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Antiviral Research 63 (2004) 1–6
Antiviral Research
www.elsevier.com/locate/antiviral

Review

HIV hollow fiber SCID model for antiviral therapy comparison with SCID/hu model

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Received 13 October 2003; accepted 6 April 2004

Abstract

Severe combined immunodeficient (SCID) mice have been evaluated for applicability as hosts for a human immunodeficiency virus (HIV) animal model, compatible with the pathogenesis of HIV disease and/or for testing compounds for antiviral efficacy. McCune et al. [Science 241 (1988) 1632] described the SCID/hu model and Namikawa et al. [J. Exp. Med. 172 (1990) 1055] and Rabin et al. [Antimicrob. Agents Chemother. 40 (1996) 755] described the SCID/hu (Thy/Liv) model which was developed for the evaluation of HIV pathogenic mechanisms and for the prioritization of antiviral compounds that were efficacious in vitro. Hollingshead et al. [Antiviral Res. 28 (1995) 265] and Xu et al. [Bioorg. Med Chem. Lett. 9 (1999) 133] described the HIV hollow fiber SCID mouse model. This model was developed to be a low cost, high throughput, time efficient, simple in vivo screening system for preliminary anti-HIV efficacy evaluation for the prioritization of antiviral compounds that demonstrated in vitro efficacy. The hollow fiber model is used as a pharmacologic tool to help separate active and inactive agents and direct the best lead compounds into additional animal model testing (e.g. SCID/hu). Compounds that are known to have an antiviral effect in man (e.g. 3′-azo-3′-deoxythymidine (AZT), dideoxyinosine (ddI) and dideoxycytidine (ddC)) were evaluated in both models. The endpoints (e.g. PCR, flow cytometry, MTT, p24, RT) evaluated in both models indicate that HIV-1 virus replicates in both models and infection is suppressed in the SCID/hu and hollow fiber SCID mouse models when treated with approved clinical antiviral agents. While both models are useful for the evaluation of antiviral therapies, there are distinct advantages (e.g. cost, time, material, equipment, expediency) with the hollow fiber assay over the SCID/hu model (Thy/Liv) for antiviral drug evaluations particularly in terms of cost effectiveness.
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Keywords: HIV; SCID model; Hollow fiber; Antiviral therapy

1. Introduction

Over the years, scientists have been searching for a small, inexpensive animal model that meets one or more needs, including studying the pathogenic mechanisms of human immunodeficiency virus (HIV) infection, preclinical testing of novel antiretroviral compounds, and evaluating vaccine efficacy. There are advantages associated with evaluating chemotherapeutics in small laboratory rodents including cost, quantities of compound needed for testing, the number of animals required per experiment and the animal housing space requirements.

Because no animal model for HIV disease satisfies all of the preclinical needs for modeling HIV-infected humans, the search for alternatives that are cost effective, expedient and physiologically compatible with the human disease are being continuously pursued. To date, the models that were developed have contributed valuable information regarding the pharmokinetics of anti-HIV agents, the impact of immunologic therapies and the pathogenesis of HIV disease in man.

Since HIV does not produce direct infection and disease in mice (Canivet et al., 1990; Chesebro et al., 1990), a variety of systems using rodents have been developed including using severe combined immunodeficient (SCID) mice for the evaluation of anti-HIV agents. Langley et al. (1998) reported that cotton rats (*Sigmodon hispidus*) do undergo HIV infection, however, the model was not developed. McCune et al. (1990) and Kaneshima et al. (1991) discussed the use of the SCID/hu mouse model as a means to evaluate the potential

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0166-3542/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2004.04.001

for antiviral efficacy against HIV. Mosier et al. (1991) reported the SCID/hu-PBL model in which human peripheral blood leukocytes (PBL), administered to SCID mice, were subsequently infected with HIV. Namikawa et al. (1990) and Rabin et al. (1996) discussed the development of the SCID/hu (Thy/Liv) model for evaluating anti-HIV agents. Hollingshead et al. (1995) and Xu et al. (1999) reported an assay using HIV-infected human cell lines packaged in polyvinylidene fluoride (PVDF) hollow fibers and cultivated in SCID mice for evaluating anti-HIV agents. Using the SCID/hu (Thy/Liv) mouse model as the representative model for the SCID/hu models, we will briefly discuss each model's methodology and its pros and cons for antiviral testing.

