Virology 405 (2010) 225–233

Contents lists available at ScienceDirect

Virology

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

Protective properties of non-nucleoside reverse transcriptase inhibitor (MC1220) incorporated into liposome against intravaginal challenge of Rhesus Macaques with RT-SHIV

Mélanie Caron [a], Guillaume Besson [a], Sonia Lekana-Douki Etenna [a], Armel Mintsa-Ndong [a], Spyridon Mourtas [b], Antonia Radaelli [c], Carlo De Giuli Morghen [c], Roberta Loddo [d], Paolo La Colla [d], Sophia G. Antimisiaris [b,e], Mirdad Kazanji [a,f,*]

[a] Unité de Retrovirologie, Centre International de Recherches Médicales de Franceville, Franceville, BP 769, Gabon
[b] Laboratory of Pharmaceutical Technology, Department of Pharmacy, Université of Patras, Rio, 2050 Patras, Greece
[c] Department of Medical Pharmacology, University of Milan, Milan, Italy
[d] Department of Biomedical Sciences and Technologies, University of Cagliari, Italy
[e] Institute of Chemical Engineering and High-Temperature Processes-FORTH, GR-26500, Patras, Greece
[f] Réseau International des Instituts Pasteur, Institut Pasteur, Paris, France

ARTICLE INFO

Article history:
Received 24 February 2010
Returned to author for revision 6 April 2010
Accepted 2 June 2010
Available online 1 July 2010

Keywords:
Microbicide
MC1220
RT-SHIV
viral load
proviral load
T-cell subset
protection
liposomal gel

ABSTRACT

In the absence of an effective vaccine against HIV, it is urgent to develop an effective alternative such as a microbicide. Single and repeated applications of MC1220 microbicide were evaluated in macaques. First, animals were given a single application of 0.5% or 1.5% MC1220-containing liposomal gel. A second group were treated with 0.5% MC1220 once a day for 4 days. The control groups were treated by liposomal gel alone. Thirty minutes after the last application, animals were challenged with RT-SHIV. In the first protocol, 2 of 4 animals treated by 0.5% of the MC1220 and 2 of 5 treated by 1.5% were protected. In the second protocol, 3 of 5 treated animals were protected and 5 of 5 controls were infected. The RNA viral load at necropsy was significantly lower ($p = 0.05$) in treated-infected animals than in controls. In both protocols, the number of CD4+ T cells was lower at viremia peak in infected than in protected animals.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the main sexually transmitted infection worldwide, affecting approximately 34 million people (Garcia-Lerma et al., 2008). About 2.5 million people are newly infected each year, and 2.1 million of them die, adding to the 19 million people who have already died from diseases associated with this infection (McGowan, 2006). In the absence of an effective HIV-1 vaccine, the virus continues to spread, with the highest prevalence of infection in developing countries, especially in sub-Saharan Africa and South-East Asia (Klausner et al., 2003). Transmission in Africa is usually heterosexual, and women now account for nearly 50% of the prevalence of HIV/AIDS worldwide (Ambrose et al., 2007) and, unfortunately, few people use condoms to limit the spread of HIV. Therefore, in addition to more effective therapeutic tools, there is an urgent need for cost-effective preventive measures to prevent the spread of HIV/AIDS.

Microbicides are user-friendly, convenient and readily available and would extend the range of means for self-protection against HIV, both in developing and industrialized countries. They limit sexual transmission of the virus (Stone, 2002) by acting on the epithelial barrier of the vaginal and cervical mucosa, which have been implicated in HIV transmission (Galvin and Cohen, 2004). Microbicides must be safe, effective, affordable and acceptable. They should not cause local irritation or epithelial damage, and they should inhibit the virus at its point of entry through the vaginal mucosa, prevent all subsequent steps leading to infection of the host, block viral replication and have a high genetic barrier to resistance (McGowan, 2006; Veazey et al., 2005). Microbicides would appear to be a good preventive method before intercourse (Shattock and Moore, 2003).

