Antiviral Research, 15 (1991) 149–160

ADONIS 016635429100028F

AVR 00467

Influence of trifluoperazine on the late stage of influenza virus infection in MDCK cells

Hiroshi Ochiai, Masahiko Kurokawa and Seihachiro Niwayama

Department of Virology, Toyama Medical and Pharmaceutical University, Toyama, Japan

(Received 16 August 1990; accepted 29 October 1990)

Summary

We investigated the influence of the anticalmodulin drug, trifluoperazine (TFP) on influenza virus growth in MDCK cells. The inhibitory effect of TFP on virus growth was observed even when TFP was added at a late stage of infection. This inhibitory effect was concentration-dependent in the concentration range of 20–35 μM. At 35 μM, TFP caused a complete alteration in the distribution pattern of hemagglutinin (HA), concomitant with a decrease in the appearance of HA on the cell surface. After removal of the drug, the HA gradually began to show a normal distribution pattern and reappeared on the cell surface. The time course of rearrangement of HA was in accord with that of the recovery of cell supernatant infectivity. Scanning electron microscopic study revealed that the drug did not cause accumulation of the progeny viruses on the cell surface. The drug effect on the virus growth was reversed by the simultaneous presence of purified calmodulin (CaM). These data suggest that TFP acts as a reversible inhibitor of influenza virus morphogenesis, but not budding, by disturbing cellular CaM and/or CaM-dependent functions.

Trifluoperazine; Calmodulin; Influenza virus; Morphogenesis

Introduction

Influenza virus is composed of at least seven structural proteins: RNA polymerases PA, PB1 and PB2; hemagglutinin (HA) and neuraminidase (NA), which

Correspondence to: Hiroshi Ochiai, Department of Virology, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan.

0166-3542/91/$03.50

are both inserted in the envelope: nucleoprotein (NP) and matrix (M) protein (Stuart-Harris et al., 1976). Influenza virions attach to their receptor(s) on the cell surface, internalize and proceed through the different stages of the virus replicative cycle, until progeny viruses are eventually released by budding (Stuart-Harris et al., 1976). Despite the extensive knowledge of the composition of the virus, the individual processes in the late stage of influenza virus infection such as viral morphogenesis and budding are not completely understood.

Since the discovery of calmodulin (CaM) as an activator of cyclic nucleotide phosphodiesterase in mammalian brain (Cheung, 1970; Kakiuchi et al., 1970), it has been demonstrated that this protein participates in various cellular processes as a modulator of many Ca^{2+}-dependent enzymes (Klee et al., 1980; Means et al., 1980). Several studies have also shown that the events on the cell membrane such as endocytosis, exocytosis, capping of ligand-receptor complex and maintenance of cell shape can be influenced by phenothiazines (Elferink, 1979; Horwitz et al., 1981; Merrit et al., 1981; Osborn et al., 1980; Salisbury et al., 1980). On the other hand, phenothiazine derivatives such as trifluoperazine (TFP) and chlorpromazine (CPZ) have also been recognized as CaM antagonists, to which they specifically bind in the presence of Ca^{2+} (Hidaka et al., 1979; Honda et al., 1968; Levin et al., 1976). These data suggest that CaM is involved in specific events on the cell membrane. Several authors pointed out that these events on the cell membrane may play an important role in virus-cell interactions, especially virus penetration, maturation and budding (Alexander, 1979; Compans, 1984; Knipe, 1990). It has been reported that CPZ inhibits influenza virus penetration into chick embryo cells without affecting virus adsorption (Křižanová et al., 1982), and phenothiazines inhibit measles virus budding from HeLa cells (Bohn et al., 1983).

We wanted to determine the experimental conditions to dissect the sequential events in the late stage of influenza virus infection. In the present study, we investigated the influence of TFP on the growth of influenza A NWS (H1N1 subtype) virus in Madin-Darby canine kidney (MDCK) cells.

Materials and Methods

Cells and virus

MDCK cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100 μg/ml). The cells were maintained in a humidified atmosphere containing 5% CO_2 at 34°C. Influenza A NWS (H1N1) virus was used throughout the experiments. In order to prepare virus stock solution, the virus was propagated in the allantoic cavity of 10-day-old embryonated chicken eggs for 48 to 72 h at 35°C. The allantoic fluids were collected and then stored in small portions at −80°C after clarification at $1000 \times g$ for 20 min. The virus titer of allantoic fluids was 1.5×10^8 plaque forming units (PFU)/ml.