2. SCID/hu (Thy/Liv) mouse model

The SCID/hu models (McCune et al., 1988; Rabin et al., 1996) were developed as preclinical models for evaluating HIV pathogenic mechanisms and for prioritizing antiviral compounds for further clinical development. These models depend upon the growth of human cell xenografts that can support HIV replication. These human xenografts (Namikawa et al., 1988; McCune et al., 1990; Kaneshima et al., 1991; Rabin et al., 1996) consist of fetal tissue (thymus, liver, or lymph nodes), peripheral blood leukocytes (Mosier et al., 1989, 1991) or bone marrow-derived cells (Kamel-Reid and Dick, 1988). For these types of studies, the xenograft may be implanted intravenously (i.v.), intraperitoneally (i.p.), subcutaneously (s.c.) or under the renal capsule. HIV infection of the SCID/hu mouse was found to be dependent upon the use of primary and molecular clone isolates of HIV. Tissue culture-adapted isolates (e.g. HIV-IIIb) were not infectious in this model (Kaneshima et al., 1991).

These studies sought to use a mouse strain that expresses a specific defect in lymphocyte maturation so that the absence of murine T and B cells might facilitate the engraftment of human hematopoietic and lymphoid tissues. The SCID mouse has a deficiency in VDJ recombinase that produces an inability to mount an effective cellular or humoral immune response to foreign antigens (McCune et al., 1988).

To reconstitute SCID mice with human T cells, a source of human hematopoietic progenitor cells is required. While such cells may be found in adult bone marrow, an alternative source of hematopoietic stem cells is the human fetal liver. This organ, a site of hematopoiesis in humans between 8 and 24 weeks of gestation, contains progenitors for all hematolymphoid cell lineages (Namikawa et al., 1986). After implantation of SCID mice with human fetal liver, thymus or lymph node tissues, it was postulated that the implant would grow and become tolerant of the mouse environment (McCune, 1997). Namikawa et al. (1990) reported the co-implantation of small fragments (approximately 1 mm × 1 mm × 1 mm) of human fetal thymus and liver under the renal capsule of SCID mice resulting in the formation of a unique structure (Thy/Liv). Rabin et al. (1996) reported a standardized SCID/hu (Thy/Liv) mouse model for preclinical efficacy testing of anti-HIV-1 compounds, using the SCID/hu model with conjoined implants of human fetal thymus and fetal liver (Thy/Liv).

