INHIBITORY ACTIVITY OF ABOB ON THE GROWTH
OF INFLUENZA VIRUS A (Hong Kong)

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Many papers report^{1) - 12)} the inhibitory effect of N¹, N¹-anhydrobis-(β -hydroxyethyl) biguanide hydrochloride (ABOB) on influenza virus multiplication and its prophylactic and therapeutic evaluation in clinical trials.

Inhibitory activity of ABOB on new type of influenza A virus (Aichi/2/68 strain), was tested in this study. This type of virus came into epidemic in Hong Kong in summer 1968. The antigenic properties of this virus were a little different from type A2 virus which is a previous epidemic strain.

MATERIALS AND METHODS

Virus: Influenza A virus (Aichi/2/68 strain)*, at its 8th egg passage, was used in this study. The strain was propagated in chorioallantoic cavity of 11 days-old egg.

Drug: N¹-N¹-anhydrobis-(β -hydroxyethyl) biguanide-HCl (ABOB)** was freely soluble in water and various concentrations of it in sterile physiologic saline were used throughout these experiments. As a rule, fresh solutions were prepared immediately before each administration.

Tissue culture: Primary monkey kidney cells, primary chick embryonal kidney cells and primary human embryonal lung cells were employed throughout this study.

Method of culture and virus inoculation were previously described.

Infectivity titration: Specimens were made in serial tenfold dilution and 0.1 ml of each dilution was inoculated into cell culture tube or chorioallantoic cavity of 11 days-old egg. Five culture tubes were used for each dilution.

Primary monkey kidney cell culture tube was considered to be infected when at least showed viral haemagglutinin in culture fluid 7 days after inocu-

* The strain was supplied by courtesy of Dr. M. Kaji, Kyushu University, Fukuoka.

** ABOB was kindly supplied from the Sumitomo Chemical CO. Osaka.

lation. Primary chick embryonal kidney cell culture tube was considered to be infected when at least 20 per cent of the cells showed viral cytopathic effect 5 days after inoculation. Primary human embryonal lung cells culture tube was considered to be infected when at least showed haemadsorption phenomenon 5 days after inoculation. The 50 per cent endpoint was calculated by the Reed and Muench's Method¹³⁾.

Test for antiviral activity in ovo: Eleven-day old embryonated eggs were treated with 0.1 ml of ABOB solution at a desired concentration via the allantoic route immediately following the inoculation of 0.1 ml of tenfold diluted solution (10^3 , 10^4 , 10^5 , 10^6 and 10^7) of the Hong Kong strain of influenza type A virus. The eggs were incubated at 34 °C for 48 hours.

Test for antiviral activity in tissue culture: Tissue culture tubes were treated with 0.1 ml of ABOB solution at a desired concentration immediately following the inoculation of 0.1 ml of tenfold diluted solution of the Hong Kong strain of influenza type A virus. The tissue culture tubes were incubated at 35 °C for 5 or 7 days.

EXPERIMENTAL RESULTS

Activity on Aichi/2/68 virus in primary cultured cells of monkey kidney (Cynomolgus irus);

The results are summarized in Table 1. At the inoculum below 30 TCD₅₀, 0.1 mg/ml of ABOB completely inhibited the infection to the cells. When the cells were challenged with 100-300 TCD₅₀ of virus, ABOB could not completely inhibit the infection but could reduce the growth of virus.

TABLE 1
*Activity of ABOB on the growth of virus
in primary monkey kidney cells*

Experiment	ABOB treated (0.1 mg/ml)	Control	Log. difference
1	4.3 *	5.7	-1.4
2	4.3	5.9	-1.6
3	4.7	5.9	-1.2

* Virus titer $\log_{10}$ TCD₅₀/0.1 ml
Virus and ABOB were added simultaneously

Activity in chorioallantoic cavity of 11 days-old egg;

The results are summarized in Table 2. Although ABOB (50 mg/egg) reduced a little the growth of the virus in the cavity from $10^{6.5}$ to $10^{5.9}$ /0.1 ml. The compound failed to show significant effects even at the dose of 50 mg/egg.

