Chinese herbal formula XQ-9302: pilot study of its clinical and in vitro activity against human immunodeficiency virus

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Objectives. To evaluate the effectiveness of XQ-9302—a purified, precise mixture of 20 Chinese herbs—against infection with human immunodeficiency virus in vitro and in the clinic.
Design. In vitro cell culture assay, heavy metal content analysis, and pilot non-randomised clinical trial.
Setting. Drug rehabilitation centre and municipal surveillance centre, Shanghai, China.
Patients. Forty-eight patients who had various clinical histories, such as drug abuse, cancer, and infection with human immunodeficiency virus, participated in the clinical study.
Intervention. During the clinical trial, multiple 15-day courses of XQ-9302 10.8 g/d were given to participants.
Main outcome measures. CD4 count, P24 antigen level, level of antibody against human immunodeficiency virus, number of copies per millilitre of human immunodeficiency virus in the plasma (viral load), and any side effects.
Results. XQ-9302 protected cultured MT4 cells from infection with human immunodeficiency virus in vitro. Clinical tests showed that the herbal formula relieved the symptoms of acquired immunodeficiency syndrome and enhanced CD4 counts in patients infected by the human immunodeficiency virus. There were no observable side effects, even after taking the drug for several months. In three patients who had acquired immunodeficiency syndrome, treatment with XQ-9302 reduced the magnitude of the viral load by more than 1 log.
Conclusion. XQ-9302 not only improves the immune function of patients infected with the human immunodeficiency virus, but also interrupts viral replication and slows the progression of the disease without detectable side effects. In addition, the heavy metal content of XQ-9302 is well within safety levels set by the Government of China.

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Key words: Acquired immunodeficiency syndrome, drug therapy; Drugs, Chinese herbal/therapeutic use; Evaluation studies; Follow-up studies; HIV antibodies/blood;Virus replication

Introduction

The medical treatment of patients who have acquired immunodeficiency syndrome (AIDS) consists of therapy against human immunodeficiency virus (HIV), the control of opportunistic infections and secondary tumours, and palliative medication. Of these interventions, anti-HIV therapy is the key element. Recent studies have demonstrated the important benefits of triple drug combinations—inhibitors and anti–reverse transcriptase drugs—in suppressing HIV replication and preventing drug resistance.[1-3] Such triple drug combinations, however, have potential adverse effects such as evoking additive toxicity and giving rise to replication-competent multidrug-resistant isolates. The high financial cost of these drugs is also an important concern. Accordingly, at the 11th International Conference on AIDS in Vancouver, Dr Peter Piot, the Executive Director of the Joint United Nations Programme on HIV/AIDS (UNAIDS), called for the development and use of traditional medicines, herbs, and other potentially cheaper curative drugs to treat patients with AIDS, especially in the developing countries.[4,5]

China is one of the few countries in the world where traditional medicine has been practiced for more than

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5000 years and where it is accorded the same acceptance as western medicine. One of the strengths of traditional Chinese medicine is that it can improve immune functions without either long- or short-term toxic effects. Herbal treatment is the most important component in traditional Chinese medicine, and recent studies have shown that the use of Chinese herbs can suppress HIV replication in vitro and in vivo.[6-8]

The Chinese herbal formula XQ-9302, developed by the Shanghai Xiong-Qi Biological Products Ltd., Shanghai, China has been studied for nearly 10 years. Before 1994, studies were centred on the physicochemical analysis of the drug, the establishment of its standardised good manufacturing practice, and bioassays to determine its immunological effects and toxicity.[9] Since 1994, research has focused on the anti-HIV properties of the drug. This paper reports the preliminary results of the use of XQ-9302 against HIV-1 both in vitro and in the clinic.

Methods

Materials

The Chinese herbal formula XQ-9302 is a light brown powder that consists of 20 types of Chinese herbs such as *Da-Huang, Huang-Bai, Huang-Lian, Kun-Bu, and Hai-Chao.* Each capsule of the formula contains 0.45 g of powder that has been purified from 3.79 g of raw herbs, according to a precise formula. The powder dissolves easily in water.

In vitro studies

An HIV-1 isolate that had come from an HIV-1–seropositive individual from the United States was propagated in MT4 human lymphocytic cells obtained from the National Hygiene Institute, Japan, and titrated to determine the median tissue culture infective dose ($TCID_{50}$) per millilitre of virus stock.