Despite more than 20 years of research, however, no microbicide is currently available to provide sterilizing immunity against HIV-1 (Duerr et al., 2006). Of the different types of microbicide, only two

* Corresponding author. Institut Pasteur de Bangui, Bangui, Central African Republic.
E-mail address: mirdad.kazanji@pasteur.fr (M. Kazanji).

0042-6822/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2010.06.008

have been tested in phase III trials, PRO2000® and Carraguard® (Abdool Karim, 2010; Skoler-Karpoff et al., 2008) but it is difficult to predict whether these microbicides will have a beneficial or a deleterious effect on HIV transmission (Teleshova et al., 2008). As a proved spermicide tested to prevent against sexually transmitted diseases, the Nonoxynol-9 reduced the susceptibility of gonorrhoea infections otherwise increased the frequency of genital ulcers and vulvitis (Kreiss et al., 1992; Roddy et al., 1998). In clinical trials, microbicides benefit is not deftly evaluated even though a moderate effect was statically monitored. The microbicides efficacy might depend on adherence variability to the trial, independently to the action mechanism of microbicides.

Microbicides that specifically inhibit HIV transmission include monoclonal antibodies against envelope glycoproteins (gp120), attachment receptors and chemokine co-receptors (CCR5 or CXCR4) (Hu et al., 2004; Jiang et al., 2005; Schols, 2004; Veazey et al., 2003); inhibitors of fusion between HIV and its target cells (Derdeyn et al., 2000; Kilby et al., 1998); and reverse transcriptase (RT) inhibitors (Van Herrewege et al., 2004a,b). In the last substance category, several have been already tested in phase II clinical trials as Tenofovir®, UC-781® or TMC-120® (McGowan, 2006); others are tested in preclinical trials (Ambrose et al., 2007). MC1220 is a non-nucleoside RT inhibitor (NNRTI) microbicide of the 3,4-dihydro-2-alkoxy-6-benzyl-4-oxo-pyrimidines (DABOs) series. Pani et al. (2001) demonstrated the ability of DABOs to suppress HIV replication for an entire experimental period of 40 days with no cytotoxicity indication. This property correlates with their ability to tightly bind to the HIV-1 reverse transcriptase. Furthermore, of these series, MC1220 was with the highest *in vitro* potential, showing a "memory effect," i.e. the capability of knocking out HIV replication in freshly infected cells after a 4-h treatment followed by incubation of the extensively washed infected culture in the continuous absence of the drug. We hypothesized that MC1220 could inhibit the intravaginal transmission of HIV by hindering the conversion of viral RNA into DNA, resulting in a reduction to viral integration. In preclinical trials, various *in vitro* models were tested and then, *in vivo*. Veazey *et al.* have demonstrated a dose-dependent protection by monoclonal antibody against vaginal HIV transmission in non-human primates (Veazey et al., 2003). Therefore, rhesus macaques seem well-established *in vivo* models for the HIV-1 transmission (Harouse et al., 2001).

In this study, we evaluated the protective efficacy of MC1220 in a non-human primate model: adult female rhesus macaques. Dose and time effects of MC1220 in preventing the vaginal transmission of SHIV89.6P were evaluated by single and repeated applications. Animals were followed up virologically and immunologically to determine the RNA viral load, the DNA proviral load (in peripheral blood mononuclear cells (PBMCs)), antibody responses, immunological changes (evolution of T-cell subsets) and the viral and proviral load in PBMCs and various lymphoid organs at necropsy.

Results

Irritation effect after intravaginal treatment

The scores for irritation, as measured by the previously described methods (D'Cruz et al., 2003), produced after two doses of MC1220 microbicide incorporated into liposomal gel are summarized in Table 1. Little irritation was seen, with mean scores of 1.08 with 0.1% and 0.56 with 0.5% MC1220. The most irritation was observed in the control group treated by the liposomal gel without microbicide (total score, 1.44), which was significantly different ($p = 0.002$) of the result from the animals group treated by liposomal gel containing 0.5% MC1220 (total score, 0.5). No erythema or edema was detected with 1.5% MC1220 in liposomal gel (data not shown).

