The AAPS Journal, Vol. 12, No. 3, September 2010 (© 2010)
DOI: 10.1208/s12248-010-9187-z

Meeting Report

AIDS Treatment with Novel Anti-HIV Compounds Improved by Nanotechnology

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Received 6 January 2010; accepted 16 February 2010; published online 6 April 2010

Abstract. The first International Symposium of Nanomedicine on AIDS "AIDS Treatment with Novel Anti-HIV compounds Improved by Nanotechnology" was held November 19–20, 2009 in Beijing, China. This symposium provided an international forum for presentation and discussion of exciting new advances in the emerging research area of nanobiomedical research on AIDS treatment as the focus point, as well as some issues in relevant fields such as nanobiomedical research on tumor treatment and safety evaluation of nanomedicines. Key highlights of the symposium include (1) reviewing current status of nanobiotechnology programs and their relations, more or less, with AIDS treatment; (2) reviewing current AIDS epidemiology in China and examining effectiveness and efficiency of current prevention and treatment strategies; (3) highlighting the obstacles to improve AIDS prevention and treatment, and (4) exploring innovative ways for nanotechnology to advance AIDS treatment, especially to combat HIV resistance to drugs.

KEY WORDS: AIDS treatment; anti-AIDS drug development; nanomedicine; nanotechnology.

INTRODUCTION

The first International Symposium of Nanomedicine on AIDS (ISNA-2009) was held in Beijing, China, November 19–20, 2009. Beijing University of Technology and the National Center for Nanosciences and Technology of China (NCNST) jointly hosted the symposium. The American Association of Pharmaceutical Scientists (AAPS), the Chinese Academy of Sciences (CAS), and the Chinese Ministry of Science and Technology (MOST) cosponsored the symposium. The symposium was designed, organized, and chaired by Dr. Xing-Jie Liang, Professor and Deputy Director of Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, NCNST/CAS, and Yi Zeng, Professor of Department of Oncogenic Virus and HIV/AIDS, Institute for Control and Prevention of Viral Disease, China CDC.

The rapidly emerging nanotechnology in China has been supported by governmental funds since the late 1990s through the National High-Tech R&D Plan (863 Plan), the National Basic Research Program (973 Program), the National Science Foundation of China (NSFC) Program, and the Torch Program—China's most important high-technology industry commercialization program, among others. These plans provide significant investments for nanotech projects from both the central and local Chinese governments. To better coordinate and harmonize the national efforts to fight AIDS and to apply nanotechnology to prevention and treatment of AIDS, the symposium was timed to occur before the World AIDS Day—the first day of December. The symposium focused on the following general themes: (1) reviewing current status of bionanotechnology programs and their relations, more or less, with AIDS treatment; (2) reviewing current AIDS epidemiology in China and examining effectiveness and efficiency of prevention and treatment strategies; (3) highlighting the obstacles to improving AIDS prevention and treatment; and (4) exploring innovative ways for nanotechnology to advance AIDS treatment, especially to combat HIV resistance to drugs.

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Through a recorded video, AAPS President Dr. Danny Shen conveyed his warm greetings to the symposium and his supports for this important public health-related program on behalf of AAPS. He pointed to one of his presidential goals, which is to advance AAPS global interaction and collaboration; the cosponsorship of this conference is a prime example of such an outreach effort.

CURRENT STATUS OF ANTI-AIDS DRUG DEVELOPMENT AND RELATED FORMULATION; ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION; AND NANOTECHNOLOGY ISSUES

