SCREENING OF ANTI-HIV ACTIVITIES IN EXISTING DRUGS WHICH ARE CAPABLE OF LONG-TERM ORAL ADMINISTRATION

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(Received August 5, 1989 · Accepted November 13, 1989)

Since drugs for the treatment of AIDS are administered over long periods, and since it usually takes a considerable time before new compounds are approved by the health authorities, we screened for anti-HIV activity 58 already commercially avail able drugs for long-term administration without major side effects. Lorazepam, calcium hopantenate, prochlorperazine maleate, amantadine hydrochloride, perphenazine and nitrazepam were found to exhibit anti-HIV activity in MT-4 cells. But only perphenazine and nitrazepam did so without cytotoxicity. In peripheral blood mononuclear cells, perphenazine exhibited only weak anti-HIV activity, while nitrazepam showed none. These results stress the importance of selection of *in vitro* screening system.

Key words : AIDS, HIV, Anti-HIV activity, Existing drugs

INTRODUCTION

In recent years, the number of patients with acquired immunodeficiency syndrome (AIDS) has been steadily increasing[1,2], and the development of anti-human immunodeficiency virus (HIV) drugs is urgent. Not only drugs for the treatment of AIDS are needed, but drugs which can be given to asymptomatic carriers for prophylaxis. Although aztreonam (AZT), which is considered the prototype of an anti-HIV drug, has brought about clinical improvement in AIDS patients[3,4], its administration to asymptomatic carriers is risky because of its side effects[5]. We believe that drugs for asymptomatic carriers should have the least possible side effects even if this means that their anti-HIV activity is low. If already existing drugs possess anti-HIV activity, they can easily be put to this clinical use with the advantage that they are available needs whereas new conpounds take a long time to develope. With this in mind, we screened for anti-HIV activity 58 commercial drugs available for long-term oral administration without significant side effects.

MATERIALS AND METHODS

Drugs

Fifty-eight commercial drugs available for long-term administration without major side effects were used (Table 1). The concentrations of the drugs were 100, 10, 1 or 0.1 μg/ml.

Cells and viruses

MT-4 cells and peripheral blood mononuclear cells (PBMC) were used in this study. MT-4 were cultured in RPMI 1640 containting 10% fetal calf serum (FCS). PBMC were separated from HIV seronegative individuals by the Ficoll-Conray method, and were activated by 0.1 % phytohemagglutinin-P (PHA-P) in RPMI 1640 containing 20 % FCS for 3 days. The virus strains used were LAV and JH/28. LAV was supplied by Dr. LUC MONTAGNIER. JH/28 was isolated from a Japanese AIDS patient in our laboratory.

Cytotoxicity assays

The effect of drugs on MT-4 and PHA-P-stimulated PBMC was determined as follows. MT

Table 11. Effect of various drugs on HIV replication

Sample	100 μg/ml	10 μg/ml	1 μg/ml
(Antiarrhythmic drugs)			
Ajmaline	Tox	$10^{4.7}$*	
(β-receptor blocking agents)			
Oxprenolol hydrochloride	$10^{4.7}$		
Propranolol hydrochloride	Tox	$10^{4.7}$	
Pindolol	$10^{4.7}$		
(Diuretics)			
Spironolactone	$10^{4.7}$		
Trichlormethiazide	$10^{4.7}$		
Furosemide	$10^{4.7}$		
Meticrane	$10^{4.3}$		
(Hypotensive drugs)			
Methyldopa	Tox	Tox	$10^{4.7}$
Rescinnamine	$10^{4.7}$		
(Antidementia drugs)			
Cinnarizine	$10^{4.3}$		
(Antiarteriosclerosis)			
Pyridinol carbamate	$10^{4.7}$		
Nicomol	$10^{4.7}$		
Pentoxifylline	$10^{4.7}$		
Meclofenoxate hydrochloride	$10^{4.3}$		
Pyrithioxin hydrochloride	$10^{4.7}$		
(Drugs for peptic ulcer)			
Sucralfate	$10^{4.3}$		
Sulpiride	$10^{4.7}$		
Aldioxa	$10^{4.7}$		
Gefarnate	$10^{4.7}$		
(Psychoneurotic drugs)			
Levomepromazine maleate	Tox	$10^{4.7}$	
Nitrazepam	$10^{2.3}$		
Diazepam	$10^{4.5}$		
Chlordiazepoxide hydrochloride	$10^{4.3}$		
Medazepam	$10^{4.5}$		
Amitriptyline hydrochloride	Tox	Tox	Tox
Perphenazine	Tox	$10^{3.5}$	
Prochlorperazine maleate	Tox	$10^{2.3}$	
Haloperidol	Tox	$10^{4.5}$	
Calcium hopantenante	$10^{4.0}$		
Lorazepam	$10^{4.0}$		