TABLE 2
*Activity of ABOB on the growth of virus in
chorioallantoic cavity of 11 days-old egg*

ABOB Dose (mg/egg)	ABOB treated	Control	Log. difference
50	5.9 *	6.5	-0.6
10	6.1	6.5	-0.4
?	6.3	6.5	-0.2

* Virus titer $\log_{10} EID_{50}/0.1\text{ ml}$
Virus and ABOB were added simultaneously

TABLE 3
*Activity of ABOB on the growth of the virus
in primary chick embryonal kidney cells*

ABOB Dose (mg/ml)	ABOB treated	Control	Log. difference
10	toxic	—	—
1	toxic	—	—
0.1	1.9*	5.7	-3.8
0.01	2.3	5.7	-3.4
0.001	4.5	5.7	-1.2

* Virus titer $\log_{10} TCD_{50}/0.1\text{ ml}$
Virus and ABOB were added simultaneously

TABLE 4
*Activity of ABOB on the growth of the virus
in primary human embryonal lung cells*

ABOB dose (mg/ml)	ABOB treated	Control	Log. difference
10	toxic	—	—
1	3.7*	7.2	-3.5
0.1	4.5	7.2	-2.7
0.01	6.7	7.2	-0.5
0.001	7.1	7.2	-0.1

* Virus titer $\log_{10} TCD_{50}/0.1\text{ ml}$
Virus and ABOB were added simultaneously

*Activity in primary cultured cells of chick embryonal kidney
from 18 days-old egg ;*

The results are summarized in Table 3. By 0.1mg/ml of ABOB virus titer was reduced at the difference of $10^{3.8}$ from control and even at 0.01mg/ml titer was reduced at $10^{3.4}$.

Activity in primary cultured cells of human embryonal lung;

The results are summarized in Table 4. The virus titer was reduced $10^{2.7}$ and $10^{3.5}$ compared with control respectively treated with 0.1 and 1 mg/ml of the drug.

In these data ABOB inhibited much effectively the growth of new type of influenza in primary cultured-monkey kidney, -embryonal chick kidney and -human embryonal lung cells but slightly in chorioallantoic cavity of 11 days-old egg. As above mentioned, ABOB was shown to have the inhibitory effect on the growth of this new type of influenza virus. It can be concluded that ABOB will be useful on chemoprophylaxis or chemotherapy of influenza Hong Kong virus that the pandemy was anticipated this winter.

TABLE 5
Summary of the inhibitory activity of ABOB in various host cells

ABOB dose (mg/ml)	logarismic difference of virus titer			
	Monkey kidney	Chorioallantoic cavity	Chick kidney	Human lung
1		-0.6		-3.5
0.2		-0.4		
0.1	-1.4		-3.8	-2.7
0.04		-0.2		
0.01			-3.4	-0.5
0.001			-1.2	-0.1

DISCUSSION

The study was carried out to know whether ABOB can inhibit the Hong Kong strain of influenza A virus multiplication or not.

Those culture cells were treated with various concentrations of ABOB which were added to culture media and its viability was examined for 12 days after the treatment primary monkey cell could be used under the concentration of 0.1 mg/ml and primary embryonal chick kidney cell could be used under the concentration of 0.1 mg/ml and primary human embryonal lung cells could be used under the concentration of 1 mg/ml of ABOB for this experiment.

Tissue culture (primary monkey kidney cell, primary embryonal chick kidney cell and primary human embryonal lung cells) could be used under the concentration of 1 mg/ml or less of ABOB for this experiments.

The results obtained suggest that influenza virus multiplication in tissue culture cell was inhibited by the treatment of ABOB with the concentration of 1 or 0.1 mg/ml to the culture media, but slightly in chorioallantoic cavity of 11 days-old egg.

As is known, the tissue culture consists of only one layers of cells, whereas the whole egg consists of a complete set of cells of an embryo.

SUMMARY

This paper reports the effect of ABOB against Hong Kong strain of influenza A virus multiplication in primary monkey kidney cell, primary embryonal chick kidney cell and primary human embryonal lung cells.

The results obtained suggest that Hong Kong strain of influenza A virus multiplication in tissue culture cells was inhibited by the treatment of ABOB with the concentration of 0.1 mg/ml to the culture media, although no direct virucidal effects were demonstrated in vitro.

In general, the earlier beginning of ABOB treatment tended to bring the stronger inhibition of Hong Kong strain of influenza A virus multiplication.

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