Dr. Lee Jia, Senior Project Officer of the Developmental Therapeutics Program at the National Cancer Institute/NIH, USA, started with a historical review of an early grassroots movement in March 1987 appealing for a cure for HIV/AIDS. The movement spurred expedited approval by the US Food and Drug Administration (FDA) of the first anti-AIDS drug AZT (retrovir) and the establishment of treatment investigational new drugs by FDA in the same month to make promising new drugs available to desperately ill patients as early in the drug development process as possible. Nowadays, there are 25 FDA approved anti-AIDS drugs, which have turned HIV infection into a manageable disease and, at times, suppress viremia to undetectable levels. Currently, anti-AIDS drugs can be divided into six classes according to their mechanisms of action: fusion/entry inhibitors, integrase inhibitors (e.g., raltegravir), protease inhibitors (e.g., indinavir, saquinavir), nonnucleoside reverse transcriptase inhibitors (e.g., entravirine, efavirenz), nucleoside reverse transcriptase inhibitors (e.g., emtricitabine, lamivudine), and multidrug combination products (e.g., efavirenz, emtricitabine). All these drugs including those being developed for the future are given orally. As a result, drug solubility and intestinal permeability as well as the related oral bioavailability are the major factors determining potential success or failure of a candidate HIV/AIDS drug. For example, the AIDS drugs indinavir (developed by Merck), ritonavir (developed by Abbott), and navirapine (developed by Boehringer Ingelheim) show oral bioavailability >39%, 60–70%, and 92%, respectively. However, saquinavir (developed by Hoffmann-La Roche) only possesses 4% of oral bioavailability. As a result, the company had to make a painful decision to withdraw the drug from market after marketing approval. Drug solubility can be improved by using solvent mixture (e.g., water/ethanol), solubilization (e.g., mixed micelles), complexation (e.g., cyclodextrins), and nanonization. Nanonization of a poorly soluble drug can be done via wet chemical processes, media milling, and high-pressure homogenization (1). In general, a compound with a high log P and high melting point tends to show poor solubility and poor permeability and, therefore, low oral bioavailability. Nanonization is primarily suitable for those compounds with a high log P and high melting point, but a high dose level is required in order to show its effectiveness. The mechanisms of drug nanonization in enhancing its oral bioavailability lie in (1) an increase in drug dissolution rate to overcome this rate-limiting step, (2) an increase in saturation solubility of the drug that could result in an increased concentration gradient between intestinal columnar cells and the beneath mesenteric circulation, and (3) an increase in adhesion surface area between the intestinal columnar cells and nanoparticles, which would enhance drug absorption into the systemic circulation (2). Dr. Jia illustrated two examples that clearly show that nanonization improves a drug's permeability and oral bioavailability: Both carbendazim and thiadiazole derivative 301029 (an antivirus drug) showed relatively low permeability and oral bioavailability. After nanonization, permeability rates of the tested drugs across caco-2 monolayers and their oral bioavailability in rats were significantly increased in addition to the short T_{max} values (2,3). Advantages of multidrug combinations for AIDS treatment include prevention of drug resistance and synergistic effects of the combination through actions at different points of the viral cycle. However, challenges remain including shared drug toxicities, drug–drug interaction involving intestinal absorption, metabolism, and plasma protein binding, as well as the immune reconstitution inflammatory syndrome. Dr. Jia suggested that future strategies should not be limited to targeting the virus; it is also desirable to enhance the host immune system. Drug resistance derived from long-term drug administration cannot be solved easily because multidrug resistance of the virus is difficult to overcome. Nonetheless, new hopes come with nanotechnology that may completely eradicate HIV from body.

PREVENTION AND TREATMENT OF HIV-1 IN CHINA

Yi Zeng, Professor of Department of Oncogenic Virus and HIV/AIDS, Institute for Control and Prevention of Viral Disease, China CDC is the first scientist and pioneer studying HIV/AIDS in China with more than 28 years of experience in this field. He started his speech by reviewing the history of the HIV/AIDS epidemic in China. He demonstrated that HIV was introduced into China as a contaminant of imported factor VIII in 1982 and infected the first Chinese in 1983. With the continual increase of annually reported HIV/AIDS cases, the number of living HIV/AIDS persons in 2009 has soared to 740,000. Heterosexual transmitted route, which accounts for more than 40% of the estimated living HIV/AIDS cases (2009) and estimated new infection cases (2009), is the main cause for the HIV/AIDS epidemic in China.

