58

Protection of hu-PBL-SCID/beige mice from HIV-1 infection by a modified oligonuleotide, RKS-1443

T. Agatsuma[a], H. Furukawa[a], K. Abe[b], R. Koga[c], M. Koizumi[c], H. Hotoda[c], K. Momota[a], T. Ohmine[a], T. Nishigaki[a], M. Kaneko[c], S. Kimura[d], K. Shimada[d]

[a]Biological Res. Labs., [b]Analytical and Metabolic Res. Labs., [c]Exploratory Chemistry Res. Labs., Sankyo Co., Ltd., Tokyo, Japan; [d]Dept. of Infectious Deseases, the Univ. of Tokyo, Tokyo, Japan

We evaluated the anti-HIV-1 activity of an oligonucleotide derivative RKS-1443 in severe combined immunodeficient (SCID/beige) mice transplanted with normal human peripheral blood leukocytes (PBLs). The human chimeric mice were inoculated with HIV-1_{CC1} three weeks after reconstitution and sacrificed two weeks later. Splenocytes were collected from the mice for determination of virus infection by coculture with human PBLs and also by detection of HIV-specific DNA sequences using PCR No evidence of infection was obtained for mice treated with RKS-1443 (100mg/kg/day) using intraperitoneal delivery by osmotic minipumps starting one day before virus challenge. In addition, partial inhibition of HIV-1 infection was obtained for mice subcutaneously treated with the same dose of RKS-1443. In contrast, virus infection was observed in over 80% of saline treated control mice. No toxicity towards the engrafted human cells was observed. Moreover, RKS-1443 did not inhibit lymphocyte proliferation (CC_{50}>400μg/ml) at concentrations which produced over 90% inhibition of HIV-1 (IC_{90}<3.2μg/ml against HIV-1_{CC1}). These results suggest the ability of RKS-1443 to protect the human chimeric mice against HIV-1 infection and, therefore, may indicate the therapeutic importance of RKS-1443 for the treatment of HIV-1 infection.

59

Phase I Clinical Trial with a Novel Protease Inhibitor for HIV, KVX-478, in Healthy Male Volunteers

Masahiko Nishiyama, Misa Koishi, Minoru Fujioka, Kazuma Hirata, Akitoshi Yamamoto, Hisao Suzuki, and Yoshikazu Kurashina (Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan), Munetetsu Tei and Kazuo Uebaba (Bio-Iatric Center, Kitazato Laboratory, Tokyo, Japan)

KVX-478, a novel, orally administered protease inhibitor for HIV, has been shown to have a potent inhibitory effect against proliferation of HIV with favorable bioavailability and tolerability in preclinical studies. We performed a phase I clinical study in healthy male adult volunteers to assess the pharmacokinetics and tolerability following a single oral administration of KVX-478 at doses of 150, 300, 600, 900 and 1200 mg (n = 6 per dose group) under fasting conditions. C_{max} and AUC of KVX-478 were directly proportional to the doses administered. Plasma levels of KVX-478 exceeded the IC_{90} of 70 nM for 24 hr at doses of 900 and 1200 mg, for 12 hr at 600 mg, and for 8 hr at 300 mg. C_{max} was 1718.6 ng/ml at 300 mg and 6268.4 ng/ml at 900 mg, demonstrating an excellent oral bioavailability in man. KVX-478 was well-tolerated, with no adverse experiences or laboratory test abnormalities observed. Influence of diet was assessed at a dose of 600 mg by comparing pharmacokinetic parameters. T_{max} was extended about 2.5 times, and C_{max} and AUC were decreased by 46% and 23%, respectively, following administration under non-fasting conditions, as compared with those under fasting conditions. Plasma levels of KVX-478 above the IC_{90} were maintained for at least 12 hr under fasting conditions and about 18 hr under non-fasting conditions, suggesting influence of diet on pharmacokinetics. In a subsequent repeated administration study, KVX-478 was administered for 5 consecutive days, at doses of 300 mg once daily on day 1 and 5, and 300 mg t.i.d. on days 2 to 4. KVX-478 was well tolerated, and displayed excellent bioavailability.