* $TCID_{50}$/ml

Table 1-2. Effect of various drugs on HIV replication

Sample	100 μg/ml	10 μg/ml	1 μg/ml
(Muscle relaxanate)			
Tolerisone hydrochroride	Tox	Tox	Tox
Amantadine hydrochloride	$10^{1.5}$*		
(Antispaspodics)			
Timepidium bromide	Tox		
N-butylscopolammonium bromide	$10^{4.7}$		
Mepenzolate bromide	$10^{4.5}$		
Piperidolate hydrochloride	$10^{4.7}$		
Valethamate bromide	Tox	Tox	$10^{4.7}$
Methixene hydrochloride	Tox	Tox	$10^{4.7}$
(Antiallergic drugs)			
Clemastine fumarate	Tox	Tox	$10^{4.5}$
Cyproheptadine hydrochloride	Tox	$10^{4.5}$	
(Antitussive drugs)			
Salbutamol hemisulfate	$10^{4.7}$		
Eprazinone dihydrochloride	Tox	Tox	$10^{4.7}$
Methylcysteine hydrochloride	$10^{5.0}$		
Bromhexine hydrochloride	$10^{4.7}$		
(Cardiotonics)			
Ubidecarenone	$10^{5.0}$		
Lanatoside	Tox	Tox	Tox
Caffeine	$10^{4.7}$		
(Multi vitamins)			
Mecobalamin	Tox	$10^{4.5}$	
Riboflavin butyrate	$10^{4.7}$		
Pantethine	Tox	$10^{4.5}$	
(Hepatotherapeutic drugs)			
Glutathione	$10^{4.7}$		
Magnesium and potassium L-aspartate	$10^{4.5}$		
(Others)			
Tiaramide hydrochloride	$10^{4.3}$		
Etilefrine hydrochloride	$10^{4.7}$		
Red ginseng	$10^{4.5}$		
Naproxen	$10^{4.7}$		
Control		$10^{4.7}$	

* $TCID_{50}$/ml

-4 were cultured in the presence of the drugs, and viable cells were counted on days 0, 3, 4, and 5 by the trypan blue exclusion method. PHA-P-stimulated PBMC were cultured in the presence of the drugs for 3 days and (^{3}H) thymidine (67.0 mCi/mM, New England Nuclear Co., Boston, MA) was added during the final 18 h of culture. The cells which were harvested onto glass fiber filter paper, were washed with 10 % trichloroacetic acied (TCA), and (^{3}H) thymidine incorporation was determined by liquid scintillation spectroscopy.

Culture system

The anti-HIV activity of the drugs was evaluated in MT-4 and PBMC. MT-4 were infected with LAV at a multiplicity of infection (m.o.i) of 0.001 or 0.0001 $TCID_{50}$/cell. After the virus was allowed to adsorb for 1 h, cells were incubated for 4 days in the presence of the drugs and the virus yield was determined using MT-4. PBMC were mixed with virus at a m.o.i. of 0.0001 and were incubated for 7 days in the presence of the drugs. One half of the medium was replaced with fresh growth medium with the drugs on day 4. The virus yield was determined using PBMC.

Estimation of the virus infectivity

The infectivity of the culture supernatant was assayed in MT-4 and PBMC. Serially, 10-fold diluted samples were inoculated quadruplicately to wells of a 96-well microplate containing MT-4 cells (2×10^4 cells/0.1 ml/well) or PBMC (4×10^6 cells/0.05 ml/well). In the case of PBMC, fresh cells (4×10^6 cells/0.1 ml/well) were added on day 4. The cytopathic effect on MT-4 and PBMC was examined on days 5 and 7, respectively, after infection, and the $TCID_{50}$ was calculated.