Professor Zeng analyzed the characteristics of the epidemic in China: (1) although the HIV/AIDS epidemic is still on the rise, the rate of escalation has begun to abate; (2) heterosexual transmission and injection drug use are two major transmission routes; (3) geographic distribution of HIV infections varies in China and some provinces have more serious problems; (4) the wide existence of principal HIV epidemic factors makes it extremely urgent to popularize HIV/AIDS knowledge among Chinese citizens. Finally, Professor Zeng gave an overview on AIDS treatment studies worldwide with special attention paid to treating AIDS with traditional Chinese medicinal herbs. In addition to the extensively studied AIDS immunotherapy, using Chinese medicinal herbs for treatment of AIDS has its unique advantages: (1) China has rich medicinal herbs resources, (2) the bioactive substances contained in medicinal herbs might be functional in treating HIV, and (3) combining usage of different medicinal herbs often gives better performance.

Professor Zeng introduced a newly made prescription named ZL-1 based on Chinese medicinal herbs. *In vitro* and *in vivo* pharmacological studies have proved the new drug to have a strong inhibitory effect on the replication of HIV virus by blocking the entry of HIV to the cells through its binding to gp41 (a glycoprotein (gp)) and inhibiting viral integration and reverse transcription.

Fujie Zhang, Clinician and Professor of the National Center for AIDS Control and Prevention, Chinese Center for Disease Control and Prevention, summarized the progress in the Chinese AIDS antiretroviral treatment from 2002 to 2009. Financial support from the central government on AIDS treatment and prevention has increased to 2 billion RMB per year, which makes it possible to give patients free antiretroviral drugs, free CD4 testing, and free viral load testing. Also, the coverage of highly active antiretroviral therapy (HAART) in patients is rapidly growing with improved follow-ups to as many as seven times a year per patient (4). The 5-year (2002–2008) outcomes of the China National Free Antiretroviral Treatment Program showed that the program has successfully reduced mortality from 22.6 deaths per 100 person-years at 3 months to about four to five deaths per 100 person-years after 6 months of HAART among adult patients in China with AIDS. Cumulative probability of immunologic treatment failure increased over time from 12% at 1 year to 50% at 5 years, which reflects the very weak immune systems some patients have early in the treatment and calls for the availability of second-line regimens (5). Professor Zhang pointed out that new anti-AIDS drugs need to be more diverse in potential targets, offer simpler combinations that have multiple mechanisms of action, have long-term effect on HIV-1 inhibition, attack new targeting sites, and boost by auxiliary agents.

DRUG DELIVERY STRATEGIES FOR TREATMENT OF AIDS

When a person is infected by the HIV virus, usually, the virus is confined to the blood or the transmission site. It proceeds quickly to the lymphoid nodes and lymphoid tissues including those in the gastrointestinal tract. HIV is a disease that can be controlled but not cured because it is difficult for anti-AIDS drugs to reach the lymphoid organs or be kept there long enough to make an impact. Therefore, a reservoir of virus remains in the lymph nodes of HIV patients on HAART even with when virus is undetectable in plasma. Rodney J. Y. Ho, Professor of Pharmaceutics, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA, USA, pointed out that the existing anti-AIDS drugs are relatively safe and very selective for viral targets; however, drugs given either orally or by IV injection often produce lower levels in lymph nodes and lymphoid tissues than in blood. As a result, lymphocytes (target of HIV infection in the lymphoid tissues) is exposed to lower drug concentration by about 67–70% than the corresponding cells found in the circulating blood. This sheltering from drug exposure in lymphoid tissue allows the virus to continue to replicate, although at a much lower rate. This hypothesis is consistent with the reports that virus in lymphoid tissues replicates at a low level and is still sensitive to highly active antiretroviral therapy. The present challenge is to devise ways to deliver anti-HIV drugs to lymphoid tissues more efficiently and achieve concentrations high enough to eradicate the viral sanctuaries. The main goal of Professor Ho's research is to use various nanoparticles as platforms to improve the therapeutic index of drugs, which includes enhancing drug localization in HIV-infected target cells and reducing drug localization in off-target "normal cells". Anti-HIV drug delivery system targeting to lymphoid tissues with pH-responsive nanoparticles was developed. Dr. Ho's study showed that the intracellular levels of anti-HIV protease inhibitor in lymph node mononuclear cells are about 25–35% of mononuclear cells in blood. To improve drug concentration in lymphoid tissues, pH-dependent indinavir-incorporated lipid–indinavir nanoparticles (50–80 nm in diameter) in suspension was given by subcutaneous injection (10-mg/kg lipid-associated indinavir) to macaques. The results indicated that ratio of indinavir concentration in lymph node/plasma ranged from 2.5- to 22.7-fold between 6 and 28 h after the administration of lipid-associated indinavir, much higher than the ratio of the soluble form of free indinavir (25 mg/kg) given orally. Also, only HIV-2-infected macaques treated with lipid-free drug showed evidence of HIV-2 RNA in lymph nodes as compared to animals treated with lipid-associated indinavir (6,7). Dr. Ho concluded that the targeting effect of nanoparticles to the lymphoid tissues is mainly particle size dependent and that is why they used lipid–drug complex in the range of 50 to 80 nm. The 50–80 nm nanoparticles are easily trapped in lymph nodes as they circulate through the lymphatic system. The strategy of delivering pH-responsive lipid-associated indinavir nanoparticles to lymph nodes described by Dr. Ho is active and could reduce the virus load in these hard-to-reach tissues. Currently, Dr. Ho and his group are attempting to arm the pH-responsive nanoparticles with multiple anti-HIV drugs such as reverse transcriptase inhibitors, protease inhibitors, and guided by attached ligands that target HIV hosts ($CD4^+$) or infected cells to simultaneously improve the loading efficiency. Additionally, preclinical studies are ongoing in his lab for clinical development of this nanoparticle targeting system.