3. A brief description of the SCID/hu (Thy/Liv) model methodology and results

C.B./17 scid/scid mice are anesthetized with 5% ketamine–2.5% xylazine. The left flank of the mouse is shaved and a 1–1.5 cm incision is made to expose the left kidney. Human fetal thymus and liver are cut into small pieces (approximately 1 mm × 1 mm × 1 mm) and one or two of each of these fragments is implanted under the kidney capsule using an 18 gauge tumor-implant trocar. Attention is given so that the fragments stay close together under the capsule (Namikawa et al., 1990). At 12–20 weeks post-transplant, randomly selected animals from each cohort (20% of the total) are examined under general anesthesia to evaluate the growth of the Thy/Liv implant. If the size of the implant is greater than or equal to approximately 30 mm^3, then the entire cohort is entered into the antiviral dosing experiment. At 1–5 days before or 1–7 days after inoculation of this tissue with virus, the test compounds are administered by the appropriate route and schedule. For viral infection, the most reliable means of infecting the mice has proven to be direct injections of small volumes of viral stock directly into the subrenal capsule implants (Rabin et al., 1996). For this, the mice are placed under general anesthesia, the left flank is shaved, and a 1–1.5 cm excision is made to expose the kidney carrying the Thy/Liv implant. Immobilizing the kidney with forceps, and using a 1 ml syringe filled with India ink and equipped with a 27 gauge sharp needle, an opening is made through the fibrous kidney capsule. Using the India ink mark as a guide, the Thy/Liv implant is injected with 25–50 ul of virus stock in one to three places with a 30 gauge blunt needle. The wound is closed by approximating the peritoneal lining with one stitch, and using one to two staples to close the skin. Antiviral compounds (e.g. 3′-azo-3′-deoxythymidine, AZT; dideoxyinosine, ddI) are administered for an additional period of time (2–4 weeks) via the appropriate route and schedule. At the end of treatment, endpoint analyses including the polymerase chain reaction (PCR) for proviral DNA, flow cytometry for the evaluation of cell subpopulations, p24 levels by enzyme-linked immunosorbent assay (ELISA), and virus isolation by co-cultivation (Rabin et al., 1996) are performed from a single cell suspension made from the transplanted human Thy/Liv implants.

Fetal tissues (thymus, liver) implanted beneath the kidney capsule of SCID mice will grow, thereby creating the SCID/hu (Thy/Liv) mouse model. This SCID/hu model supports HIV-1 viral replication when infection is achieved by intravenous, or preferably, by direct intra-implant inoculation of small volumes of standardized viral stock.

Administration of antiviral agents such as AZT to the SCID/hu mouse suppresses HIV-1 replication (Kaneshima et al., 1991; Rabin et al., 1996). In summary, the SCID/hu (Thy/Liv) model is a reliable preclinical animal model for the evaluation of anti-HIV efficacy of various compounds and it may help guide the selection of effective drugs to treat HIV-1 disease in human (Rabin et al., 1996).

4. The HIV hollow fiber SCID mouse assay

The hollow fiber assay (Hollingshead et al., 1995) was developed as a low cost, high throughput, expedient, simple in vivo screening system for preliminary anti-HIV efficacy evaluations to prioritize antiviral compounds. The hollow fiber assay was not developed to characterize or simulate the pathogenesis of the human infection process. Rather, it is a pharmacologic tool that helps to separate active from inactive agents. This model can direct the best lead compounds into additional animal model testing (e.g. SCID/hu). The hollow fiber assay is conducted by implanting hollow fibers, filled with uninfected or HIV-infected human cells, into two physiological compartments (i.p. and s.c.) of the SCID mouse.

We have confirmed the methodology of Hollingshead et al. (1995), using HIV-IIIb infected human CD4+ CEM-SS cells. Preliminary experiments with this model confirm that:

(1) CEM-SS cells are capable of in vitro and in vivo replication within PVDF hollow fibers.
(2) This growth can be accompanied by replication of HIV, indicating that the hollow fiber material is compatible with virus replication as demonstrated by Hollingshead et al. (1995) and Xu et al. (1999).
(3) Virus replication is suppressed by known anti-HIV agents (e.g. AZT; dideoxycytidine, ddC).
(4) Replication of HIV in cells within hollow fibers can be demonstrated using various endpoints, e.g. assessment of cell viability using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay conducted directly on cells within the hollow fibers, and measuring reverse transcriptase (RT) activity and p24 antigen concentrations in lysates of cells collected from the hollow fibers as well as in serum and peritoneal wash samples from mice.

The assay is technically simple because of the ease of infecting continuously passaged human CD4+ lymphoid lines (e.g. CEM-SS) and the simplicity of hollow fiber sample preparation. The hollow fibers are prepared and implanted on the same day (day 0) so the cells, whether infected or not, are exposed to antiviral therapy early in the infection process.