Effects of TFP on the virus growth

Monolayer cultures of MDCK cells grown in 35-mm plastic dishes were washed twice with phosphate-buffered saline (PBS) and then inoculated at a multiplicity of infection (moi) of 0.1 PFU/cell in 0.2 ml of PBS containing 1% bovine serum albumin per culture. After a 45-min adsorption period at room temperature, the cultures were washed four times with PBS and then incubated in 2 ml of MEM supplemented with 2% FBS (maintenance medium) at 37°C (0 h post infection). After incubation for appropriate periods at 37°C, the drug was added to the cell culture medium at a final concentration of 10 to 35 μM. As a control, the infected cells were processed in the absence of the drug. In some experiments, a mixture of 35 μM TFP and purified CaM at various concentrations was used instead of TFP alone. At 15 h post infection (p.i.), half of the cell culture fluid (1 ml) was collected and centrifuged at 500 × *g* for 15 min to determine the supernatant virus yield. To determine total virus yield, the remaining cells and cell culture fluid were treated with ultrasonication for 10 s at middle range (Micro-Ultrasonic Cell Disrupter: Kontes) on melting ice, followed by the centrifugation as described above. The virus yields in both samples were assayed by plaque titration on MDCK cells in the presence of a low concentration of trypsin (Tobita et al., 1975). Finally, cell-associated virus yield was calculated by subtracting the supernatant virus yield from the total virus yield in the culture. To examine reversible drug effect on the influenza virus growth, the cells were infected at an moi of 5 PFU/cell and treated with 35 μM TFP for different time periods, as shown in Fig. 3. After the replacement of the medium at 4.5 h p.i., supernatant virus yields were assayed (in duplicate) at 30 min intervals.

Immunofluorescence and scanning electron microscopy

MDCK cells grown on coverslips were infected at an moi of 5 PFU/cell, as described above. After 1 h incubation at 37°C, the coverslips were transferred to medium containing 35 μM TFP and further cultured for 3.5 h. For the cytoplasmic immunofluorescence, the cell monolayers were washed with cold PBS and fixed in acetone-methanol (1:1 v/v) for 30 min at −20°C. For the cell surface immunofluorescence, the cell monolayers were fixed with 1% paraformaldehyde in 0.072 M cacodylate buffer (pH 7.5) and 0.72% sucrose for 30 min at 4°C (Pattaik et al., 1986). Indirect immunofluorescence staining was carried out by using anti-HA monoclonal antibody B-4 (Ochiai et al., 1988) and FITC-labeled rabbit anti-mouse IgG antiserum (Cappel, Cochranville, PA, USA), as described elsewhere (Pattaik et al., 1986). The immunofluorescence was examined with a transmission fluorescence microscope (Olympus BH-2, Tokyo, Japan).

For scanning electron microscopy (SEM), cultures were washed twice with PBS at 7.5 h p.i., fixed with 1.5% glutaraldehyde in PBS for 60 min at 4°C, and then fixed with 1% OsO_4 in PBS for 90 min at 4°C. After thorough washing with PBS, the cultures were dehydrated in ethanol, treated with isoamyl acetate and dried with JCD-5 (JEOL, Tokyo, Japan). The cultures were mounted on stubs, gold sput-

tered at 3 cm with 1400 V and 6 mA for 5 min, and examined in a Hitachi X-650 scanning electron microscope.

Drugs and chemicals

TFP dihydrochloride and CaM purified from bovine brain were purchased from Sigma (St. Louis, MO, U.S.A.). The drug solutions were freshly prepared from a stock solution of 10 mM dissolved in dimethylsulfoxide and protected against light throughout the experiments.

Results

Effect of TFP on influenza virus growth in MDCK cells

To obtain some indication of a drug-sensitive stage during infection, we examined the time-related drug effect on both supernatant and cell-associated virus yields by treatment of infected cells with 35 μM TFP for different times. To compare cell-associated virus yield with supernatant virus yield, each sample had to be collected before the complete cytopathology appeared. As a result, the samples were collected at 15 h p.i. and, therefore, virus yields in the drug-untreated cells were relatively low: 1.1×10^6 and 9.1×10^5 for the supernatant and cell-associated virus yields, respectively (Table 1). As shown in Table 1, as 35 μM TFP was added earlier, the reduction in both supernatant and cell-associated virus yields at 15 h p.i. increased. However, even when the drug was added as late as 7.5, 10 or 12.5 h p.i., both supernatant and cell-associated virus yields at 15 h p.i. were decreased as compared to those of drug-untreated cells. This inhibitory effect was observed at both 30 and 25 μM TFP, but not at 10 μM TFP. These data suggest that the drug acts on some events in the late stage of influenza virus infection, when the nascent viruses are formed and released by budding. It is noteworthy that the drug affects both supernatant and cell-associated virus yields; i.e., the reduction of supernatant virus yield is not accompanied by compensatory increase in cell-associated virus yield. Moreover, when 35 μM TFP was added at 7.5 h p.i., the supernatant virus yield increased whereas cell-associated virus yield (at 15 h p.i.) decreased, as compared to the supernatant and cell-associated virus yields for untreated cell cultures. When the drug was added at 10 and 12.5 h p.i., a similar phenomenon was observed. These findings suggest that the drug does not affect budding per se, but a certain event before budding.