Viral replication assay

Fifty microlitres of a suspension MT4 cells (20 000 /mL) were placed in each well of a 96-well microculture tray containing 100 μL of XQ-9302 in RPMI-1640 tissue culture medium supplemented with 10% newborn calf serum. Serial dilutions of the drug were added; the dose ranged from 8.00 mg/mL to 31.25 mg/mL. The wells were then divided into two groups: in one group, each well received 50 μL of 100x$TCID_{50}$ HIV-1; in the other group, each well received only 50 mL RPMI-1640 medium as a control. The cytopathic effect (CPE) of HIV infection and toxicity of XQ-9302 was examined daily from the third day after viral infection until the sixth day by using the monotetrazolium (MTT) assay: 20 μL of MTT solution (7.5 mg/mL) were added to each well and the trays were incubated at 30°C for 2 hours; after removing of 150 μL of fluid from each well, cells were disrupted by adding 100 μL of 10% Triton X-100 solution and the optical density at 550 nm was read using an LP400 enzyme-linked immunosorbent assay reader (Sanofi Pasteau Diagnostics, Paris, France). The concentration of XQ-9302 that provided 50% protection against the CPE of HIV infection and the concentration that caused 50% cytotoxicity were then calculated.

Metal analysis

To analyse the metal content of XQ-9302, 2 g of the formula were dissolved in 50 mL (0.01 M) nitric acid. For mercury and arsenic analysis, 5 g and 1 g of XQ-9302, respectively, were placed into the nitric acid. Each mixture was then ashed prior to testing. A total of 27 metal elements were analysed: potassium, sodium, calcium, magnesium, boron, mercury, arsenic, lead, cadmium, strontium, barium, manganese, silicon, iron, aluminum, chromium, copper, nickel, zinc, antimony, tin, beryllium, cobalt, titanium, lithium, lanthanum, and vanadium. Mercury was analysed by using the F732-S Double Wavelength instrument (Shanghai Hua Guang Instrunment and Equipment Factory, Shanghai, China), and lead and cadmium levels were determined by using the PE-2100 Atomic Absorption Spectrovac (Perkin Elmer Co., Norwalk, United States). The other elements were measured by using the PS-6 ICP Plasma Spectrovac (Baird Co., Boston, United States).

Quality control of XQ-9302

The bioactivity of each lot of XQ-9302 that was produced by Shanghai Xiong-Qi Biological Products Ltd., was examined using the HIV viral replication assay that was described above. The concentration of XQ-9302 required to provide 50% protection of MT4 cells against the CPE of HIV infection was used as the quality control measurement. The bioactivity was regarded as acceptable if 50% protection of MT4 cells against the CPE of an HIV dose of 100x$TCID_{50}$ was achieved by less than 0.30 mg/mL XQ-9302.

Monitoring the safety and clinical effects of XQ-9302 in humans

The study of the safety and immunostimulatory effects, and the pilot study to clinically evaluate XQ-9302 in humans was performed in a special drug rehabilitation centre and a municipal surveillance centre of Shanghai. A total of 48 participants who had various clinical histories such as drug abuse, cancer, and HIV infection were selected. All cases of AIDS had been strictly diagnosed using the criteria developed by the

Ministry of Health of China[10] and classified in accordance to the categories defined by the United States Centers for Disease Control and Prevention. All participants gave their consent and approval was given by the Shanghai Clinical Trail Ethics Committee of the Shanghai Municipal Bureau of Health. The CD4 count of each patient before the trial was measured. The formula was administered in 0.45 g capsules: one course consisted of six capsules per dose (ie 2.7 g) at four doses per day for 15 days. The minimum dose was two courses, given with no interval and without any other Chinese herb or antiretroviral drug. In addition, the following special requirements were strictly adhered to: (1) medication was taken at 3:00 am, 9:00 am, 3:00 pm, and 9:00 pm each day; (2) the consumption of cold food and beverage (ie freshly removed from the refrigerator) was avoided, as dictated by traditional Chinese medicine; and (3) using liquorice root was avoided because it interferes and reduces the efficacy of *Sargassum fusiforme*, which is one of the herbal components of XQ-9302.

All participants in the trial were periodically monitored with respect to their clinical features; any side effects were noted. Blood was withdrawn and placed into tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant; the plasma was used in tests and some was also stored at -70°C. Laboratory tests, including routine blood tests, tests to detect P24 antigen and HIV antibody, and CD4 counts, were performed at the Shanghai Municipal AIDS Surveillance Center. The HIV load was determined in the HIV Laboratory, Laboratory Services Branch, Ontario Ministry of Health in Toronto, Canada, which used a new method referred to as the 'Paediatric protocol of branched DNA' (in which the sensitivity cut-off value is 10 000 HIV copies of RNA per millilitre).[11] All data and clinical records were kept confidential.