RESULTS

Inhibitory effect of various drugs on HIV replication

First, 58 commercial drugs which allow long-term administration without major side effects were assayed for anti-HIV activity in MT-4 cells. When the virus yield was reduced to ≦1/5 of the control, the drug was judged to have anti-HIV activity. The psychotropic drugs, nitrazepam (100 μg/ml), perphenazine (10 μg/ml), prochlorperazine maleate (10 μg/ml), calcium hopantenate (100 μg/ml), and lorazepam (100 μg/ml), and a muscle relaxant, amantadine hydrochlorid (100 μg/ml), had anti-HIV activity (Table 1). There was no anti-HIV activity in antiarrhythmic drugs, β-receptor blocking agents, diuretics, hypotensive drugs, antidementia drugs, antiarteriosclerosis drugs, drugs for peptic ulcer, antispasmodics, antiallergic drugs, antitussive drugs, cardiotonics, multi-vitamins, hepatotherapeutic drugs and others.

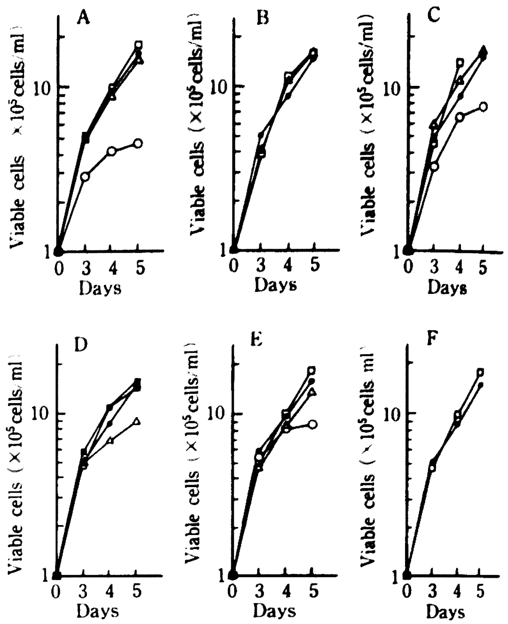

Fig. 1. The growth of MT-4 in the presence of drugs

A. nitrazepam, B. perphenazine, C. amantandine hydrochloride, D. lorazepam, E. calcium hopantenate, F. prochlorperazine maleate

MT-4 were cultured in the presence of drugs, and viable cells were counted on day 0, 3, 4 and 5 by trypan blue dye exclusion method.

Represent -●- control (No addition), -○- 100 μg/ml, -△-10 μg/ml, -□- 1 μg/ml and -■-0.1 μg/ml of drugs respectively.

Relationship between cytotoxicity and anti-HIV activity

We studied whether or not the anti-HIV activity of the drugs at various concentrations was concordant with their cytotoxicity. The anti-HIV

Table 2. Effect of various concentrations of drugs on HIV replication

	m.o.i. 0.001				m.o.i. 0.0001			
	100 μg/ml	10 μg/ml	1 μg/ml	0.1 μg/ml	100 μg/ml	10 μg/ml	1 μg/ml	0.1 μg/ml
Amantadine hydrochloride	$\leq 10^{1.5}$*	$10^{4.0}$	$10^{4.5}$	—	$\leq 10^{1.5}$	$10^{2.6}$	$10^{2.5}$	—
Lorazepam	insoluble	$10^{4.0}$	$10^{4.7}$	$10^{4.4}$	insoluble	$10^{2.0}$	$10^{2.8}$	—
Nitrazepam	$10^{2.3}$	$10^{5.0}$	$10^{4.9}$	—	$\leq 10^{1.5}$	$< 10^{1.6}$	$10^{3.0}$	—
Perphenazine	ND**	$10^{3.5}$	$10^{4.2}$	—	ND	$\leq 10^{1.5}$	$10^{2.9}$	—
Calcium hopantenate	$10^{4.0}$	$10^{4.5}$	$10^{4.7}$	—	$10^{2.0}$	$10^{3.0}$	$10^{3.3}$	—
Prochlorperazine maleate	$\leq 10^{1.5}$	$10^{2.5}$	$10^{4.7}$	—	$\leq 10^{1.5}$	$\leq 10^{1.5}$	$10^{2.4}$	—
Control	$10^{4.7}$				$10^{2.9}$			

ND : no data
* TCID50/ml
** Cells died completely
m.o.i. : multiplicity of infection

Table 3. Effect of drugs on DNA synthesis of PBMC

Concentration (μg/ml)	Uptake of (^{3}H) thymidine (cpm)	
	perphenazine	nitrazepam
100	596±6	12,962±1,610
80	548±47	14,687±4,172
50	26,888±2,209	20,629±4,192
0	22,190±383	
Background	292±10	

