extract.

Conclusions: The crude extract from aerial parts of the medicinal plant *Tanacetum vulgare* L. demonstrated low cytotoxicity in Hep-2, MDBK and moderate cytotoxic effects in MDCK cells. It exerted significant antiviral activity against HSV-1 as determined by the recorded

Antiviral Chemistry and Chemotherapy
2020, Vol. 28: 1–6

DOI: 10.1177/2040206620906061
journals.sagepub.com/home/avc
SAGE

Genome analysis of coxsackievirus B1 isolates during the consecutive alternating administration course of triple antiviral combination in newborn mice

Petar Grozdanov[1], Marie-Line Joffret[2], Adelina Stoyanova[1], Patsy Polston[2], Emna Achouri[2,3], Ivanka Nikolova[1], Francis Delpeyroux[2] and Angel S Galabov[1]

Abstract

Background: We developed a new approach for the treatment of enterovirus infections, the consecutive alternating administration (CAA) of a combination of enterovirus inhibitors. On the model of coxsackievirus B1 (CVB1) in mice, two phenomena were observed: absence of drug resistance and increased susceptibility to the antivirals. This study aims to clarify the genetic basis of these phenomena.

Methods: Brain samples from CVB1-infected mice subjected to a CAA course with the combination pleconaril/MDL-860/oxoglaucine were used for viral RNA extraction and next generation sequencing. In parallel, samples from monotherapeutic courses of the three substances included in the combination were studied. Whole genome sequence analysis was carried out on all samples.

Results: Samples of pleconaril monotherapy showed mutations in 5′untranslated region, VP3, 2C, 3C and 2A regions of viral RNA, translated in amino acid substitution of the 2A protein. The MDL-860 course induced changes in CVB1 RNA in the VP3 and 2C regions. The oxoglaucine monotherapy samples showed RNA mutation and amino acid substitution in the VP1 region and nucleotide substitution in the 3D region. In the specimens taken from mice subjected to the CAA course with pleconaril/MDL-860/oxoglaucine, the following RNA mutations were established: 5′ untranslated region, 2A, and 2B, and amino acids substitutions in VP3 and 2A, which differ from those mentioned above. These changes could be the reason for the prevention of drug resistance development and also to be considered as the basis for the phenomenon of increased drug susceptibility.

Conclusions: The results reveal that the high anti-enteroviral efficacy of the CAA course is substantiated by the appearance of specific changes in the viral genome.

Keywords

Drug combination, drug resistance, enteroviruses, genomic analysis

Date received: 11 September 2019; accepted: 8 January 2020

Introduction

Enterovirus (EV) infections are a significant cause of morbidity and mortality throughout the world. EVs have been associated with many human diseases, including myocarditis; pericarditis; dilated cardiomyopathy; Bornholm disease; aseptic meningitis; poliomyelitis; juvenile insulin-dependent diabetes; hand, foot, and mouth disease; common cold; uveitis; and so on.

[1]Department of Virology, The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria
[2]Department of Virology, Institut Pasteur, Paris, France
[3]Department of Computational Biology, Institut Pasteur, Paris, France

Corresponding author:
Angel S Galabov, Department of Virology, The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria.
Email: galabov@microbio.bas.bg