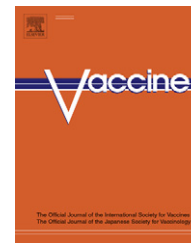




available at www.sciencedirect.com



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



Genetic diversity-independent neutralization of pandemic viruses (e.g. HIV), potentially pandemic (e.g. H5N1 strain of influenza) and carcinogenic (e.g. HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs)

Girish J. Kotwal^{a,b,c,*}

^a Division of Infectious Diseases, Department of Medicine, University of Massachusetts, Worcester, MA 01605, USA

^b Kotwal Bioconsulting, LLC, Louisville, KY 40241, USA

^c Kbiotech Pvt. Ltd., Cape Town, South Africa

Received 28 October 2007; received in revised form 3 December 2007; accepted 5 December 2007

Available online 26 December 2007

KEYWORDS

Entry inhibitor;
Fulvic acid;
Hemagglutinin;
Inactivation;
H5N1;
Influenza subtypes;
Pomegranate juice;
Treatment;
Genetic diversity;
Human
Immunodeficiency
virus;
Enveloped virus;
Neutralizing
compounds

Summary Genetic diversity and hypermutation contribute to difficulties in developing a vaccine against viruses like HIV and influenza. There are currently no known immune correlates of protection against HIV. This has made the development of a vaccine against HIV that would provide sterilizing immunity in the near future an impossible task. The abandonment of a recent AIDS vaccine human trial due to a failure to elicit a protective sterilising immune response confirms that empirical attempts to develop a vaccine may result in failures. Also the difficulty in predicting the next pandemic strain of influenza may make it difficult to respond rapidly should there be an outbreak. Therefore, it is time to explore broad spectrum agents that can target either the lipid portion of the envelope or the sugar moieties of the glycoproteins or the rafts (regions within viral and cell envelopes where a higher concentration of the glycoproteins exist). Broad spectrum agents that can serve as disrafters or neutralize the viral infectivity by binding to the envelope lipid or sugar moieties will not be affected by the vagaries of hypermutation of surface antigens. This is because the post-translation modification is a host function. Presented here is a review of recently reported agents present in pomegranate juice (polyphenols, beta-sitosterol, sugars and ellagic acid) and fulvic acid, described here as the envelope virus neutralising compounds (EVNCs) and

Abbreviations: EVNCs, enveloped virus neutralising compounds; HA, haemagglutinin; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; KSHV, kaposi sarcoma herpes virus.

* Correspondence address: Kotwal Bioconsulting, LLC, 4664 Shenandoah Drive, Louisville, KY 40241, USA.

E-mail address: GJKOTW01@gmail.com.

0264-410X/\$ – see front matter © 2007 Elsevier Ltd. All rights reserved.

doi:10.1016/j.vaccine.2007.12.008

complex molecules like lectins and mucins. Pomegranate juice was previously reported to inactivate HIV and further shown by our group to inactivate influenza, herpes viruses and poxviruses. A formulation consisting of fulvic acid, a complex mixture of compounds was previously reported to render vaccinia virus, HIV and SARS virus non-infectious. Recently, both fulvic acid and pomegranate juice have been shown to inactivate genetically diverse strains of influenza including H5N1, further confirming the broad spectrum nature of these agents. How EVNCs will be used in developing a vaccine achieving sterilizing immunity or prophylaxis needs to be researched.

© 2007 Elsevier Ltd. All rights reserved.

