Virulence

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: http://www.tandfonline.com/loi/kvir20

Ozonation of human HIV-infected plasmas for producing a global vaccine: How HIV-patients may help fight the HIV pandemia

Velio Bocci, Iacopo Zanardi & Valter Travagli

To cite this article: Velio Bocci, Iacopo Zanardi & Valter Travagli (2010) Ozonation of human HIV-infected plasmas for producing a global vaccine: How HIV-patients may help fight the HIV pandemia , Virulence, 1:3, 215-217, DOI: 10.4161/viru.1.3.11830

To link to this article: http://dx.doi.org/10.4161/viru.1.3.11830

Virulence 1:3, 215-217; May/June, 2010; © 2010 Landes Bioscience

Ozonation of human HIV-infected plasmas for producing a global vaccine

How HIV-patients may help fight the HIV pandemia

Velio Bocci,[1,*] Iacopo Zanardi[2] and Valter Travagli[2]

[1]Department of Physiology; and [2]Department of Pharmaceutical Sciences; University of Siena; Siena, Italy

Key words: HIV, ozone, vaccine, human albumin, adjuvant, protocol

A vaccine against HIV able to generate properly neutralizing antibodies and an efficacious cytotoxic T-lymphocyte response is of paramount importance. We are proposing a novel approach based on the collection of thousand small HIV-infected human plasma samples for preparing a global vaccine, able to counteract HIV diversity and mutagenicity. The pooled plasmas will undergo several steps for sterilizing and inactivating HIV, possibly other contaminant viruses and other pathogens. The critical step is the prolonged and controlled exposure of plasmas to ozone so that finally each ml of plasma has interacted with a precise dose of ozone. To inactivated plasma, both therapeutic human albumin and ozonated ethyl oleate are added for enhancing a proficient absorption and reaction with the immune system of the vaccine. The need of a partner collaboration for developing the production and the preliminary testing of the vaccine is essential.

Introduction

A recent publication[1] reports that a further refinement of the highly antiretroviral therapy (HAART), by using a median of novel drugs for almost a year, can almost delete HIV plasma viral load in already heavily treated patients. These news, though good, must be tempered by the risk of drug resistance, metabolic disorders and the risk of malignancy. Thus, in spite of a continuous medical progress,[2] the role of prevention and of an effective vaccine remain of fundamental importance. Sexual drive tends to neglect the necessary precautions in both advanced and third-world countries and, even the best thought vaccines[3-5] have yielded disappointing results because HIV not only presents a genomic heterogeneity but undergo frequent antigenic variations precluding an efficacious result.

History of Medicine shows that vaccines against either bacterial or viral diseases have represented one of the most significant medical progress. From the initial heroic times, when a vaccine was simply prepared by inactivating bacteria with formic aldehyde, there had been a tendency to isolate the most relevant antigens, which after inactivation, have shown to be active and relatively free of side effects. After clarifying the genome and HIV proteins, the strategy of selecting one or only a few HIV components was adopted in the belief of achieving an effective vaccine but time has revealed that a poor or inadequate immune response[3,6] accompanied by the HIV diversity and mutagenic capabilities,[7] leads to an incompetent defence. Moreover the problem has become more complex because each continent has HIV variants, which challenge the valence of a global vaccine.

Recently we became interested in this problem and made the proposal of evaluating a HIV autovaccine, which, in still responsive patients, may be able to reduce the viral daily replication as well as a further variation of antigenic constituents.[8] This concept is under evaluation but the relevance of the autovaccine remains limited to the single donor patient. It has been frequently said that in the awkward field of HIV vaccination, unorthodox thinking may be helpful and we would like to propose a new scheme that may have a general use. This idea has grown out from the previous one and it could become useful if the vaccine will prove to be efficacious, safe and without side effects.

Time Required

The time required to perform a preliminary evaluation is estimated in about two and a half years.

Expected Results and Discussion

To the best of our knowledge, the idea of using the HIV virions present in HIV patient's plasma has been not yet evaluated. So far a great number of vaccines for preventing HIV infection have been made of either the components of the viral envelope, or HIV subunits made by genetic engineering or of HIV genes free, or carried by recombinant vectors. One of the most disheartening disappointments has been to observe the failure of the generated antibodies to either fully prevent or block HIV invasion or/and enhance and maximize the cytotoxic T-lymphocyte