RNA NANOTECHNOLOGY AND NANOMEDICINE

Peixuan Guo, Director of the NIH Nanomedicine Development Center of Phi29 DNA Packaging Motor for Nanomedicine, Endowed Chair in Biomedical Engineering of the University of Cincinnati, USA, discussed the possible combination of RNA nanotechnology with HIV infection detection and anti-HIV treatment. Professor Guo first introduced the most powerful biomotor constructed to date—an *in vitro* bacteriophage phi29 DNA packaging motor first reported by him in 1986 (8). He discovered the motor pRNA (small packing RNA) in 1987 (9) and elucidated the formation of hexameric pRNA in 1998 (10). In 2004, he first proposed the concept of RNA Nanotechonolgy in a paper published in *Nano Letters*.

The 30-nm imitating DNA packing motor is driven by a hexameric ring composed of six synthetic ATP-binding pRNAs. Another essential component of the nanomotor is a 3.6-nm central channel formed by 12 copies of protein gp10. This protein nanopore allows double-stranded DNA to pass through during the packing process via sequential action of

the motor components using ATP hydrolysis energy. In 1995, his lab first realized the assembly of infectious virion of double-stranded DNA phage phi29 with purified recombinant proteins and artificially synthesized nucleic acids *in vitro*, which was much more difficult than the *in vitro* construction of RNA viruses. Professor Guo presented the monomer, dimer, trimer, tetramer, rods, triangles, 3D arrays, and some other extraordinary versatile bionanostructures and shapes formed by pRNAs through intermolecular base pairing between pRNA's right loop (bases 42–25) and the left loop (bases 82–85) during its programmable self-assembly process. The application of such RNA nanotechnology in the treatment of cancer and viral (11–15) infection has been demonstrated in both cell culture and animal trials. The application of pRNA nanoparticle in HIV therapy is in progress (unpublished data). Professor Guo explained that the applications of pRNA nanoparticles for nanotechnology and therapeutic gene and drug delivery have special advantages: (1) the multivalent nanoparticle can be controllably synthesized with defined structure and stoichiometry, which ensures the amount of nanoparticle-loaded molecules to be controllable; (2) targeted delivery and detection can be achieved by end conjugation on pRNA with cell receptor binding ligands; (3) the nonprotein antigenicity-free nanoparticles, with the advantageous size of 10–100 nm, have extended *in vivo* half-life, which allow repeated long-term administration in chronic disease treatment; and (4) RNA nanoparticles are treated as chemical drugs rather than biological entities. Therefore, this classification will facilitate FDA approval.