Scanning electron microscopy (SEM) of infected cells following treatment with TFP

We examined by SEM infected cells that had been cultured either in the presence or absence of 35 μM TFP from 1 h to 7.5 h p.i. As shown in Fig. 1b (arrow), numerous budding virus particles (about 120 nm in diameter) were observed on the

TABLE 1

Effect of TFP on influenza virus replication in MDCK cells

Onset of drug treatment (p.i.)	Virus yield (PFU/ml) at 15 h p.i. at concentration of TFP (μM)				
	35	30	20	10	0
0 h ↓	$\frac{2.7\times10^{3a}}{2.0\times10^{3b}}$	n.t.[c]	n.t.	n.t.	$\frac{1.1\times10^{6}}{9.1\times10^{5}}$
1 h ↓	$\frac{6.7\times10^{3}}{4.3\times10^{3}}$	n.t.	n.t.	n.t.	
$\frac{2.5\times10^{1}}{6.5\times10^{2}}$ 5 h ↓	$\frac{6.8\times10^{3}}{4.5\times10^{3}}$	$\frac{2.1\times10^{4}}{1.6\times10^{4}}$	$\frac{1.8\times10^{5}}{1.1\times10^{5}}$	$\frac{9.1\times10^{5}}{6.1\times10^{5}}$	
$\frac{2.3\times10^{4}}{5.6\times10^{4}}$ 7.5 h ↓	$\frac{5.0\times10^{4}}{1.9\times10^{4}}$	$\frac{8.8\times10^{4}}{4.5\times10^{4}}$	$\frac{2.9\times10^{5}}{2.0\times10^{5}}$	$\frac{1.1\times10^{6}}{8.2\times10^{5}}$	
$\frac{1.2\times10^{5}}{7.5\times10^{4}}$ 10 h ↓	$\frac{5.1\times10^{5}}{3.5\times10^{4}}$	n.t.	n.t.	n.t.	
$\frac{5.4\times10^{5}}{4.8\times10^{5}}$ 12.5 h ↓	$\frac{6.7\times10^{5}}{1.1\times10^{5}}$	n.t.	n.t.	n.t.	

p.i. = post infection; [a]supernatant virus yield; [b]cell-associated virus yield; [c]not tested. Virus growth in the drug-free culture is expressed in bold figures.

surface of the drug-untreated cells, whereas few budding virus particles were detected on the surface of drug-treated cells (Fig. 1c, arrow). These findings suggest that TFP may influence a certain event before budding rather than budding itself or infectivity of progeny viruses. This study also revealed that 35 μM TFP suppressed the appearance of microvilli on the surface of MDCK cells (see arrow heads in Fig. 1a and b). We then examined the effect of TFP on the viability of the uninfected cells by the dye exclusion test. When subconfluent cultures were treated with TFP at various concentrations for 48 h at 37°C, cell viability was not affected by TFP up to 35 μM. Cell viability was 68.0, 98.0, 98.2 and 98.1% for 40, 35, 30, and 0 μM TFP, respectively. These data suggest that the presence of 35 μM TFP suppresses the appearance of microvilli without affecting cell viability.

Reversible effects of TFP on the distribution pattern of HA and virus growth

We examined the distribution pattern of HA by the immunofluorescence method, as described in the Materials and Methods. As shown in Fig. 2B, the cyto-

