**In Vivo Kinetics and Biodistribution of a HIV-1 DNA Vaccine after Administration in Mice**

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In this study we have investigated the pharmacokinetics and tissue distribution of GX-12, a multiple plasmid DNA vaccine for the treatment of HIV-1 infection. Plasmid DNA was rapidly degraded in blood with a half-life of 1.34 min and was no longer detectable at 90 min after intravenous injection in mice. After intramuscular injection, plasmid DNA concentration in the injection site rapidly declined to less than 1% of the initial concentration by 90 min post-injection. However, sub-picogram levels (per mg tissue) were occasionally detected for several days after injection. The relative proportions of the individual plasmids of GX-12 remained relatively constant at the injection site until 90 min post-injection. The concentration of plasmid DNA in tissues other than the injection site peaked at 90 min post-injection and decreased to undetectable levels at 8 h post-injection. The rapid in vivo degradation of GX-12 and absence of persistence in non-target tissues suggest that the risk of potential gene-related toxicities by GX-12 administration, such as expression in non-target tissues, insertional mutagenesis and germline transmission, is minimal.

Key words: GX-12, Plasmid DNA vaccine, HIV-1, Pharmacokinetics, Biodistribution

**INTRODUCTION**

Two decades after of the first clinical reports of acquired immunodeficiency syndrome (AIDS) were published, the cumulative number of worldwide HIV infections has exceeded 60 million with the estimated number of mortalities due to AIDS reaching 13 million (UNAIDS, 2001). Unfortunately, a cure of the viral disease has yet to be realized despite the active researches currently on-going in the field.

Combination drug regimens using antiretroviral agents such as protease inhibitors and reverse transcriptase inhibitors are currently adopted for the treatment of HIV infection and AIDS. Highly active antiretroviral therapy (HAART) has enabled the reduction of plasma HIV titer to below detection limit, and its availability has led to a substantial decrease in AIDS-related mortalities in developed countries (Palella et al., 1998). However, antiretroviral therapies are subject to several concerns such as acute and chronic toxicities (Yeni et al., 2002), potential generation of resistant strains (Little et al., 2002), inability of complete virus eradication (Finzi et al., 1999) and a high cost which limits its applications in developing countries where the impacts of the AIDS epidemic are the most devastating (UNAIDS, 2001). In view of the clinical and economical limitations of antiretroviral therapy, it is generally perceived that the development of an effective and economical vaccine could be an attractive alternative for slowing down the AIDS epidemic (Gottlieb, 2001). Conventional vaccination approaches such as inactivated whole virus, attenuated virus or subunit vaccines have proven to be ineffective or pose an unacceptable risk to the person receiving the vaccine. A more recent and promising approach is DNA vaccination, which has several advantages such as the ability to induce both humoral and cellular immune responses, flexibility of design, safety, ease of manufacture, and suitability for prime-boost strategies in combination with viral vectors (Fomsgaard, 1999).

Genexine Co. and Dong-A Pharmaceutical Co. have...
jointly developed GX-12, a novel candidate vaccine for the treatment of HIV-1 infection and AIDS. GX-12 is a DNA vaccine that consists of four plasmid vectors encoding major (env, pol, gag) and accessory genes (rev, vif, nef, tat, vpu) of HIV-1 subtype B as well as a human interleukin-12 mutant (hIL-12m) gene. Due to the inclusion of multiple genes, the vaccine stimulates a broad immune response against various humoral and cellular epitopes, which is potentially advantageous in controlling viral immune escape. Expression of the IL-12 gene enhances the proliferation of peripheral T lymphocytes and HIV-1 specific CTL responses to increase the therapeutic efficacy of the vaccine (Ha et al., 2002). Previously, a SIV vaccine analogue of GX-12 elicited protective immunity against SIV infection in monkey experiments, thus demonstrating the potential efficacy of the HIV vaccine candidate (Hunsmann, 2001).