One of the next intriguing steps in the development of the phi29 motor is to add more diverse molecules such as receptor-binding aptamers, therapeutic siRNAs, drugs (water soluble or water insoluble), ribozymes, detection molecules, chemical ligand groups, and some other molecules to the bionanomotor to use it as a polyvalent carrier for targeting, therapy, and detection (11–14,16). Professor Guo's presentation aroused great interest among scholars from multiple disciplines attending the meeting, and there was discussion of collaboration using pRNA nanoparticles as delivery vehicles of therapeutic genes in HIV genic therapy and the use of the membrane-embedded nanochannel (15) of phi29 DNA packaging motor for nucleic acids sequencing and HIV therapy.

APPLICATION OF NANOMATERIALS AS AIDS DRUGS CARRIERS

Xing-Jie Liang, Professor and Deputy Director of CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, and Aoneng Cao, Professor of Institute of Nanochemistry and Nanobiology, Shanghai University, presented recent advances in the construction of carriers for delivery of anti-AIDS compounds in their research. Professor Liang's talk focused on nanoparticle-based anti-HIV siRNAs delivery. He introduced two systems currently used in his lab: the electrostatic and covalent layer-by-layer assembled nanoparticles and the biologically safe core–shell structural polyplexes. AuNPs surface-modified with alternating anionic, cationic, and pH-sensitive polyelectrolyte multilayers have been synthesized in Liang's lab. siRNAs and anti-HIV drugs can be loaded in the same nanoparticle between the charge-shifting polymers via electrostatic adsorption. Once the nanoparticle enters lysosome, siRNAs and drugs can be efficiently released due to the properties of pH-sensitive polymers. This system made it possible to monitor the therapeutic nanoparticle's cell entry process, accumulation, and metabolism in the cell, making use of AuNPs plasma resonance absorption. Polymeric nanoparticle delivery system can effectively protect bioactive molecules from degradation in physiological environment and increase cell uptake of molecules being delivered by incorporating targeting groups on the polymer. Particularly noteworthy is their enhanced permeability and retention effect, which makes polymer nanoparticles highly potential candidates for siRNAs delivery. The biodegradable amphiphilic multiblock copolymer PCL-*g*-PDMAEMA synthesized by Liang's group can form stable cationic core–shell micellar nanoparticles in aqueous solution by self-assembly process. This nanoparticle can be used as a dual gene and drug delivery vehicle by loading hydrophobic drugs in the core and negatively charged siRNAs on the shell. Experiments have proved that the transfection efficiency of PCL-*g*-PDMAEMA NPs complexed with anti-HIV shRNAs in 293T cell line is statistically higher than Lipofectamine 2000 mediated shRNA gene transfection and with lower cytotoxicity. The PCL-*g*-PDMAEMA nanoparticle is also a pH and thermosensitive material. The loaded drugs release faster at acid pH (5.0) than near neutral pH (7.4), and the nanoparticle solution transforms to gel when temperature rises from 25°C to 37°C. Professor Liang explained that the next progression of this PCL-*g*-PDMAEMA nanoparticle is to use it as an *in vivo* slow release drug dosage form based on its pH and thermosensitivity. Such studies are underway.

The packaging efficiency and release capacity of different nanomaterials toward the same drug is quite different; therefore, an important aspect of nanoparticle-based drug delivery system research is to pair the right nanomaterials with the right drugs. Professor Cao presented elegant studies on silica and polymer nanomaterial-based drug delivery systems and the possibility of using graphene for drug delivery. There are two strategies to use silica nanoparticles in drug packaging: (1) package the drug simultaneously with the formation of silica nanoparticles by inverse microemulsion technique and (2) adsorb drugs into silica mesoporous nanomaterials. Some attempts have been made by Cao's group in encapsulating protein drugs using silica nanoparticles. Silica nanoparticle shell helps to maintain the conformation of the enclosed proteins as well as provide protections against protease, chemical denaturant, and thermal stress, which gives hope to long-term storage of protein drugs. The silica nanoparticle-encapsulated protein drugs can be vacuum dried and then stored in dry conditions. Experiments have proved that, after rehydration, protein activity can be regained.