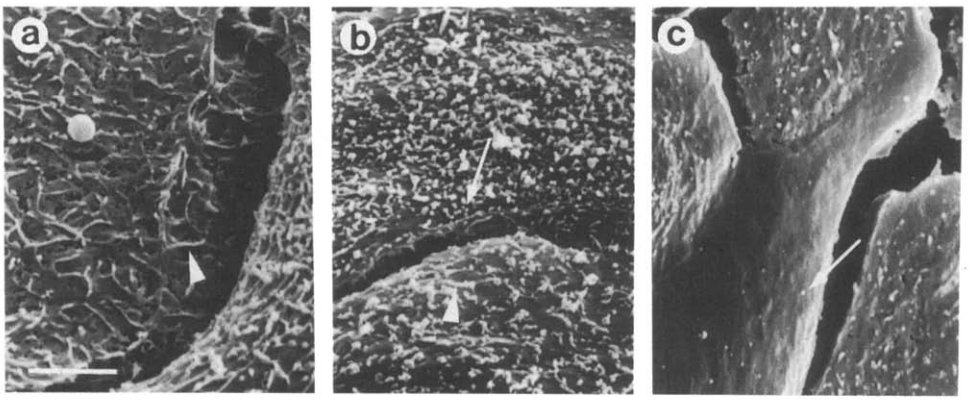

Fig. 1. Scanning electron micrograph (SEM) of the infected cells cultured either in the presence or the absence of 35 μM TFP. MDCK cells grown on the cover slips were infected at an moi of 5 PFU/cell and cultured in drug-free medium at 37°C. At 1 h p.i., the cells were transferred to medium with (c) or without (a and b) 35 μM TFP and cultured for up to 7.5 h p.i. Thereafter, the cells were processed for SEM as described in Materials and Methods. Fig. 1a indicates the uninfected control cells. Arrows and arrow heads indicate virus particles and microvilli on the cell surface, respectively. Magnification of each photograph is 3800 ×. Bar in Fig. 1a indicates 3 μm.

plasmic immunofluorescence method revealed for untreated infected cells a finely dotted distribution of HA. In the presence of 35 μM TFP, the HA distribution pattern was completely altered; that is, aggregated spots of fluorescence were observed (Fig. 2C). On the other hand, the cell surface immunofluorescence method revealed for untreated cells a linear pattern of fluorescence along the cell surface (Fig. 2G), whereas the appearance of fluorescence was markedly reduced on the drug-treated cell surface (Fig. 2H).

When the medium was replaced by drug-free medium, the aggregated spots of HA became smaller within 30 min (Fig. 2D), and the distribution pattern became similar to that of the untreated cells within 60 min (Fig. 2E). Concomitantly with the rearrangement of the cytoplasmic HA after the removal of the drug, HA gradually reappeared on the cell surface within 30 min (Fig. 2I) and finally assumed the same linear pattern of fluorescence within 60 min (Fig. 2J). These findings suggest that the presence of 35 μM TFP influenced the distribution pattern of HA in a reversible manner.

As shown in Fig. 3A, we also examined the influence of drug removal on the supernatant infectivity. Recovery of virus yield began at 60 min after removal of TFP (Fig. 3B, c) and the rate at which virus yield increased within the 90 to 150-min period after removal of TFP was similar to that of the untreated cells (compare a and c in Fig. 3B). These data suggest that the time course of rearrangement of HA after removal of the drug is correlated with the recovery of the supernatant infectivity. On the contrary, following replacement of drug-free medium by drug-containing medium supernatant, infectivity levelled off within 90 min (Fig. 3B, b).