60

Antiviral Effects of Plasma and Milk Proteins: Lactoferrin shows Potent Antiviral Activity on both HIV and HCMV Replication *in vitro* in the Same Concentration Range

P.J. Swart[1], M.C. Harmsen[2], M.P de Béthune[3], R. Pauwels[3], E. De Clercq[3], T.H. The[2] and D.K.F. Meijer[1]

[1]Department of Pharmacokinetics and Drug Delivery, University Centre for Pharmacy, A. Deusinglaan 2, 9713 AW Groningen, [2]Department of Immunology, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Neherlands, [3]Rega Institute for Medical Research, Katholieke Universiteit, B-3000 Leuven, Belgium

A series of native and chemically derivatized proteins, purified from serum or milk were assayed in-vitro for their anti-HIV-1- and anti-HCMV-cytopathic effects in MT4 cells or fibroblasts respectively. Of all proteins tested only native lactoferrin was able to completely block HCMV replication. The IC_{50} values ranged between 35 and 100 $\mu g.ml^{-1}$. Native lactoferrin also inhibited HIV-1 induced cytopathic effect (IC_{50} = 40 $\mu g.ml^{-1}$). Charge-modified lactoferrin in which additional negatively charged groups were introduced by succinylation exhibited a 4-fold stronger antiviral effect on HIV-1 than the parent compound but was without activity on HCMV. Charge-modified HSA (NCAs) had a very strong inhibitory effect on HIV-1 infection in-vitro (IC_{50}s: 0.04 $\mu g.ml^{-1}$ for Aco-HSA to 0.2 $\mu g.ml^{-1}$ for Suc-HSA) in contrast to HSA itself. Earlier studies indicated a predominant effect of the negatively charged albumins on HIV/cell fusion. In contrast, the NCAs had no detectable effect on HCMV. Linearization of the NCAs reduced the anti-HIV activity about 10-fold. Linearization of lactoferrin completely abolished the anti-HIV-1 and anti-HCMV activity, indicating that the protein molecule needs to be correctly folded to be antivirally active. Lactoferrin did not exhibit any activity if added two hours after HCMV infection was initiated, a time period which is sufficient for the virus to penetrate all target cells. It is concluded that lactoferrin likely exerts its effect at the level of virus adsorption and/or penetration.

61

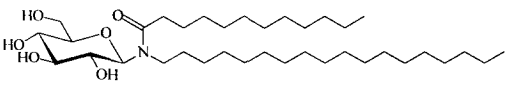
Synthesis, Biological and Physical Evaluation Of Some Novel Glycolipid Analogues

W.P. Blackaby[1], C. McGuigan[1], G.S. Attard[2], N. Mahmood[3], A.J. Hay[3], J. Balzarini[4] and E. De Clercq[4]

[1]Welsh School of Pharmacy, University of Wales Cardiff, Cardiff CF1 3XF; [2]University of Southampton, Southampton S09 5NH; [3]MRC Collaborative Center, London W1N 4AL, United Kingdom; [4]Rega Institute for Medical Research, K.U.Leuven, B-3000 Leuven, Belgium

Glycoconjugates of carbohydrates and lipids are commonly encountered in mammalian cell membranes. By virtue of their exposed nature they serve as binding sites for antibodies, toxins, bacteria, or viruses and are therefore important in mediating communication with the cells' external environment.(Fietz, Nature 314, 53-57, 1985).

Recently, a new class of these conjugates, 'glycosylamides', having antiherpes and adjuvant properties were discovered (Lockhoff, Angew. Chemie Int. Ed. Engl. 30, 1611-1620, 1991).



N-(β-D-glucopyranosyl)-N-octadecyldodecanamide

Here we report on the synthesis and biological evaluation of a number of structural analogues of the above compound. All compounds were evaluated for their *in vitro* antiviral activity against HIV-1, HSV-1 and their anti-neoplastic activity.