Professor Cao presented the extraordinary story of three reversible addition–fragmentation chain transfer (RAFT) polymerization systems that have been developed and multifunctional nanoparticles with selectively defined heterofunctional groups in the core and on the surface prepared via cascade aminolysis/Michael addition coupled with alkyne-azide click reaction (17). Polymer nanoparticles synthesized in this way falls into the range of 50–80 nm, which is favorable

for the loaded drugs to take effects. The biocompatible nanoparticles formed via RAFT dispersion polymerization with hydrophobic core and poly(ethylene glycol) shell were tested for anti-HIV drug loading efficiency and *in vitro* release performance. Recent results showed the anti-HIV drug loaded nanoparticles presented slow-release property suggesting their possible usage as long-acting dosage form.

The main preparation methods of graphene include mechanical exfoliation, epitaxial growth, catalytic chemical vapor deposition methods, bottom-up organic synthesis, liquid-phase exfoliation of graphite, and reduction of graphene oxide. Professor Cao's research focuses on the last two methods for their manipulation simplicity in solution phase and suitability for mass production. Common shortcomings of the production methods mentioned above are as follows: (1) the yield of single-layer graphene sheets is very low and (2) graphene sheets are not stable in solution and easily aggregate back to graphite and lose the single-layer based superior physiochemical properties. The strategy Cao's group used to produce single-layer graphene nanocomposite started from the soluble graphene oxide, which is stable in a single-layer state. A one-step reaction was applied to reduce graphene oxide and deposit CdS quantum dots on graphene simultaneously. This graphene-based optoelectronic system with well distributed CdS showed efficient electron-transport property: Graphene significantly quenched the fluorescence of the CdS quantum dots (18). Although there have been many reports on using graphene sheets detection sensors, there is still a long way to go in applying graphene for drug packaging. Professor Cao pointed out that control of the solubility, size, and uniformity of graphene sheets are key factors to consider. Exploratory works using water-soluble graphene oxide as possible drug carriers are underway now.

CHEMICAL COMPOUNDS EMPLOYED FOR HIV-1 INHIBITION

The HIV envelope glycoprotein Env consists of two noncovalently associated gp subunits: gp120 and gp41. Entry of HIV-1 into macrophages and $CD4^+$ T cells requires the formation of entry complexes involving Env, the target cell receptor CD4, and either the G-protein-coupled chemokine coreceptor CCR5 (for macrophage tropic or dual tropic strains of HIV) or CXCR4 (for T-cell tropic or dual tropic strains of HIV). Conformational changes within HIV-1 surface gp120 resulting from binding to the lymphocyte surface receptors expose its hidden hydrophobic center and trigger the highly hydrophobic N terminus of gp41 ectodomain to insert into the target membrane, thereby fusing the virus into the target cell. Professor Cunxin Wang and Professor Liming Hu from the College of Life Science and Bioengineering, Beijing University of Technology gave an intriguing presentation on (1) computer simulation of the structural and kinetic properties of HIV-1 target acting with drug molecules and elucidating the relationships between drug molecules and their receptors, the function of active site, and the role of key residues and (2) computer-aided drug design, synthesis of HIV target agents, screening the active ingredient of natural products for inhibitors aiming at one or several steps of the HIV-1 life cycle, and the inhibitors' biological activities evaluation.

Among the 25 anti-AIDS drugs approved by FDA, most anti-AIDS drugs are reverse transcriptase inhibitors and protease inhibitors (19). Drug resistance is a serious but common problem for reverse transcriptase inhibitor-based therapy since this kind of inhibitor has been used for more than 20 years and is widely used in cocktail therapy in different forms and combinations. In this context, Professor Wang and Professor Hu focus their research on integrase inhibitors, gp41 inhibitors, and CCR5 inhibitors.