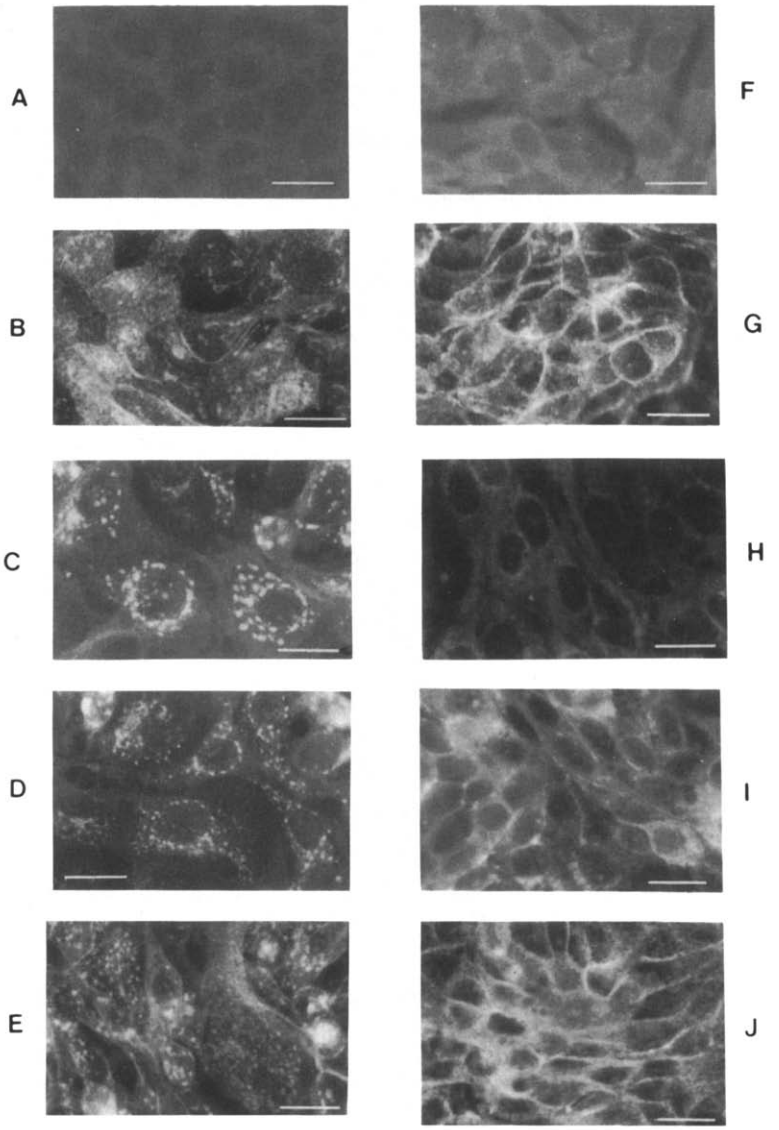

Fig. 2. Drug effect on the distribution pattern of HA. MDCK cells grown on the cover slips were infected and cultured either in the presence (C and H) or absence (B and G) of 35 μM TFP in the same way as described in the legend to Fig. 1. At 4.5 h p.i., the cells were fixed and stained with anti-HA monoclonal antibody and FITC-labeled anti-mouse IgG. In some experiments, before being fixed, the cells were transferred from drug-containing medium to drug-free medium and further cultured for 30 min (D and I) or 60 min (E and J). The photographs on the left side (A–E) and the right side (F–J) indicate the cells fixed for cytoplasmic and cell surface fluorescence, respectively. A and F indicate the uninfected cells cultured in the absence of drug. Magnification of each photograph is 400 ×. Bars indicate 25 μm.

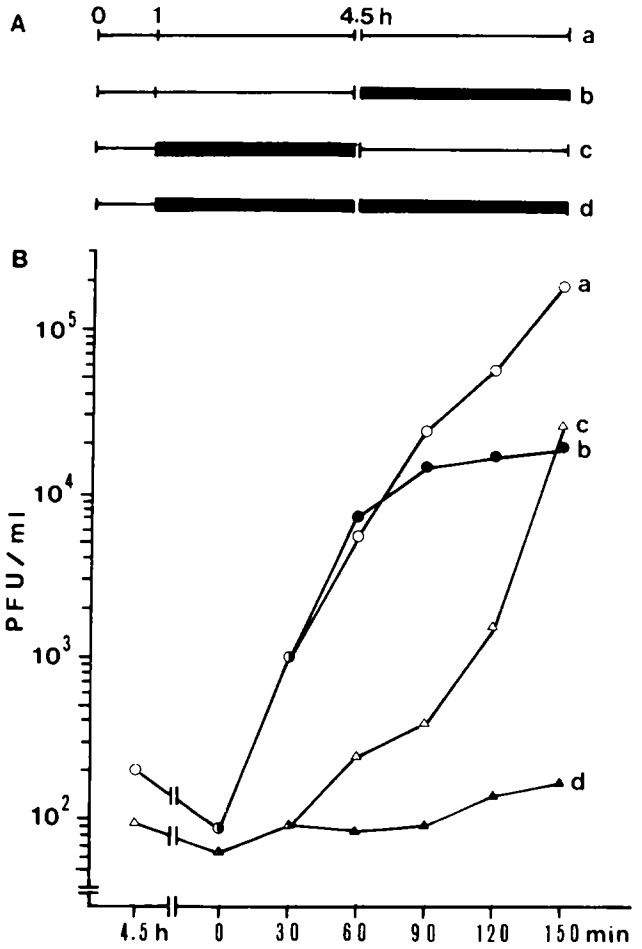

Fig. 3. Reversible drug effect on influenza virus replication. MDCK cells were infected at an moi of 5 PFU/cell and treated with 35 μM TFP for different time periods (A, a–d: thick lines indicate the presence of the drug). The letters a–d represent the different experimental systems. After the removal of the medium, followed by washing with PBS at 4.5 h p.i., medium with or without the drug was added. After this treatment, the supernatant virus yields were assayed at 30 min intervals.

