EFFECT OF N\textsuperscript{1}-ISOBUTYLBIGUANIDE HYDROCHLORIDE AGAINST ADENOVIRUS IN HELA CELL CULTURE

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It has been reported that N\textsuperscript{1}-isobutylbiguanide hydrochloride and N\textsuperscript{1}-benzylbiguanide hydrochloride were more effective than N\textsuperscript{1},N\textsuperscript{1}-anhydrobis (β-hydroxyethyl) biguanide hydrochloride (ABOB) against certain myxoviruses among the biguanide derivatives studied on the antiviral activity\textsuperscript{1}).

Since the inhibitory activity of ABOB against adenovirus in HeLa cell culture and its therapeutic effect in the treatment of pharyngoconjunctival fever due to adenovirus type 3 were reported\textsuperscript{2,3)}, N\textsuperscript{1}-isobutylbiguanide hydrochloride was tested for its activity for adenovirus in series of chemotherapeutic studies on adenovirus infection.

The present paper reports the results of preliminary study on the effect of the compound against adenovirus in HeLa cell culture.

Materials and Methods

Virus Adenovirus type 3, Koito strain**, and type 8, Kanhisa strain\textsuperscript{4}) propagated in HeLa cell culture were used.

Tissue Culture HeLa cells were employed throughout the experiment. Method of culture and virus inoculation were described previously.

N\textsuperscript{1}-isobutylbiguanide hydrochloride\textsuperscript{5} The compound was dissolved in distilled water to give the concentration of 0.5% and autoclaved at 1 lb for 20 minutes. Prior to the experiment the solution was diluted to the appropriate concentrations with culture media.

Infectivity Titration, Complement Fixation and Hemagglutination Tests were carried out by the method described elsewhere\textsuperscript{3}).

Eosin Exclusion Test To examine the percentage of living HeLa cells in culture tubes with various concentrations of N\textsuperscript{1}-isobutylbiguanide hydrochloride was done by the method described elsewhere\textsuperscript{3}).

Results

Viability of HeLa cell treated N\textsuperscript{1}-isobutylbiguanide hydrochloride

Viability of HeLa cells treated with various concentrations of compound for 7 days was examined by eosin exclusion test (Fig. 1). At all concentrations tested, no significant differences of viability were observed until 72 hours after treatment. A marked cell damage was seen in the concentrations of 100 mcg/ml 120 hours after treatment. The percentages of living cells treated with 50 mcg/ml or less of concentrations of the compound were quite similar to that of the control cells for 120 hours after treatment. Accordingly, the concentrations of 50 mcg/ml or less were used throughout the following experiments.

Effect of N\textsuperscript{1}-isobutylbiguanide hydrochloride on adenovirus in HeLa cells

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Viability of HeLa cells treated with N\textsuperscript{1}-isobutylbiguanide hydrochloride}
\end{figure}
Fig. 4. Correlation of onset of treatment with N-salicyloylglycine hydrochloride (N-salicyloylglycine hydrochloride) and the time of treatment (56 days after inoculation of the same time of treatment).

Fig. 5. Correlation of onset of treatment with N-salicyloylglycine hydrochloride and the time of treatment (56 days after inoculation of the same time of treatment).
HeLa cells were infected with \(10^{8.6}\) TCID\(_{50}\)/ml of type 3 adenovirus and the treatment with N\(^1\)-isobutylbiguanide hydrochloride was started 24 hours before virus inoculation and continued throughout the experiment. As shown in Figure 2, inhibition of cytopathic effect (CPE), infectivity and complement fixing (CF) antigen production were noted under the presence of N\(^1\)-isobutylbiguanide hydrochloride in culture media. The inhibition was more marked in the concentration of 50 mcg/ml than 25 mcg/ml.

The results in type 8 infection were quite similar to those of type 3 infection (Fig. 3). In addition to lesser CPE, lower infectivity and CF antigen titer, production of hemagglutinin was inhibited when treated with the compound.

To examine the correlation of initiation of the treatment to inhibition of virus production, the treatment of N\(^1\)-isobutylbiguanide hydrochloride was started 24 hours before, at the same time and 24 hours after virus inoculation.

As seen in Figs. 4 and 5, the degree of inhibition of CPE, infectivity and CF antigen production was most apparent when the treatment was started 24 hours before virus inoculation. In general, the earlier beginning of N\(^1\)-isobutylbiguanide hydrochloride treatment tended to bring the stronger inhibition at the concentrations of 50 mcg/ml or 25 mcg/ml.

**Discussion and Summary**

N\(^1\)-isobutylbiguanide hydrochloride was tested for anti-adenovirus activity in HeLa cell culture. N\(^1\)-isobutylbiguanide hydrochloride was more toxic for HeLa cells than ABOB and the concentrations of 50 mcg/ml or less were used in the experiment, whereas ABOB was tested at the concentrations of 1.0 mg/ml or less. The inhibition of adenovirus multiplication by 50 or 25 mcg/ml of the compound was nearly similar to that by 1.0 or 0.5 mg/ml of ABOB.

The beginning of treatment of HeLa cells with N\(^1\)-isobutylbiguanide hydrochloride was earlier, inhibitory effect for adenovirus was stronger under the condition tested.

Further study on the mechanism of the inhibitory effect will be continued.

**References.**


2) **KAJI, M., KAMIYA, S., TATEWAKI, E., NAGAFUCHI, J. and FUJIWARA, N.** Effect of N\(^1\),N\(^1\)-anhydrobis(8-hydroxyethyl) biguanide hydrochloride (ABOB) against adenovirus. Chemotherapy 14, 66, 1966.