Results

Activity of XQ-9302 against human immunodeficiency virus

From 1994 to 1997, 10 batches of XQ-9302 were produced by Shanghai Xiong-Qi Biological Products Ltd. In our level-3 laboratory analysis, XQ-9302 was found to display potent in vitro anti–HIV-1 activity, as measured by monitoring the inhibition of syncytium formation of infected MT4 cells. The various batches of XQ-9302 provided 50% protection to MT4 cells against the CPE of 100x$TCID_{50}$ of HIV-1 at drug concentrations of 0.147 to 0.261 mg/mL, and 90% protection at drug concentrations of 0.781 to 2.992 mg/mL. The cytotoxicity assay showed that XQ-9302 effected 50% toxicity in MT4 cells only at the relatively high drug concentrations of 2.609 to 2.992 mg/mL.

Metal content of XQ-9302

After analysing the metal content of XQ-9302, only the following seven of the 27 elements tested for were found: potassium, sodium, calcium, magnesium, boron, mercury, and arsenic. The amounts found were 3200 to 3500 mg/kg, 1400 to 1600 mg/kg, 63 to 120 mg/kg, 42 to 76 mg/kg, 2.0 to 2.2 mg/kg, 0.014 to 0.136 mg/kg, and 1.0 mg/kg, respectively. Lead and cadmium were not detected.

Clinical treatment of acquired immunodeficiency syndrome

In this study, 48 patients were treated with XQ-9302; they included 24 intravenous drug users, 16 patients with HIV infection or AIDS, two cancer patients, and six patients with other viral infections. The treatment period was at least 1 month; for some patients, it was nearly 1 year. There were no side effects reported. The CD4 counts of patients with HIV infection or AIDS were measured both before and after the XQ-9302 treatment period (Table 1). The case reports of three patients with AIDS who have been under observation for more than 10 months are presented below.

Case 1

The patient in case 1 was a 27-year-old woman with no history of intravenous drug use, blood transfusion, or blood donation, but who had had sexual contact with an HIV-positive visitor from abroad. The patient tested positive for HIV antibodies in 1992 and was followed up by the Shanghai Municipal AIDS Surveillance

Table 1. Levels of CD4 before and after XQ-9302 treatment

Case code	Duration of treatment (months)	CD4 level (counts per mL)	
		Before	After
004	2.5	394	773
005	2.5	394	292
006	2.5	394	424
007	2.5	350	452
008	2.5	350	525
009	2.5	229	408
010	2.5	394	949
011	2.5	365	277
012	2.5	350	817
013	2.5	321	277
014	2.5	263	306
015*	2.0	190	325
016*	3.0	285	827
017*	3.0	390	542
018	1.0	120	284
019	2.5	110	400

* Cases of acquired immunodeficiency syndrome

Center. Since August 1996, she had had a continuous fever, diarrhoea, fatigue, night sweats, menstrual disturbance, and weight loss. Her CD4 count was 190 /mL. Results from the blood lymphocyte culture for HIV, P24 antigen test, and HIV antibody test were positive. After one 15-day course of the XQ-9302 medication, the fever subsided, diarrhoea disappeared, and her appetite returned. After the fourth course of XQ-9302 medication, the results from blood lymphocyte culture for HIV and the P24 antigen test were negative and the CD4 count increased to 325 /mL. The patient has completed 10 courses and so far has not experienced any side effects.

Case 2

A 41-year-old man with no history of intravenous drug use, blood transfusion, or blood donation had been sexually active in brothels overseas. The patient tested positive for HIV antibodies in the winter of 1991 and was followed up by the Shanghai Municipal AIDS Surveillance Center. Symptoms of AIDS appeared in 1996 and included continuous fever, diarrhoea, extensive weight loss, fatigue, fungal infection, herpes infection, and respiratory tract infections. Blood lymphocyte culture was positive for HIV and tests for the presence of P24 antigen and HIV antibodies gave positive results. The CD4 count was 285 /mL. After two 15-day courses of XQ-9302, most of the symptoms started to disappear and after six courses, the CD4 count increased to 827 /mL. Twenty courses of treatment have since been completed and the CD4 count has been stable, being between 750 and 1180 /mL. The test for P24 antigen and blood culture for HIV have since remained negative.

Case 3

A 42-year-old woman who had no history of intravenous drug use, blood transfusion, or blood donation had probably been infected between 1993 and 1994 by her husband, who had been infected with HIV by blood transfusion, as was confirmed by the Japanese Ministry of Health; the husband had died from AIDS in 1994. She returned to Shanghai in October 1996, and physical examination revealed lymphadenopathy, fever, diarrhoea, dyspepsia, weight loss, and pronounced liver enlargement to four finger-widths below the ribs. Blood lymphocyte culture did not detect HIV and the tests for, P24 antigen and HIV antibody gave positive results. The CD4 count when she was first followed up by the Shanghai Municipal AIDS Surveillance Center was 390 /mL. After receiving two courses of XQ-9302, her condition improved and most symptoms disappeared. After six courses of the herbal formula, the liver returned to normal size, as indicated by ultrasonography, and the CD4 count increased to 542 /mL. The patient has completed 15 courses of the XQ-9302, and to date has not experienced any side effects.