Influence of purified CaM on the inhibitory effect of the drug on the influenza virus growth

It was further examined whether the effect of TFP could be reversed by the simultaneous presence of purified CaM. The cells were infected at an moi of 0.1 PFU/cell and cultured in drug-free medium at 37°C. At 5 h p.i., 35 μM TFP, 5 μM purified CaM, or a mixture of 35 μM TFP and CaM at various concentrations was added to the medium, and the cells were cultured until 15 h p.i. As shown in Table 2, virus yields increased to attain 47.2% and 12.8% of the control values, when, in

TABLE 2

Reversal of TFP-induced inhibition of virus growth in the presence of purified CaM

CaM (μM)	35 μM TFP	PFU/ml	(%)
–	–	1.8×10^7	100.0
5.0	–	1.9×10^7	105.6
5.0	+	8.5×10^6	47.2
2.0	+	2.3×10^6	12.8
0.5	+	2.7×10^4	0.15
–	+	9.0×10^3	0.05

Infected MDCK cells were cultured in drug-free medium for 5 h and then cultured in the presence of drug or a mixture of drug and CaM. At 15 h p.i., virus yields in the supernatants were assayed.

addition to TFP, CaM was present at 5 or 2 μM, respectively, whereas the effect of 0.5 μM CaM was negligible. The presence of 5 μM CaM alone did not have a significant effect on virus yields. These data indicate that the inhibitory effect of TFP on virus replication can be reversed by the simultaneous presence of purified CaM.

Discussion

The effect of phenothiazine on the early stage of influenza virus infection has been reported by Křižanová et al. (1982). At 20 to 50 μM, CPZ inhibited virus penetration into chick embryo cells, without affecting virus adsorption, when drug was added during the virus adsorption period. In our study, 35 μM TFP had the highest inhibitory effect on the virus growth when it was added immediately after adsorption, suggesting that TFP might inhibit virus penetration into MDCK cells, which is in agreement with the above cited report. The drug was therefore added later than 1 h p.i. in most experiments to localize the drug-sensitive event. In addition, we used a low moi (0.1 PFU/cell) for infection in the experiments reported in Tables 1 and 2. It has been demonstrated that the post-translational proteolytic cleavage of HA by trypsin-like enzymes is essential for infectivity of influenza virus (Klenk et al., 1975). The NWS virus used in this study did not form plaques on MDCK cells in the absence of trypsin (data not shown).

When the time-related growth inhibition of 35 μM TFP was examined, the earlier the addition of the drug, the more pronounced its effect on both supernatant and cell-associated virus yields. More importantly, the drug affected cell-associated virus yields as well as supernatant virus yields when it was added at a late stage of infection (later than 7.5 h p.i.). This inhibitory effect was also observed at 30 and 20 μM TFP, but the effect of 10 μM TFP was negligible. In the presence of 35 μM TFP, the distribution pattern of HA was completely altered, concomitantly with a decrease in the appearance of HA on the drug-treated cell surface. Scanning electron microscopy revealed that drug treatment did not lead to accumulation of progeny viruses on the cell surface. These findings indicate that TFP does not affect the budding process per se but some event before budding.

After removal of the drug, the time course of rearrangement of HA paralleled that of recovery of the supernatant infectivity. The lag time observed in this reversible effect of TFP was much shorter than the eclipse phase of influenza virus, suggesting that all virus components including virus-specific RNAs and proteins were synthesized in the presence of 35 μM TFP. In fact, when we labeled the infected cells with [^{35}S]methionine (5 μCi/ml) between 4.5 and 5 h p.i. either in the presence or the absence of 35 μM TFP, no qualitative or quantitative differences were noted in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of the immunoprecipitates (data not shown). Our data suggest that TFP is a reversible inhibitor of influenza virus morphogenesis in MDCK cells. The most likely drug-sensitive event in the influenza virus replicative cycle is different from that in measles virus infection, where TFP inhibits the budding process (Bohn et al., 1983).

Krĭzanová et al. (1982) reported that 20 to 50 μM CPZ had no effect when the drug was added after the 1 h adsorption period, which contrasts with our results. This may be attributed to differences of drug doses and cell types (chick embryo cells versus MDCK cells) used in the studies. Under our experimental conditions, CPZ showed a relatively weak effect on virus growth compared to TFP, that is, 60 μM CPZ was required to reduce virus yield by 3 log when added 1 h p.i. (data not shown). In addition, it has been demonstrated that the distribution pattern of CaM in fibroblasts is quite different from that in epithelial cells: the former shows a stress-fiber-like distribution of CaM (Dedman et al., 1978), whereas the latter shows a diffuse distribution of CaM (Andersen et al., 1978). From these facts, the distribution pattern of CaM could be considered as a possible factor influencing the drug effect.