Enveloped viruses like HIV, HBV, HCV, influenza and herpes viruses together contribute annually to over a billion infections and significant mortality worldwide. HIV remains a major killer of young people in sub-Saharan Africa [1], the success of antiretrovirals (ARVs) and prevention education in the west has not been fully replicated in Africa and South Asia. With ARVs beyond the reach of the overwhelming majority of infected individuals in these regions, and with side effects (lactic acidosis, peripheral neuropathy and adipose deposition) difficult to manage, there is an urgent need for alternate prophylactic and treatment approaches that can be easily delivered, are nontoxic, and are unaffected by the rapid mutation rates of the virus. In addition to the many established challenges, Africa may soon have to deal with the spread of bird flu [2,3], which had emerged in the western part of the continent. Africa, with a large immunosuppressed and malnourished population, must be considered particularly vulnerable to this threat. Asia and South America are only slightly better off but would also be devastated by a pandemic flu. The few effective treatments of acute influenza include oseltamivir phosphate (Tamiflu), a neuraminidase inhibitor [4], but its availability in Africa is very limited. Shikimic acid, one of the main starting materials of Tamiflu is obtained from star anise, a cooking spice from a tree grown in China but other sources of shikimic acid are being explored and fairly high concentrations of it are found in natural sources, e.g. pine, spruce and fir trees. In a recent article, additional problems related to the use of shikimic acid, developments and the problems toward the synthesis of Tamiflu and the use of other starting materials have been discussed [5]. Furthermore, any influenza outbreak is likely to precede the availability of protective vaccines by a long period. Clearly, there is an urgent need to bring anti-flu drugs like relenza "back to life" [6], and to investigate promising antivirals from all possible sources [7]. Coal-derived humic acids and oxyfulvic acid, which are complex mixtures formed during the decomposition of organic matter including cellulose found naturally have been previously shown to have anti-HIV properties [8,9]. A formulation called Secomet V has been reported to have a broad spectrum antiviral properties [10] and was subsequently revealed a year later by Stefan Coetzee from Fulvimed Pvt. Ltd. to have fulvic acid as the major active ingredient. Complex molecules like lectins and mucins which can interfere with attachment of enveloped viruses also seem to have a broad spectrum antiviral activity against HIV and poxviruses [11–13]. These agents can be predicted to be effective against influenza but that remains to be proved and they may not be too practical because of their molecular size and due to interference of normal tissue functions, other than as possible additives to microbicides.

A network of researchers have been investigating the antiviral properties of a formulation called Secomet V, whose active ingredient is fulvic acid, which is a complex mixture of compounds arising from decomposing organic matter. Antiviral activity of fulvic acid containing Secomet V against poxviruses and SARS has been demonstrated in our laboratory and that of a German group [10]. The bioactive fulvic acid found in the formulation was separated by ultrafiltration using a 3000 Da filter and column chromatography and found to be in the range of 200–600 Da; with the peak activity observed around fractions corresponding to 113, 226 and 452 Da indicating that the active compounds maybe repeats of a monomeric unit of 113 Da (Kotwal, unpublished). The mechanism of action and structure of fulvic acid and bioactive compounds from pomegranate juice are currently being fully elucidated but hemagglutinating activity is inhibited indicating that fulvic acid possibly blocks entry of the virus by interacting with the sugar or lipid moiety or both of surface glycoproteins of enveloped viruses. Previously, pomegranate juice was shown to provide an HIV-1 entry inhibitor [14]. Our studies suggest that the bioactive molecule renders the virus non-infectious by inhibiting entry into the cells. Once a virus enters a cell, the agent has no effect on viral replication. Short- and long-term toxicity studies *in vitro* and *in vivo* show that the bioactive agent is safe. Physical and chemical analyses show that the formulation is acidic (around pH 2) and heat stable (survives autoclaving), and that the levels of iron and arsenic are well below the permissible levels. In order to test the hypothesis that the formulation containing fulvic acid or pomegranate juice can neutralize the infectivity of a wide range of genetically diverse strains of a given enveloped virus, possibly by interacting with sugar chains on the viral surface protein and serving as an entry inhibitor, we tested its activity against influenza A/HK/x31(H3N2), influenza A/Vietnam/1203/04 (H5N1), and a reassortant x31 containing the NS gene segment of an H5N1 isolate (Sangster, Sandbulte, Webby and Kotwal, unpublished). The reassortant x31 was described before [15]. All viruses were inactivated in a dose-dependent manner when treated for 5 min at room temperature with the fulvic acid containing formulation or for the same period at 37°C with pomegranate juice. To obtain further evidence for the antiviral action of fulvic acid, mice were intranasally administered a normally highly lethal dose of A/x31 that had been treated for 5 min with fulvic acid. Control mice received an equivalent dose of mock-treated virus. Mice that received the treated virus showed no signs of disease, whereas the mock-treated virus produced extensive weight loss (due to diminished water consumption) and 75% mortality. In addition, the duration of the survival of the infected untreated