The HIV-1 integrase can be divided into three functional domains: the N-terminal domain, the catalytic core domain, and the C-terminal domain. Normally, the integrase exists and functions in the form of tetramer. There are three approaches to designing integrase inhibitors: (1) inhibit the 3′-processing and the strand transfer reactions by targeting the catalytic core domain, (2) inhibit the formation of integrase polymer by targeting the N-terminal domain and catalytic core domain, and (3) inhibit integrase's nuclear localization by targeting the N-terminal domain and catalytic core domain. The binding modes and inhibitory mechanism of the 4-hydroxycoumarin integrase (IN) inhibitor NSC158393 with the wide type and IN mutant were studied with molecular docking method, molecular dynamics (MD) simulations, and the molecular mechanics generalized born surface area approach. Combination of NSC158393 with wide-type IN constrained the flexibility of the functional 140s loop of IN, which affected its catalytic activity. The single substitution mutants—W132G and C130S—showed drug resistance to NSC158393 partly because the binding of NSC158393 did not significantly affect the flexibility of the functional loop of these mutants (20).

Two small-molecule N-substituted pyrroles, NB-2 and NB-64, display antiviral activity against HIV-1 infection by specifically targeting the hydrophobic pocket of gp41 and consequently prevent the successful completion of gp41 zipping. Professor Wang's group investigated the binding affinities and calculated the binding free energy between the N-peptide of gp41 and NB-2/NB-64 via the flexible docking method, MD simulation, and the molecular mechanics Poisson–Boltzmann solvent accessible surface area method. The study found that the nonpolar interactions between the inhibitor NB and the gp41 hydrophobic pocket residues largely contributed to their combination, while the strong polar interactions between the inhibitor carboxyl group and Arg579 of gp41 played a key role in inhibiting gp41's activity (21).

One aspect of Professor Wang's research is to use the method of three-dimensional quantitative structure–activity relationship analyses, comparative molecular field analysis (CoMFA), and its extension comparative similarity indices analysis (CoMSIA) to investigate the structural features and binding characteristics of CCR5 receptor with a series of 1,3,4-trisubstituted pyrrolidine-based CCR5 receptor inhibitors. CoMFA and CoMSIA contour maps generated in the study provided a visual representation of contributions of steric, electrostatic, hydrogen bond and hydrophobic fields, and the prospective binding modes and showed crucial structural regions where any change in the steric, electrostatic, hydrophobic, and hydrogen bond fields may affect the inhibitor's biological activity. The result is useful for future rational drug design and structural optimization of potent CCR5 inhibitors (22).

Using liquid chromatography–mass spectrometry, two compounds named E809 and E823 that have inhibitory activity against both gp41 and integrase were identified as the bioactive ingredients of anti-AIDS Chinese medicinal herbs by Hu's group. E809 and E823, with molecular weight of 809 and 823 respectively, are both flavonoid glycosides and differ only in one substituent. However, the inhibitory activity of E809 is ten times higher than that of E823. Therefore, molecular simulation was performed to study the core site of compound E809. Possible combination model of E809 with gp41 was constructed according with the structure–activity relationship. They discovered that intermolecular hydrogen bonding and hydrophobic interactions were important factors determining E809's activity. Hu's group are now using the natural product quercetin as a substrate to synthesize E809 mimics in the hope of developing potential lead compounds of gp41 and integrase inhibitors with superior inhibitory activities. Professor Hu also shared their experience in synthesizing a series of quinoline, quinolizine, and naphthyridine based HIV-1 integrase inhibitors, for example, the 6-sulfamoyl-4-oxoquinoline-3-carboxylic acids derivatives (23), 4-oxo-4*H*-quinolizine-3-carboxylic acid derivatives (24), and 1,2,3-trizole-substituted 1,4-dihydro-4-oxo-1,5-napthyridine-3-carboxylic acids. He also introduced the BIAcore method and pseudovirus-based method for *in vitro* screening of HIV integrase inhibitory activity.