Although it has been reported that phenothiazines might act as non-specific inhibitors against CaM-independent cellular factors (Levin et al., 1976), the drug doses used in this study (10 to 35 μM) correspond closely to those used by others (Hidaka et al., 1979; Honda et al., 1968; Levin et al., 1976; Weiss et al., 1980) as an in vitro antagonist of CaM-dependent enzymes. In addition, the inhibitory effect of TFP was partially reversed by purified CaM. Taking into account the drug dose used in this study and the reversible drug effect following removal of the drug or in the simultaneous presence of purified CaM [as revealed in this study and as reported by others (Bohn et al., 1983; Krĭzanová et al., 1982)], we suggest that inhibition of influenza virus replication in TFP-treated MDCK cells does not result from the direct interaction of TFP with the virus, but the interaction of the drug with cellular CaM.

Furthermore, TFP not only inhibits influenza virus replication, but also suppresses the appearance of microvilli in MDCK cells (this study) and HeLa cells (Bohn et al., 1983). The function of the cytoskeleton is regulated by Ca^{2+} and CaM in nonmuscle cells (Adelstein, 1982; Knipe, 1990; Yerna et al., 1979). Actin plays an important role in the morphogenesis of some viruses (Stallcup et al., 1979; Tyrrell et al., 1979) and the M protein of paramyxoviruses is the probable component interacting with actin (Damsky et al., 1977; Giuffre et al., 1982; Mountcastle et al., 1977; Rutter et al., 1977; Wang et al., 1976; Yoshida et al., 1979).

In conclusion, TFP might be useful for obtaining better insight into the mechanism of morphogenesis of influenza virus and the interaction of CaM-dependent cellular factors with influenza virus components involved in this process.

References

Adelstein, R.S. (1982) Calmodulin and the regulation of the actin-myosin interaction in smooth muscle and nonmuscle cells. Cell 30, 349–350.

Alexander, K. (1979) Early interactions of viruses with cellular membranes. In: M.A. Lauffer, F.B. Bang, K. Maromorosch and K.M. Smith (Eds), Advances in virus research, Vol. 24, pp. 223–276. Academic Press, London and New York.

Andersen, B., Osborn, M. and Weber, K. (1978) Specific visualization of the distribution of the calcium dependent regulatory protein of cyclic nucleotide phosphodiesterase (modulator protein) in tissue culture cells by immunofluorescence microscopy: mitosis and intercellular bridge. Cytobiologie 17, 354–364.

Bohn, W., Rutter, G., Hohenberg, H. and Mannweiler, K. (1983) Inhibition of measles virus budding by phenothiazines. Virology 130, 44–55.

Cheung, W.Y. (1970) Cyclic 3′,5′-nucleotide phosphodiesterase. Demonstration of an activator. Biochem. Biophys. Res. Commun. 38, 533–538.

Compans, R.W. (1984) Enveloped virus maturation at restricted membrane domains. In: N.A. Louis and M.B.A. Oldstone (Eds), Concepts in viral pathogenesis. pp. 123–129. Springer-Verlag, New York.

Damsky, C.H., Sheffield, J.B., Tusznski, G.P. and Warren, L. (1977) Is there a role for actin in virus budding? J. Cell. Biol. 75, 593–605.

Dedman, J.R., Welsh, M.J. and Means, A.R. (1978) Ca^{2+}-dependent regulator. Production and characterization of a monospecific antibody. J. Biol. Chem. 253, 7515–7521.

Elferink, J.G.R. (1979) Chlorpromazine inhibits phagocytosis and exocytosis in rabbit polymorphonuclear leukocytes. Biochem. Pharmacol. 28, 965–968.

Giuffre, R.M., Tovell, D.R., Kay, C.M. and Tyrrell, D.L.J. (1982) Evidence for an interaction between the membrane protein of a paramyxovirus and actin. J. Virol. 42, 963–968.

Hidaka, H., Naka, M. and Yamaki, T. (1979) Effect of novel specific myosin light chain kinase inhibitors on Ca^{2+}-activated Mg^{2+}-ATPase of chicken gizzard actomyosin. Biochem. Biophys. Res. Commun. 90, 694–699.

Honda, F. and Imamura, H. (1968) Inhibition of cyclic 3′,5′-nucleotide phosphodiesterase by phenothiazine and reserpine derivatives. Biochim. Biophys. Acta. 161, 267–269.

Horwitz, S.B., Chia, G.H., Harracksingh, C., Orlow, S., Pico-Hirst, S., Schneck, J., Sorbara, L., Speaker, M., Wilk, E.W. and Rosen, O.M. (1981) Trifluoperazine inhibits phagocytosis in a macrophagelike cultured cell line. J. Cell. Biol. 91, 798–802.

Kakiuchi, S. and Yamazaki, Y. (1970) Calcium dependent phosphodiesterase activity and its activating factor (PAF) from brain. Studies on cyclic 3′,5′-nucleotide phosphodiesterase (III). Biochem. Biophys. Res. Commun. 41, 1104–1110.