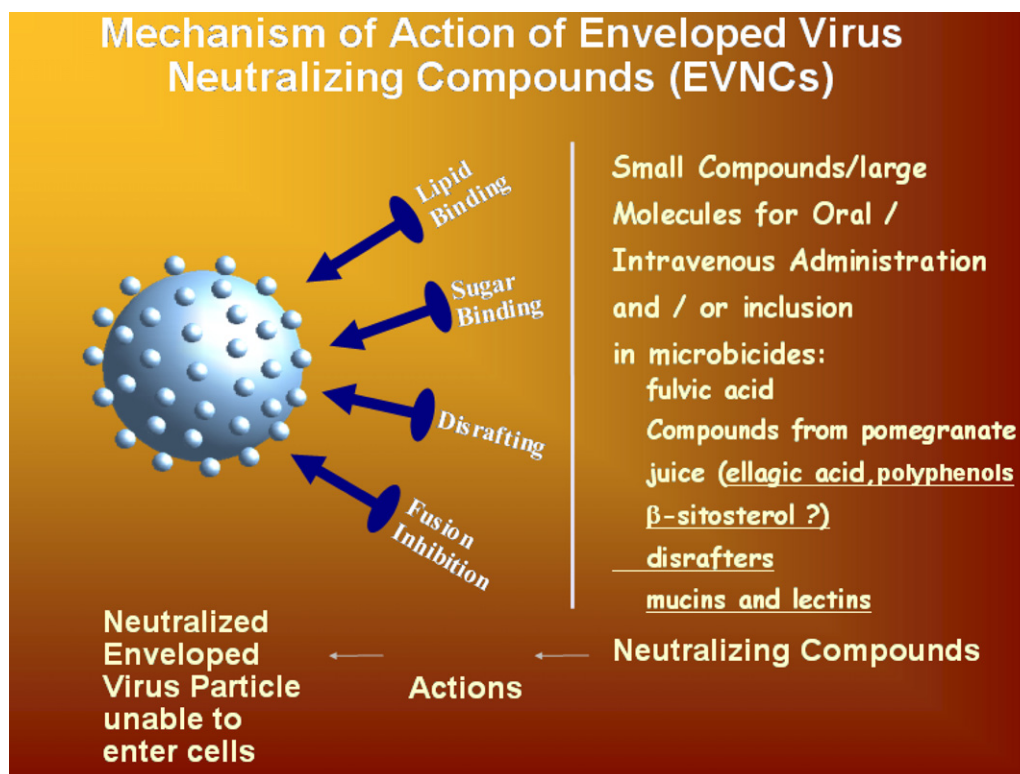


Figure 1 Mechanism of Action of Enveloped virus neutralizing compounds (EVNCs).

mice mimics the duration of the period of survival from fever to death in H5N1 infected humans [16].

We suggest that compounds with antiviral activity from fulvic acid and from pomegranate juice neutralize the infectivity of diverse enveloped viruses and a number of subtypes of a given enveloped virus, indicating potential for development as a treatment option that can be broadly effective against pandemic viruses like HIV potentially pandemic viruses like influenza and carcinogenic viruses like HBV and HCV. Although it remains to be proved that the carcinogenic viruses and potentially dangerous bioweapon like variola can be neutralized by the EVNCs, one can predict with a fair bit of confidence that demonstration of routine experimental neutralization of a live attenuated vaccinia virus, vGK5 [17] with the greatest structural robustness compared to all known enveloped viruses and similar to variola can be extrapolated to the carcinogenic hepatitis viruses and variola. These findings also raise the possibility that certain plant acids, sugars and complex carbohydrates may have an application in the production of inactivated whole viral vaccines as well as microbicides (see Fig. 1). The EVNCs could be administered orally in concentrated capsules or administered intravenously after extensive research into the bioavailability and proper well conceived clinical trials. Until this can be accomplished, universally available pomegranate juice or the pomegranate fruit known for centuries could be consumed regularly and in significant amounts, may provide benefit against enveloped viral infections but should not be seen as a substitute for other well established means of infection prevention such as effective vaccines against HBV, seasonal influenza and condoms against HIV. The EVNCs in the body fluid could neutralize

viruses in the blood stream and elicit an immune response to the neutralized authentically folded virus particle and thus facilitate development of a potentially protective immunity [18].

Acknowledgement

The authors would like to thank Dr. Siddarth Sharma of the Institute of Science Bangalore in India and Professors Paul Barlow and Dr. Dusan Uhrin of the Chemistry Department, University of Edinburgh, Scotland for assistance with molecular weight analysis of the compounds.