A number of theoretical safety concerns that are not encountered in conventional pharmaceutical agents exists with DNA vaccines, including toxicity associated with the expression of the encoded genes, autoimmune diseases and the potential for chromosomal integration. To address these safety issues, the in vivo fate of the DNA compound has to be characterized in preclinical studies, usually by polymerase chain reaction (PCR) or related gene amplification techniques. In this study, we have investigated the in vivo kinetics and biodistribution of GX-12 using PCR. The study was conducted as part of the preclinical evaluation of GX-12 to support regulatory submission.

MATERIALS AND METHODS

Test substance

GX-12 consists of a mixture of four plasmids: pGX10-GEhx, encoding HIV-1 gag and env; pGX10-dpol jr encoding HIV-1 pol; pGX10-VN/TV jr encoding HIV-1 vif, nef, tat and vpu; and pGX10-hIL-12m encoding a human IL-12 mutant gene. The plasmids are formulated in 150 mM phosphate buffer, pH 7.0, each at a concentration of 0.5 mg/mL. The plasmid vectors were constructed by cloning the respective genes into the eukaryotic expression vector pGX10. The pGX10 backbone carries a prokaryotic origin of replication (ColE1), a bacterial kanamycin resistance gene and a eukaryotic expression cassette consisting of a human CMV promoter, adenovirus tripaltparte leader sequence, SV40 late polyA and SV40 enhancer. The vaccine was produced to clinical grade according to a proprietary process established at Dong-A Pharm. Co. Briefly, E. coli DH5α cell lines carrying the plasmids were grown in kanamycin-containing medium in a 15 L fermentor. The fermentation broth was subjected to a series of purification steps including alkaline lysis, PEG precipitation, anion exchange chromatography and gel filtration chromatography. The purified plasmids were mixed to the final composition and dialyzed against formulation buffer.

Intravenous administration of GX-12

GX-12 was administered to male ICR mice (Charles River Laboratories), 6 weeks of age, as a single injection of 50 µL volume (100 µL total plasmid DNA) via the tail vein. Control mice received a 50 µL injection of vehicle control (150 mM phosphate buffer, pH 7.0). Five mice were used per time point. Blood samples were drawn from the retro-orbital plexus at 1, 5, 15, 30, 45, 60, 90, 120 min and 8 h post-administration. The samples were immediately frozen by immersion in liquid nitrogen after addition of EDTA. The blood samples were stored at -75°C until further analysis.

Intramuscular administration of GX-12

Single 50 µL doses of GX-12 (100 µL total plasmid DNA) were injected into the left femoral muscles of male ICR mice. Control mice received a 50 µL injection of vehicle control. Five mice were used per time point. At 5 min, 15 min, 30 min, 90 min, 120 min, 8 h, day 1, day 3, day 7, day 14 and day 30 mice were sacrificed and tissue samples (brain, heart, stomach, intestines, liver, lung, spleen, kidney and testis) were taken for subsequent analysis. The tissue samples were frozen immediately by immersion in liquid nitrogen and stored at -75°C until analysis. Blood samples were drawn from the retro-orbital plexus and rapidly frozen by immersion in liquid nitrogen after the addition of EDTA. The blood samples were stored at -75°C until further analysis.

Isolation of GX-12 from blood samples

Fifty µL of blood was diluted 20-fold in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and extracted with equal volumes of phenol/chloroform. After addition of 1 µL of salmon sperm DNA as a co-precipitation agent, the DNA was precipitated by the addition of 1 volume of 3 M sodium acetate and 2 volumes of ethanol. The precipitated DNA was separated by centrifuging at 15,000 rpm for 10 min and dissolved in nuclease-free water.

Isolation of GX-12 from tissue samples

About 50–200 mg of tissue was minced and suspended in 600 µL of resuspension buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 100 µL/mL RNase A) and homogenized using a tissue homogenizer (Bellco). After addition of 600 µL of lysis buffer (0.2 M NaOH, 1% SDS) and 600 µL of neutralization buffer (1.32 M potassium acetate, pH 4.8) the cell debris was removed by centrifugation at 15,000 rpm for 10 min. The supernatant was extracted with equal volumes of phenol/chloroform. DNA was precipitated by adding 2 volumes of ethanol. After separation by centrifug-