Table 1
Mean vaginal irritation scores for monkeys treated with the microbicide MC1220 incorporated in liposome or with the liposomal gel alone (controls).

Vaginal component (range of possible score)	Control liposome alone	MC1220	
		0.1% gel + liposome	0.5% gel + liposome
Erythema	1.02 ± 0.3	0.78 ± 0.36	0.4 ± 0.26
Oedema	0.42 ± 0.32	0.3 ± 0.24	0.16 ± 0.17
Total score	1.44	1.08	0.56

The individual irritation score was assigned on the basis of a semi-quantitative scoring system for inflammation: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = intense. The cumulative scores for erythema and Oedema formation were: < 4 = acceptable, 5–6 = marginal and > 6 = unacceptable

Plasma viral load and PBMC proviral load after challenge

In Protocol 1 (single application, Fig. 1), four of five control animals became infected after challenge, with high plasma viremia (10^7–10^8 copies/ml) and a high proviral load at week 2 after challenge (Fig. 2A). In contrast, no virus was detected in PBMCs of two of the four animals (#R08 and #R09) treated by 0.5% MC1220 (Fig. 2B) and two of the five animals (#R11 and #R13) treated by 1.5% MC1220 (Fig. 2C).

In Protocol 2 (repeated applications), all five control animals became infected after challenge, with high plasma viral load (10^6–10^9 copies per ml) and high proviral load (10^4–10^6 copies per 10^6 PBMCs) (Fig. 2D). RT-SHIV was not detected, however, in the plasma or PBMCs of three of the five animals treated four times by 0.5% MC1220 during the 8 weeks after challenge (Fig. 2E).

Virus isolation from PBMCs of treated and challenged animals

In order to confirm the above observations, we isolated the virus from PBMCs at various times after challenge. As seen in Fig. 3, the results confirmed those obtained for the plasma viral load and the proviral load. RT-SHIV could not be isolated from one control animal in Protocol 1, but all the other animals were positive. In the groups treated by 0.5% and 1.5% MC1220, RT-SHIV was isolated from two of four and three of five animals, respectively (Fig. 3A, B, C). RT-SHIV was isolated from one animal (#R14) at week 3 after challenge but not thereafter (Fig. 3C).

In Protocol 2, RT-SHIV was isolated from all the control animals (Fig. 3D) but from only two of five animals treated four times by 0.5% MC1220 (Fig. 3E). The virus was not found in PBMCs of the remaining three animals (#R21, #R22, #R24) during the 14 weeks of this study (Fig. 3E).

Evaluation of antibody responses after challenge

The antibody response to RT-SHIV was evaluated by ELISA at various times after challenge. All RT-SHIV-infected animals in both protocols developed antibodies to RT-$SHIV_{SIVmac239}$ proteins; however, no antibodies were detected in protected animals, i.e. animals #R08 and #R09 treated by 0.5% MC1220, #R11 and #R13 treated by 1.5% MC1220 in Protocol 1 and animals #R21, #R22 and #R24 treated four times by 0.5% MC1220 in Protocol 2 (online supplementary data 1).

RT-SHIV isolation and proviral load in organs after necropsy

In order to evaluate the efficiency of MC1220 in inducing complete protection, all animals were necropsied 14 weeks after challenge. RT-SHIV was isolated by co-culture from various organs of control group, although animal #R04 appeared to be negative in Protocol 1. No RT-SHIV was found in 50% of animals treated by 0.5% or 1.5% MC1220. Furthermore, in the group treated by 1.5% MC1220, the cell-associated viral load in lymph nodes was lower than in the other groups or undetectable. In Protocol 2, RT-SHIV was detected in all organs of