A surgical procedure is required only at the beginning of the experiment for implantation of the fibers. At the end of the in-life phase of the experiment, the mice are sacrificed and the desired samples (blood, peritoneal wash, hollow fibers) for endpoint analyses are collected. The time required for completion of an experiment from receipt of the test animals to endpoint assays (MTT, p24 and RT) requires less than 4 weeks.

5. A brief description of the HIV hollow fiber SCID mouse assay methodology

CEM-SS human lymphoid CD4+ cells (NIH AIDS Research and Reference Reagent Program, Bethesda, MD, USA) are cultivated in continuous passage as a suspension culture and used at a density of 1×10^7 cells/ml. Viral stocks were prepared as described previously (Buckheit and Swanstrom, 1991) and CEM-SS cells are acutely infected with cell culture isolates of HIV-IIIb (HTLV-IIIB/H9 from Gallo's original stock cat.# 398 NIH AIDS Research and Reference Reagent Program, Bethesda, MD, USA) at a multiplicity of infection (moi) of 0.0001.

Polyvinylidene fluoride hollow fibers (Spectrum Medical Corp., Los Angeles, CA, USA) with a molecular weight cut-off of 500,000 and an internal diameter of 1 mm are conditioned to optimize cell growth as described previously (Hollingshead et al., 1995). Briefly, the fibers are flushed and soaked in ethanol followed by a water rinse and sterilization by autoclaving. After conditioning, the fibers are maintained in sterile containers at 4 °C until used.

The conditioned hollow fibers are filled with the cell inoculum (uninfected or acutely infected cells) on a sterile field within a biological safety cabinet using a 20-gauge Teflon catheter to fill the fibers. The fibers are heat sealed at 2 cm intervals with hot needle holders (250–280 °F) or with a FS-2 Mark I Hollow Fiber Sealer (OUTSOURCE 2000, Huntsville, AL, USA). The fibers are separated at the center of the heat seals thereby producing fiber samples 2 cm in length and each containing approximately 20 μl of inoculum (2×10^5 cells/fiber). The fiber samples are maintained in medium at 4 °C until they are implanted into mice (within 1 h).

Hollow fibers samples are implanted into anesthetized SCID mice. Each mouse receives three s.c. fibers, and three i.p., thus providing six experimental data points per experimental animal. The s.c. fibers are implanted via an 11 gauge tumor implant trocar through a small incision in the dorsal cervical skin. The hollow fibers for i.p. implantation are introduced into the peritoneal cavity through a small incision in the dorsolateral skin and abdominal wall. After the implants are inserted, the incision sites are closed using one or two surgical staples.

Treatment groups include drug vehicle controls as well as mice receiving the test agents (e.g. AZT, ddC, etc.). The routine dosing schedule used for test agents is every 8 h starting on day 0 immediately prior to fiber implantation and continuing through day 6. Generally, the route of administration is i.p. although oral dosing is also very amenable to this assay; however, it is important to know the oral bioavailability prior

to pursuing extensive oral dosing experiments. In addition to vehicle-treated controls, uninfected cells served as drug toxicity controls so that any reduction in viral burden can be attributed to an antiviral rather than an anticellular effect.

Endpoint analysis: On day 7, animals are sacrificed and samples (blood, peritoneal wash and the hollow fibers) are collected and analyzed (Hollingshead et al., 1995; Xu et al., 1999). A stable endpoint MTT assay as described by Alley et al. (1991) using the modifications of Hollingshead et al. (1995) is used to assess cell viability. Fibers for reverse transcriptase assays and p24 antigen quantitation are placed into 1.5 ml tubes and lysing buffer (250 ul) is added. The fibers are cut in half and incubated in the lysing buffer at room temp for 30 min. After incubation, the samples are vortexed and 150 ul of the lysate are transferred to 96-well plates to serve as the sample source for the RT and p24 antigen assays. A standard HIV reverse transcriptase assay is conducted on the collected lysates, as previously described (Buckheit and Swanstrom, 1991). For P24 antigen determination, commercially available ELISA-based assay kits (Coulter Corp., Hialeah, FL, USA) are used with the assays conducted according to the manufacturer's instructions.