*Correspondence to: Velio Bocci; Email: bocci@unisi.it
Submitted: 10/20/09; Accepted: 06/12/09
Previously published online: www.landesbioscience.com/journals/virulence/article/11830

response. It is known that a very small percentage of humans is almost naturally HIV-resistant but the responsible factor(s) remains elusive. Our project envisages the use of presenting the immune system all the necessarily inactivated viral components to the immune system as it happens during the natural infection, a strategy that may give time to the normal immune system to organize a series of defensive reactions without undergoing any immunosuppressive activity by the normal HIV. It is known that it is not possible or too risky trying an attenuated immunodeficiency virus. The inactivation has been carried out during the ozonation step, and ozone was selected in comparison to heat, UVA, radiation and chlorine owing to long experience in ozonating human blood by using an ample range of ozone concentrations.[8,11,12] The bland ozonation normally carried out on blood of HIV patients is unable to have a sterilizing and therapeutic effect.[20] This is so because ozonated autohemotherapy successfully carried out in vascular diseases[11,12] must use far lower levels of ozone, which are not deleterious for blood cells and also unable to inactivate HIV in the circulation. We are well aware that ozone oxidation may modify some epitopes and it remains to be seen if and which antigens will remain active or silent. A potential advantage of this procedure is that a vaccine made of virions obtained from a great variety of patients will contain all possible HIV genetic diversity at the present and possibly in future times. If the immune system is able to efficiently respond to the ozonated antigens, the vaccine could be used as preventive and possibly curative vaccine.

The development of the vaccine requires a considerable financial commitment and we will be glad to collaborate with an eventual partner interested in pursuing this novel strategy.

Materials and Detailed Procedure

Owing to technical and logistical difficulties, it will be necessary to perform a preliminary evaluation in Italy and, if results are promising, a second phase by collecting HIV-infected plasma samples in five continents will be entertained. Nonetheless the technical procedure will probably remain unvaried but it will be easily scaled up.

(a) After giving the most ample information on the finality of the project and if the HIV patient will sign the informed consent, during one of the periodic control at the HIV clinic, he may agree to donate 20 ml of blood in a sterile syringe containing 3 ml of Na-citrate at 3.8% concentration. A basic limitation is that HIV patients' donors, on the basis of previous tests, should be free of concomitant diseases such as chronic hepatitis (A, B, C, D), bacterial infections, tbc, malaria and other parasitosis and haematological diseases. Owing to the fact that a great number of individual plasma will be pooled, it is necessary to limit the infection to HIV, although it remains impossible to avoid other latent viral infections.

After appropriate mixing, blood will be centrifuged at 3,000 xg for 20 min to sediment all blood cells. About 10–12 ml of the supernatant plasma containing a virions at a median level of about 30,000–50,000 copies/ml will be stored in a half-litre plastic container at -20°C, which within about a month will eventually collect another 35–40 plasma samples of HIV patients. It is planned to collect five containers collected in different Italian hospitals (possibly Milan, Bologna, Florence, Rome and Naples) to yield a total volume of about 2,100–2,200 ml of plasma. This phase will take approximately two months to be completed.

(b) After thawing and mixing the content of the five containers, pooled human HIV plasma will be centrifuged at 3,000 xg for 15 min and the clear plasma will undergo filtration through a 0.22 μm filter to eliminate possible large size, pathogenic contaminants. This and the following steps will be performed under sterile and safety conditions. A small aliquot will be tested for assessing the HIV viral concentration and for other potentially present virus contaminations.

(c) The plasma will undergo five successive stages of overnight freezing at -196°C by liquid nitrogen with successive thawing next morning. This step is performed for at least partially breaking the viral envelope and releasing the content of the viral core in order to facilitate the following step.

(d) Two litres of plasma will now undergo ozonation in a closed neutral glass container. Pure medical oxygen will be filtrated (0.2 μm) before entering a modern medical ozone generator showing by photometry, in real time, the generated ozone concentration. The actual ozone concentration is periodically checked by the iodometric method.[9] The oxygen-ozone gas mixture (about 95 and 5%) with a precise flow of 1 l/min and an ozone concentration of 50 μg/ml will finely bubble through a sintered glass into the plasma continuously mixed with a magnetic stirrer. The ozonation process will proceed for 32 min until each ml of plasma had been treated with an ozone dose of 800 μg. The ozonation vessel has an outlet tubing connected with a suitable ozone destructor to prevent air contamination.

On the basis of almost twenty years experience,[10-13] viral inactivaction should be total without compromising the structural composition of several viral components. Far lower ozone concentrations would have been sufficient for inactivating both viruses and bacteria in saline solution because plasma antioxidants are protective and must be totally oxidized.[14] Murray et al.[15] have also reported the ozone capability of inactivating a number of viruses either in phosphate buffer solution alone or with the addition of calf serum.

(e) The ozonated plasma will then undergo a further centrifugation at 3,000 xg for 15 min under sterile conditions. An equal volume (2 l) of therapeutic human albumin 20% (Uman Albumin, Kedrion—Italy) will be added with 100 ml of ozonated ethyl oleate with a peroxide number of about 3,000 mEq/1,000 g, equivalent to about 20 μg/ml ozone.[16] After the intramuscular injection, human albumin at high concentration is intended to increase the oncotic interstitial pressure and act as an expander for enhancing absorption of the viral components via the lymphatic system.[17] The ethyl oleate containing ozone as a trioxolane,[18] bound to albumin, will act as a mild adjuvant and inducer of heme-oxygenase-1.[19] After appropriate mixing, 2.1 ml of the plasma-oil suspension will be closed in vials for the successive testing. Just before the injection, the content will be mixed and aspirated in a 2.5 ml syringe via a G21 needle. About

1,000 vials will become available for preliminary studies.