Table 4. Effect of drugs on JH/28

Concentration (μg/ml)	Perphenazine	Nitrazepam
50	$10^{5.0}$*	$10^{5.5}$
30	$10^{5.5}$	$10^{5.5}$
20	$10^{5.5}$	$10^{5.5}$
10	$10^{5.5}$	$10^{5.5}$
0	$10^{5.5}$	

* TCID50/ml

activities of prochlorperazine maleate, calcium hopantanate, lorazepam and amantadine hydrochloride were observed together with their cytotoxicity. In two cases, 10 μg/ml of nitrazepam at an m.o.i. of 0.0001 and 10 μg/ml of perphenazine at an m.o.i. of 0.001 or 0.0001, there was anti-

HIV activity without cytotoxicity (Fig. 1 and Table 2).

Anti-HIV activity of nitrazepam and perphenazine in PBMC

Nitrazepam and perphenazine, which exhibited anti-HIV activity in MT-4 without cytotoxicity at certain concentrations, were also tested for anti-HIV activity in PBMC. Their cytotoxicity was tested for the inhibition of (^{3}H) thymidine incorporation into a cold 10% TCA-insoluble fraction of the cells. At 50 μg/ml, perphenazine exhibited weak anti-HIV activity without cytotoxicity, while 50 μg/ml of nitrazepam showed no anti-HIV activity (Tables 3 and 4).

DISCUSSION

Recently, various compounds have been reported to have an inhibitory effect on HIV replication *in vitro*[6,7]. But new compounds take a long time to be registered with health authorities for administration to humans, whereas if any existing commercially available drugs have anti-HIV activity, they could readily be applied in clinical trials for AIDS. Accordingly, we screened for anti-HIV activity 58 commercial drugs available for long-term administration without major side effects. Lorazepam, calcium hopantenate, prochlorperazine maleate, amantadine hydrochloride, perphenazine and nitrazepam possessed anti-HIV activity in MT-4 cells. But only perphenazine and nitrazepam exhibited anti-HIV activity without cytotoxicity. Their anti-HIV activity was retested in PBMC, which seemed to mimic *in vitro* situations better than the MT-4 assay system. In this system, nitrazepam showed no anti-HIV activity, and that of perphenazine was very weak. MT-4 cells were established from PBMC of adult T cell leukemia patients[8]. The cause of the difference between anti-HIV activity in MT-4 and PBMC was not clear, but we offer the following explanation. The benzodiazepines, nitrazepam and perphenazine, as well as their metabolites, have a hypnotic effect. One possibility is that their major anti-HIV activity is based on their metabolites, and the difference in anti-HIV activity between MT-4 and PBMC may depend on the amount of these metabolites. Since the growth of MT-4 is more rapid than that of PBMC (unpublished data), the amount of metabolites in MT-4 may be much greater. By analogy, the cellular metabolites of AZT have anti-HIV activity[9]. Since there is no animal model of AIDS except HIV infection in chimpanzees[10], compounds with anti-HIV activity *in vitro* could be used in clinical trials for AIDS without animal experiments if the compounds are not toxic for humans. In our results, anti-HIV activity was different in MT-4 and PBMC, showing the selection of *in vitro* screening systems is very important. When we screened 58 commercial drugs, we discovered anti-HIV compounds among them. If screening for anti-HIV activity in registered drugs available for long-term administration is continued, it should be possible to discover more efficient anti-HIV compounds and to gather information for new drug design by studying their activity-structure relationship.

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長期服用しうる医薬品の抗エイズ活性

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エイズ患者に対する抗エイズ薬の投与は長期にわたるため，当然のことながら抗エイズ効果が強いことと ともに副作用の少ない薬剤が望まれる。そこで今回我々は，すでに国内で医薬品として認められている薬剤の中から，比較的毒性が低く長期間投与しうる薬剤58コを選び，抗エイズ効果の有無をスクリーニングした。その結果，ロラゼパム，ホパンテン酸カルシウム，マレイン酸プロクロルペラジン，塩酸アマンタジン，ペルフェナジン，ニトラゼパムなどの薬剤に活性が認められた。しかし，ペルフェナジンとニトラゼパムを除いては細胞毒性が見られた。また，末梢血を用いて同様にスクリーニングをしたところ，ペルフェナジンのみに活性が認められた。

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