In vivo studies demonstrated that HIV-infected CEM-SS cell can be grown in PVDF hollow fibers while maintaining active virus replication. It is possible to monitor cell viability via the MTT dye conversion assay and to measure p24 antigen and RT activity in samples of the fiber lysates. The results indicate that HIV-IIIb can replicate in CEM-SS cells in the i.p. and s.c. hollow fibers. This is measured as increases in RT activity for infected control samples and decreases in viable cell mass compared to uninfected cell controls. In addition, there is evidence of antiviral activity in the mice receiving clinically approved antiviral agents (e.g. ddC, AZT). In fact, ddC treatment protects against decreases in cell viability and prevents increases in RT activity and p24 antigen levels compared to infected controls. These results suggest that the hollow fiber assay can be used for evaluating the anti-HIV efficacies of various compounds against virally infected human target cells.

6. Discussion

The SCID/hu (Thy/Liv) model requires several survival surgeries (Rabin et al., 1996) during the course of the experiment: (1) to implant tissue fragments; (2) to examine animals (~20% of total) for evaluation of the growth of the Thy/Liv implant (not all implants are successful); and (3) to infect the Thy/Liv implant with HIV by direct intra-implant injection. The HIV hollow fiber assay only requires one surgery at the beginning of the experiment for implantation of the fibers. Thus, from an animal manipulation perspective, the hollow fiber assay requires less time and surgical skill than does the SCID/hu (Thy/Liv) model.

For the SCID/hu (Thy/Liv) model, a minimum of 4 months is required for conducting an experiment (Fig. 1). This represents the time required for tissue implantation and establishment of the graft (3–4 months), viral infection (2 weeks), antiviral treatment (14–28 days) and endpoint analyses. In contrast, the hollow fiber assay can be completed within 10 days since the in-life phase of the experiment only requires 7 days to conduct and the endpoint analyses can be completed within a few days. This difference significantly impacts the costs associated with conducting these two assays since the experimental mice must be held for protracted periods of time in biologically controlled environments for the SCID/hu (Thy/Liv) model. In contrast, the hollow fiber assay requires less than 2 weeks of holding for the experimental animals.

While the SCID/hu (Thy/Liv) model can be extremely useful for evaluating antiviral therapies, as well as for studying pathogenesis and immunomodulating therapies, they are costly, time consuming, technically complex and require specialized equipment (e.g. PCR, flow cytometry) and material (e.g. fetal tissues) that are not readily available to all laboratories (Hollingshead et al., 1995).

Unlike the SCID/hu (Thy/Liv) model, the hollow fiber assay does not reproduce the complex intercellular interactions occurring during HIV infection, but it does allow evaluation of the pharmacology of potential new antiviral agents

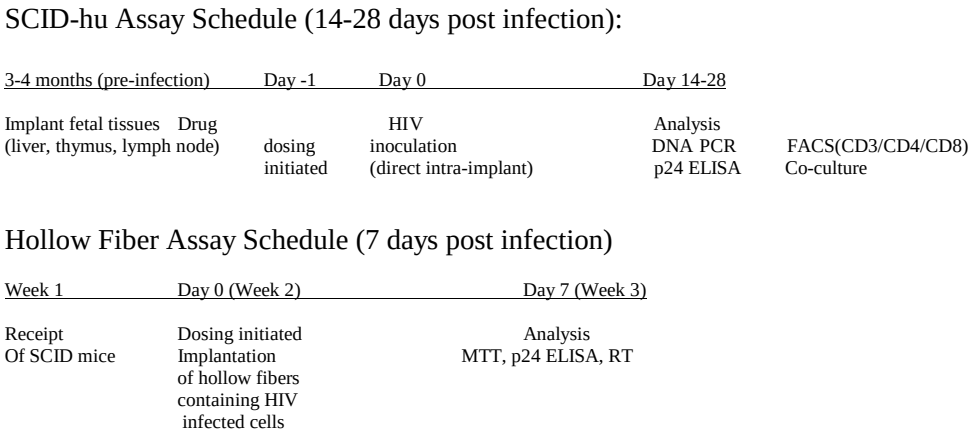

Fig. 1. Schematic of protocol for evaluation of antiviral compounds in the SCID/hu (Thy/Liv) mouse model vs. the HIV hollow fiber assay.