(f) Laboratory tests will ascertain the sterility and any possible pyrogenicity. Further tests in rabbits and monkeys will be indispensable to evaluate the full viral inactivation and if the intramuscular injection of the plasma sample causes a local reaction. If preclinical results will show that the vaccine is safe, it will be necessary to perform a phase 1 study in human volunteers. It will be indispensable to firstly evaluate the safety and lack of side effects using a mock vaccine by using plasma from healthy donors (absolutely HIV free) subjected to all the outlined steps. Only after this initial investigation, it will be possible to evaluate the vaccine as a therapeutic vaccine in HIV subjects before testing it in healthy individuals.

(g) The vaccination programme foresees four IM (glutei muscle) injections, each one every three months followed by the evaluation of both local and general side effects, possibly similar to a flu-like syndrome. Moreover it will be necessary to evaluate in all volunteers both humoral and cell-mediated responses.

Procedure at a Glance

(a) Collection of human HIV plasma samples;

(b) Centrifugation of the pooled plasma samples and filtration through an antibacterial filter;

(c) Five cycles of freezing (-196°C) and thawing (+4°C) within a week;

(d) Inactivation of HIV with ozonation (800 μg ozone per ml of plasma);

(e) Addition of both human albumin (200 mg/ml) and ozonated ethyl oleate, corresponding to 20 μg of ozone;

(f) Laboratory tests. Preclinical evaluation in animals. Evaluation in volunteers of a mock vaccine and of the actual ozonated vaccine; and

(g) Vaccination schedule.

Acknowledgements

The linguistic revision by Mrs. H. Carter is gratefully acknowledged.

References

1. Steigbigel RT, Cooper DA, Kumar PN, Eron JE, Schechter M, Markowitz M, et al. BENCHMRK Study Teams. Raltegravir with optimized background therapy for resistant HIV-1 infection. N Engl J Med 2008; 359:339-54.
2. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338:853-60.
3. Montefiori D, Sattentau Q, Flores J, Esparza J, Mascola J. Antibody-based HIV-1 vaccines: recent developments and future directions. PLOS Medicine 2007; 4:348.
4. Watkins DI. Basic HIV vaccine development. Top HIV Med 2008; 16:7-8.
5. Plotkin SA. Sang Froid in a time of trouble: is a vaccine against HIV possible? J Int AIDS Soc 2009; 12:2.
6. Blish CA, Nedellec R, Mandaliya K, Mosier D, Overbaugh J. HIV-1 subtype A envelope variants from early in infection have variable sensitivity to neutralization and to inhibitors of viral entry. AIDS 2007; 21:693-702.
7. Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med 2008; 358:1590-602.
8. Bocci V, Travagli V, Zanardi I. The failure of HIV vaccines: a new autovaccine may overcome some problems. Med Hypotheses 2009; 72:662-4.
9. Masschelein W. Jodometric method for the determination of ozone in a process gas. In: Viebahn-Hänsler R, Knoch HG, eds. Ozon-Handbuch. Grundlagen. Prävention. Therapie. Vol. IX-8. Landsberg: Ecomed 2001; 1-3.
10. Bocci V. Ozonization of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis. Med Hypoth 1992; 39:30-4.
11. Bocci V. Is it true that ozone is always toxic? The end of the dogma. Toxicol Appl Pharmacol 2006; 216:493-504.
12. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev 2009; 29:646-82.
13. Travagli V. Evaluation of the effects of ozone blood treatment on the metabolite profile of human blood. Toxicol Sci, submitted.
14. Burgassi S, Zanardi I, Travagli V, Montomoli E, Bocci V. How much ozone bactericidal activity is compromised by plasma components? J Appl Microbiol 2009; 106:1715-21.
15. Murray BK, Ohmine S, Tomer DP, Jensen KJ, Johnson FB, Kirsi JJ, et al. Virion disruption by ozone-mediated reactive oxygen species. J Virol Methods 2008; 153:74-7.
16. Zanardi I, Travagli V, Gabbrielli A, Chiasserini L, Bocci V. Physico-chemical characterization of sesame oil derivatives. Lipids 2008; 43:877-86.
17. Bocci V. Physicochemical and biologic properties of interferons and their potential uses in drug delivery systems. Crit Rev Ther Drug Carrier Syst 1992; 9:91-133.
18. Travagli V, Zanardi I, Bocci V. Topical applications of ozone and ozonated oils as anti-infective agents: an insight into the patent claims. Recent Pat Antiinfect Drug Discov 2009; 4:130-42.
19. Bocci V, Aldinucci C, Mosci F, Carraro F, Valacchi G. Ozonation of human blood induces a remarkable upregulation of heme oxygenase-1 and heat stress protein-70. Mediators Inflamm 2007; 2007:26785.
20. Bocci V, Venturi G, Catucci M, Valensin PE, Zazzi M. Lack of efficacy of ozone therapy in HIV infection. Clin Microbiol Infect 1998; 4:667-9.