Table 2. Plasma loads of human immunodeficiency virus in three patients with acquired immunodeficiency syndrome before and after treatment with XQ-9302*

Time	Viral load (per mL)		
	Case 1	Case 2	Case 3
Before treatment	226 800	184 200	184 200
After treatment (months)			
2	36 100	-	-
3	-	<10 000	<10 000
4	17 660	<10 000	<10 000
5	25 240	-	<10 000
6	17 420	<10 000	-
7	-	<10 000	20 100
8	22 140	-	14 200
9	-	-	12 060
10	<10 000	<10 000	-
11	-	10 280	-
12	-	<10 000	-

*Plasma viral load = No. of copies of human immunodeficiency virus RNA per mL

The plasma HIV loads of the patients in these three cases before treatment and during follow-up are shown in Table 2; XQ-9302 substantially reduced the viral load in the blood in each case.

Discussion

The Chinese herbal formula XQ-9302 has been shown to be effective against HIV and to play an important role in relieving the symptoms of AIDS. After an average of two 15-day courses of XQ-9302, some of the symptoms of AIDS became undetectable. No side effects, even after many months of XQ-9302 use, were reported. In addition, the level of metals in the herbal preparation were either undetectable or well within safety limits of those allowed in nutritional supplements by the Ministry of Health in China.[12] The CD4 counts in the 16 patients with HIV infection or AIDS increased after the use of XQ-9302 in most cases (Table 1; $P<0.01$, Student's *t* test). In particular, patients with AIDS had increases in CD4 levels that ranged from 135 to 512 /mL, after taking the herbal formula. Furthermore, the patient in the case 016 had severe herpes, which disappeared 7 days after taking XQ-9302. Recently, doctors in Los Angeles have given XQ-9302 to six patients with herpes and found that the drug can cure the infection quickly and without relapse (Abravanel ED, written communication, 1997). Furthermore, the patient in case 017 had hepatomegaly, which resolved after treatment with XQ-9302. Hence, the herbs in this formula might promote liver function and thereby assist liver recovery. These clinical findings, together with the laboratory findings that

immunoregulatory function is enhanced and tumour growth is inhibited in mice,[13] encourage further investigation of the possible additional roles of the drug. These studies also suggest that inhibition of HIV replication may not be the sole factor in the improvement of the clinical conditions of AIDS patient by XQ-9302.

The inhibition of viral replication

The ability of XQ-9302 to directly inhibit HIV replication is most clearly demonstrated by its protective effect in MT4 cells following incubation with HIV in vitro. The dose required to achieve 50% protection was more than 10-fold lower than the dose that caused 50% cellular toxicity in these cells. This property reflects a substantial therapeutic window at the cellular level. Using the reduction of HIV load in the blood as a marker of anti-HIV drug efficacy,[14,15] the extensive reduction in HIV load in three patients with AIDS who were given XQ-9302 and the absence of side effects clearly indicate that the efficacious dosage is also below the toxic dosage for humans.

Quality control of XQ-9302

All the herbal components used to prepare XQ-9302 are rigorously standardised with regard to the locale of their origin, season of harvest, and procedures for their extraction and processing. Nonetheless, a strict criterion of quality control is necessary to ensure the uniformity of different batches of the formula. The protection of MT4 cells against HIV in vitro provides a particularly relevant and convenient test to control the quality of XQ-9302. Since 1994, the Shanghai Xiong-Qi Biological Products Ltd., has produced 10 batches of XQ-9302. Each of these batches has yielded 50% protection of MT4 cells from infection by 100x$TCID_{50}$ HIV-1; the XQ-9302 concentration has consistently been less than 0.30 mg/mL. This result indicates that uniform potency of XQ-9302 is being achieved. In addition, providing 50% protection at a dose of no more than 0.30 mg/mL represents a useful criterion for the quality control of XQ-9302.

In conclusion, the Chinese herbal formula XQ-9302 is a very effective drug for relieving the symptoms of AIDS. Although a larger-scale clinical evaluation of the drug and more insight into its mechanism of action are needed, the potency of XQ-9302 is consistent with the requirements of a basic medication in both the enhancement of immunoregulation and inhibition of HIV regulation for the treatment of AIDS.[16] The lack of any side effects is an important property of XQ-9302. Its herbal nature means that large-scale manufacture is possible, such that the cost of the drug might be affordable to the 90% of patients with AIDS who live in developing countries.

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