The figure showing the proposed mechanism of action was prepared by Mr. Amod Kulkarni, a Ph.D. student at the University of Cape Town, Cape Town, South Africa.

The author thanks Dr. Mark Sangster of the University of Tennessee, Knoxville, USA for helpful discussion and suggested changes in the manuscript. Information on disrafters can be accessed via the website of JADO Technologies <http://www.jado-tech.com/technology/raftintervention.asp>.

References

- [1] Kotwal GJ. HIV treatment and eradication in South Africa. *J R Soc Med* 2004;97:1–2.
- [2] Palese P. Influenza: old and new threats. *Nat Med Rev* 2004;10(Suppl. 12):S82–7.
- [3] Webby R, Hoffmann E, Webster R. Molecular constraints to interspecies transmission of viral pathogens. *Nat Med* 2004;10(Suppl. 12):S77–81.

- [4] De Clercq E. Antiviral agents active against influenza A viruses. *Nat Rev Drug Discov* 2006;12:1015–25.
- [5] Farina V, Brown JC. The supply problem. *Angew Chem Int* 2006;45:7330–4.
- [6] Cyranoski D. Threat of pandemic brings flu drug back to life. *Nat Med* 2005;11:909.
- [7] McCullers AJ. The clinical need for new antiviral drugs directed against influenza virus. *J Infect Dis* 2006;193:751–3.
- [8] Van Rensburg CEJ, Dekker J, Weis R, Smith T-L, Rensburg EJ, Schneider J. Investigation of anti-HIV properties of oxihumate. *Exp Chemother* 2002;48:138–43.
- [9] Van Rensburg A, van Straton J, Dekker J. An in vitro investigation of the antimicrobial activity of oxifulvic acid. *J Antimicrob Chemother* 2000;46:853–4.
- [10] Kotwal GJ, Kaczmarek JN, Leivers S, Ghebremariam YT, Kulkarni AP, Bauer G, et al. Anti-HIV, anti-poxvirus, and anti-SARS activity of a nontoxic, acidic plant extract from the trifolium species Secomet-V/anti-Vac suggests that it contains a novel broad-spectrum antiviral. *Ann N Y Acad Sci* 2005;1056:293–302.
- [11] Habte HH, de Beer C, Lotz ZE, Tyler MG, Kahn D, Mall AS, et al. The role of crude human saliva and purified salivary MUC5B and MUC7 mucins in the inhibition of human immunodeficiency virus type I in an inhibition assay. *Virology* (Biomed Central) 2006; 3:99.
- [12] Habte HH, Kotwal GJ, Lotz ZE, Tyler MG, Abrahams M, Rodrigues J, et al. Antiviral activity of purified human breast milk mucin. *Neonatology* 2007;92(2):96–104.
- [13] Kaur A, Kamboj SS, Singh J, Singh R, Abrahams M, Kotwal GJ, et al. Purification of 3 monomeric monoco mannose-binding lectins and their evaluation for anti-poxviral activity: potential applications in multiple viral diseases caused by enveloped viruses. *Biochem Cell Biol* 2007;85(1):88–95.
- [14] Neurath AR, Strick N, Li YY, Debnath AK. Punica granatum (pomegranate) juice provides an HIV-1 entry inhibitor and candidate topical microbicide. *Ann N Y Acad Sci* 2005;1056:311–27.
- [15] Lipatov AS, Andreansky S, Webby RJ, Hutse DJ, Rehg JE, Krauss S, et al. Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses. *J Gen Virol* 2005;86:1121–30.
- [16] Yu H, Shu Y, Hu S, Zhang H, Gao Z, Chen H, et al. The first confirmed human case of avian influenza A (H5N1) in Mainland China. *Lancet* 2006;367:84–5.
- [17] Kotwal GJ, Hugin AW, Moss B. Mapping and insertional mutagenesis of a vaccinia virus gene encoding a 13,800-Da secreted protein. *Virology* 1989;171(2):579–87.
- [18] Kotwal GJ, Kulkarni AP. Antigenic variation in microbial evasion of immune responses. *Encyclopedia of LifeSciences*, John Wiley & Sons, Ltd., Chichester. <http://www.els.net/>, doi:10.1002/9780470015902.a0001207.pub2.