CHALLENGE AND FUTURE FOR ANTI-AIDS RESEARCH

HIV was the first infectious virus targeted by RNAi. Synthetic siRNAs and expressed shRNAs have been used to inhibit HIV-encoded RNAs in cell culture with superior specificity to the infected cells. Using lentiviral vectors to transduce anti-HIV shRNA genes into the patient's hematopoietic stem cells for anti-HIV treatment has been proposed. CD34+ hematopoietic stem cells are collected from blood and transduced with a lentiviral vector harboring the anti-HIV shRNA genes. The transduced cells are then reinfused into patients (25). However, the systemic and viral delivery of siRNAs and shRNAs to HIV-infected cells was problematic. The easy degradation of siRNAs meant that immense number of cells has to be delivered. In addition, immunogenicity of the viral vectors is a challenge (26). Zhiping Teng, professor of Laboratory of HIV and Tumor, Institute of Viral Disease Control and Prevention, China CDC proposed that nanocarriers may offer a solution to this issue. Professor Teng's group has succeeded in transducing isolated telomerase-related gene-modified stem cells with nanoparticle carriers harboring anti-HIV siRNAs. By using telomerase-related gene-modified stem cells, they extended the longevity of stem cells and solved the problem of rapid loss of the transgrafted stem cells in patients. It is expected that infusion of the *ex vivo* transduced stem cells back into the donor patient would allow the anti-HIV siRNAs to interrupt HIV gene expression.

Gengmei Xing, professor of CAS Key Lab for Biomedical Effects of Nanomaterials and Safety, Institute of High Energy Physics, Chinese Academy of Sciences, pointed to the importance of assessing the toxicology and metabolism of engineered nanomaterials that have potential applications in anti-HIV treatment. Some nanoparticles, upon entering the body, may stimulate the migratory ability of certain cells such as macrophages; the subsequent inappropriate localization of the migrating cells can result in life-threatening consequences. Certain nanomedicines tend to be eliminated by the immune system when applied to the body. If so, how quick is the process? Is it before or after the nanomedicine takes effect? All these questions need to be answered. Speaking about techniques to achieve this goal, synchrotron radiation circular dichroism has been applied in her lab to assay the metallofullerene nanoparticles induced secondary structure change of protein and DNA. The Boyden chamber method was used to explore how the chemotaxis ability of macrophages was affected by nanoparticles of different sizes. A high-throughput nanoparticle toxicity detection system is under construction now, which includes a cytotoxicity detection system, a cell function detection system, and a genetic toxicity detection system.

The meeting concluded with a productive brainstorming session led by Dr. Yi Zeng. The attendees discussed the next steps and challenges in anti-HIV research. Professor Changxiao Liu, Academician of Chinese Academy of Engineering, Director of Research Center for New Drug Evaluation, Tianjin Institute of Pharmaceutical Research, commented on the drug discovery and pharmacodynamic evaluation of nanomedicines. The research and development of new drugs is a systematic project, which includes an upstream or basic and mechanistic research foundation phase, drug discovery phase, preclinical studies, clinical trial, and postmarketing evaluation of the drug. We should bear in mind that bioactive molecules themselves are not medicines, so neither do nanomaterials constitute delivery systems. Drug research and development must meet the three essential requirements: safety, effectiveness, and industrial scale-up and usability. Some of the current challenges of existing drugs include side effects, poor efficiency and compliance, poor selectivity of transport and action, and drug resistance. Nanotechnology is expected to enhance drug dissolution, absorption, bioavailability, tissue targeting, and controlled release ability. The application of nanotechnology in pharmaceutical research is likely to make a significant impact on improving drug dosage forms. The nanotechnology-based drug delivery system holds the promise of achieving improved pharmacokinetics (PK), improved pharmacodynamics (PD), lower probability for drug–drug interaction, less variable PK–PD, improved overall safety, and improved physicochemical properties. Pharmacokinetics is central to the evaluation of nanocarrier drug delivery. An important challenge of nanotechnology-facilitated AIDS treatment lies in the cooperation and integration across a broad spectrum of scientific disciplines.

ACKNOWLEDGMENTS

This conference is sponsored by Ministry of Science and Technology (MOST), Chinese Academy of Sciences (CAS), National Center for Nanoscience and Technology (NCNST), and the American Association of Pharmaceutical Scientists (AAPS). The authors also thank Chinese Natural Science Foundation project (No.30970784), National Key Basic Research Program of China (2009CB930200), Chinese Academy of Sciences (CAS) "Hundred Talents Program" (07165111ZX), and China–Finland Nanotechnology (No. 2008DFA01510) for financial support.

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