as demonstrated with the clinically approved agents. Since the hollow fibers provide a physical barrier between the host and the target cell and the fiber implants are of low reactivity to the host, it is possible to use other strains of mice in this model (Hollingshead et al., 1995). While this can further reduce the costs associated with conducting these studies, it is important to note that human hematopoietic cells are considered to have superior growth in the SCID mouse compared to other strains (Hollingshead et al., 1995).

The advantages of the hollow fiber assay compared to the SCID/hu (Thy/Liv) model for antiviral drug comparisons include:

(1) The hollow fiber assay requires significantly less time to conduct the complete assay (less than 3 weeks versus 4 months).
(2) The hollow fiber assay has a simple cell infection strategy in which acutely infected cells are loaded into hollow fibers and immediately implanted on the first day of treatment whereas the SCID/hu models require 3–4 months for xenograft growth with subsequent direct intra-tissue infection which lasts for several weeks.
(3) The hollow fiber assay can be conducted with continuous passage human cell lines and laboratory-adapted viruses while the SCID/hu model requires access to fetal tissues and clinical viral isolates.
(4) The hollow fiber assay endpoints (MTT, RT and p24) are readily available to all laboratories conducting HIV related studies. In contrast, the SCID/hu model requires access to flow cytometry and PCR.
(5) The hollow fiber assay requires less test compound since the assay and requisite drug treatments are of short duration (7 days). In contrast, the SCID/hu mouse model requires 14–28 days of drug treatment (Fig. 1). This is important during the preliminary evaluations of a test compound since the compounds must be synthesized for the in vivo studies in amounts that are challenging for many academic or small biotechnology companies.
(6) With the hollow fiber assay, there are two sites of viral replication (i.p. and s.c) while there is a single site in the SCID/hu model. Two sites of viral replication provides an opportunity to administer a test agent at one site (e.g. i.p.) and observe its' effect on the same site (i.p.) and a distance site (s.c.). This aids in determining the effect of distribution and metabolism on compound activity without requiring the development of an accurate, quantitative pharmacologic assay for the test agent.
(7) In the hollow fiber assay, the host mouse cells cannot migrate into the fibers and mix with the human target cells. In contrast, McCune et al. (1990) and Rabin et al. (1996) report that host cells migrate into the xenograft tissues of the SCID/hu model. One advantage of recovery of the human cells without contamination by mouse cells is that the cytotoxicity of the test agent for the human cells, as well as the antiviral activity can be determined.

The hollow fiber assay described here allows multiple treatment permutations in a single assay, the potential for improved safety by sequestering pathogenic viruses within controlled 'packets' and the quantitative retrieval of the fiber implants. Compounds that pass this pharmacologic "prescreen" can then be evaluated to a greater extent in other models of HIV infection, including the xenograft-based SCID mouse models. By selecting only the pharmacologically active agents for additional testing, the resources required for compound re-synthesis as well as in vivo testing can be minimized. The hollow fiber assay methodology was initially developed for anticancer drug screening (Casciari et al., 1994; Hollingshead et al., 1995a) and subsequently adapted to use as an HIV model (Hollingshead et al., 1995). It has been used for over 9 years (Hollingshead et al., 1995a) as a prescreen for anti-tumor efficacy testing in mice. We are convinced that others will find this methodology adaptable to growth of a wide selection of cells as well as other virus families.

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