Klee, C.B., Crouch, T.H. and Richman, P.G. (1980) Calmodulin. Ann. Rev. Biochem. 49, 489–515.

Klenk, H.-D., Rott, R., Orlich, M. and Blödorn, J. (1975) Activation of influenza virus by trypsin-treatment. Virology 68, 426–439.

Knipe, D.M. (1990) Interactions between viruses and the cytoskeleton. In: B.N. Fields (Ed), Virology (2nd edit.), pp. 304–306. Raven Press, New York.

Křižanová, O., Čiampor, F. and Veber, P. (1982) Influence of chlorpromazine on the replication of influenza virus in chick embryo cells. Acta Virol. 26, 209–216.

Levin, R.M. and Weiss, B. (1976) Mechanism by which psychotropic drugs inhibit adenosine cyclic 3′,5′-monophosphate phosphodiesterase of brain. Mol. Pharmacol. 12, 581–589.

Means, A.R. and Dedman, J.R. (1980) Calmodulin – an intracellular calcium receptor. Nature 285, 73–77.

Merrit, J.E., Tomlinson, S. and Brown, B.L. (1981) Phenothiazines inhibit prolactin secretion in vitro. A

possible role for calmodulin in stimulus-secretion coupling in the pituitary. FEBS Lett. 135, 107–110.

Mountcastle, W.E. and Choppin, P.W. (1977) A comparison of the polypeptides of four measles virus strains. Virology 78, 463–474.

Ochiai, H., Kurokawa, M., Hayashi, K. and Niwayama, S. (1988) Antibody-mediated growth of influenza A NWS virus in macrophagelike cell line P388D1. J. Virol. 62, 20–26.

Osborn, M. and Weber, K. (1980) Damage of cellular functions by trifluoperazine, a calmodulin-specific drug. Exp. Cell. Res. 130, 484–488.

Pattaik, A.K., Brown, D.J. and Nayak, D.P. (1986) Formation of influenza virus particles lacking hemagglutinin on the viral envelope. J. Virol. 60, 994–1001.

Rutter, G., Mannweiler, K. (1977) Alterations of actin-containing structures in BHK21 cells infected with Newcastle disease virus and vesicular stomatitis virus. J. Gen. Virol. 37, 237–242.

Salisbury, J.L., Condeelis, J.S. and Satir, P. (1980) Role of coated vesicles, microfilaments, and calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. J. Cell. Biol. 87, 132–141.

Stallcup, K.C., Wechsler, S.L. and Fields, B.N. (1979) Purification of measles virus and characterization of subviral components. J. Virol. 30, 166–176.

Stuart-Harris, C.H. and Schild, G.C. (1976) The molecular virology and replication of influenza viruses. In: C.H. Stuart-Harris and G.C. Schild (Eds), Influenza. The viruses and the disease, pp. 22–50, Edward Arnold, London.

Tobita, K., Sugiura, A., Enomoto, C. and Furuyama, M. (1975) Plaque assay and primary isolation of influenza A viruses in an established line of canine kidney cell (MDCK) in the presence of trypsin. Med. Microbiol. Immunol. 162, 9–14.

Tyrrell, D.L.J. and Ehrnst, A. (1979) Transmembrane communication in cells chronically infected with measles virus. J. Cell. Biol. 81, 396–402.

Wang, E., Wolf, B.A., Lamb, R.A., Choppin, P.W. and Goldberg, A.R. (1976) The presence of actin in enveloped viruses. In: R.D. Goldman, T. Polland and J. Rosenbaum (Eds), Cell Motility, Book B: Actin, myosin, and associated proteins. Cold Spring Harbor Conference on Cell Proliferation, Vol. 3, pp. 589–599. Cold Spring Harbor Laboratory, New York.

Weiss, B., Prozialeck, W., Cimino, M., Barnette, M.S. and Wallance, T.L. (1980) Pharmacological regulation of calmodulin. Ann. N.Y. Acad. Sci. 356, 319–345.

Yerna, M.J., Dabrowska, R., Hartshorne, D.J. and Goldman, R.D. (1979) Calcium-sensitive regulation of actin-myosin interactions in baby hamster kidney (BHK-21) cells. Proc. Nat. Acad. Sci. USA 76, 184–188.

Yoshida, T., Nagai, Y., Maeno, K., Iinuma, M., Hamaguchi, M., Matsumoto, T., Nagayoshi, S. and Hoshino, M. (1979) Studies on the role of M protein in virus assembly using a ts mutant of HVJ (Sendai virus). Virology 92, 139–154.