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Effects of procaine and oxyphenylbutazone on interferon-mediated inhibition of murine leukemia virus production

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Treatment of murine NIH/MOL, C cells which are chronically infected with Moloney murine leukemia virus (MuLV), with mouse interferon (IFN), causes inhibition of extracellular MuLV production. We tested the effect of procaine, a drug that is known to expand cellular membranes and to increase their fluidity, on IFN-mediated inhibition of MuLV production. We observed that procaine did not alleviate this inhibition when IFN was present during procaine treatment. However, if IFN was removed from the culture medium before procaine treatment, the inhibition of MuLV production was partially reversed. We also observed that procaine treatment caused an increase in the amount of MuLV production by cells which had not been treated with IFN. Oxyphenylbutazone (OPB) is an inhibitor of cyclooxygenase, a key enzyme in prostaglandin biosynthesis. New synthesis of prostaglandins is thought to be needed for the antiviral actions of IFN. We observed that OPB did not prevent the antiretroviral action of IFN on NIH/MOL, C cells. OPB also failed to alleviate the IFN-mediated inhibition of replication of vesicular stomatitis virus either in NIH/MOL, C cells or in the L929 cells.

interferon; murine leukemia virus; procaine; oxyphenylbutazone; prostaglandins; membrane fluidity

Extracellular virus production by cells that are chronically infected with retroviruses is inhibited by treatment with interferons (IFN) [7,10,19]. This inhibition is not caused by an inhibition of retroviral RNA and protein synthesis in IFN-treated cells, but it is a consequence of an impairment of retroviral assembly and release from such cells [1,3,8,17,22,26]. More virus particles accumulate on the plasma membrane of IFN-treated cells as compared to untreated cells [2,4,22]. This arrest of retroviral particles is probably not due to any defect in the different steps of viral morphogenesis that precede budding but it is most probably due to changes in some properties of the plasma membrane of the IFN-treated cell [21]. IFN-treatment is known to change

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some properties of the cell membrane; for example, fluidity of the cell membrane is reduced by IFN-treatment of the cells [5,12]. The fusibility of these cells is decreased [5,23] and the lateral mobility of specific receptors on the cell surface is also reduced [12]. The antiretroviral effect of IFN may be mediated through some of these changes in the properties of the plasma membrane.

NIH/MOL, C is a NIH/3T3 cell line that is chronically infected with Maloney murine leukemia virus (MuLV). IFN treatment of these cells causes inhibition of extracellular MuLV production. Replication of vesicular stomatitis virus (VSV) in these cells is also inhibited by IFN but that of encephalomyocarditis virus (EMCV) is not [20]. In a sister line, NIH/MOL, B, replication of both VSV and EMCV is insensitive to IFN although MuLV production is sensitive [6,20]. As in many other cell lines, inhibition of MuLV production by IFN-treated NIH/MOL, C cells is caused by an arrest of virus release from the cell surface [15]. In the experiments summarized in Table 1, we tested the effects of procaine on MuLV-release from IFN-treated and untreated cells. Procaine, a local anesthetic, is known to increase the fluidity of cell

TABLE 1

Effect of procaine and IFN on MuLV production[a]

Expt. no.	IFN treatment (U/ml)	Time (h)	Procaine treatment (M)	Time (h)	MuLV production (cpm/ml × 10^{-3})	(%) Inhibition $\left(1-\frac{+IFN}{-IFN}\right) \times 100$
1	–	–	–	–	71.3	
	50	0–48	–	–	15.5	78.3
	–	–	10^{-5}	24–48	108.5	
	50	0–48	10^{-5}	24–48	19.3	82.2
	–	–	10^{-3}	24–48	97.5	
	50	0–48	10^{-3}	24–48	18.0	81.5
2	–	–	–	–	63.0	
	100	0–9	–	–	18.9	70.0
	–	–	10^{-5}	9–29	81.6	
	100	0–9	10^{-5}	9–29	47.1	42.3

[a] NIH/MOL, C cells were 50% confluent at the beginning of the treatments. A partially purified mouse IFN type I ($\alpha + \beta$) (spec. act.: 10^7 U/mg protein) was used for these experiments. Procaine hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO. In experiment 1, IFN was added to the culture medium. After 24 h the medium was replaced by fresh medium containing IFN and procaine as indicated in the table. After 48 h, the culture medium was collected and MuLV released in the medium was assayed by measuring virion-associated reverse transcriptase activity. Reverse transcriptase activity in the culture medium was measured using poly A. (dt) [12–18] as the template primer, as described in detail elsewhere [20]. 100 000 cpm of activity is equivalent to 67 moles of TTP incorporated in 20 min. In experiment 2, IFN was added to the culture medium: after 9 h the medium was removed, the cells were washed and fresh medium containing procaine but no IFN was added as indicated in the table. After 29 h the medium was collected for reverse transcriptase assay. Procaine of IFN present in the medium does not directly inhibit or enhance reverse transcriptase activity.

membrane. It also expands cell membranes either through increase in the fluidity or through other effects such as displacement of Ca^{2+} ions from phospholipid-bound sites [18]. If impaired release of MuLV from IFN-treated cells is a result of decreased fluidity of the plasma membranes of such cells, one would expect an alleviating effect of procaine on IFN-mediated arrest of retrovirus release.

In the first experiment shown in Table 1, the effect of procaine on MuLV production by NIH/MOL, C cells was tested. At a concentration of 10^{-5} M, procaine caused an increase in the production of MuLV by both IFN-treated and untreated cells. Increasing the concentration to 10^{-3} M did not augment this effect any further. In this experiment cells were exposed to IFN both before and during procaine treatment. Although procaine enhanced the amount of MuLV production it did not increase the production by IFN-treated cells preferentially. The extent of IFN-mediated inhibition of MuLV production therefore remained relatively unchanged. In the second experiment, shown in Table 1, IFN was not present during procaine treatment. Cells were treated with IFN for 9 h and then IFN was removed from the culture medium. IFN-pretreated and untreated cells were incubated further in the presence or absence of 10^{-5} M procaine. Due to the shorter duration of IFN treatment in this experiment, the degree of inhibition of MuLV production by IFN was lower than that in the first experiment. As in the first experiment procaine enhanced MuLV production both by IFN-treated and untreated cells. However, the increase was more pronounced for IFN-treated cells, implying a partial reversal of IFN-mediated inhibition of MuLV production.

These experiments demonstrated that a membrane-acting drug such as procaine can increase the amount of MuLV production by both IFN-treated and untreated cells. Moreover, under proper conditions, it also seemed to be able to partially reverse the antiretroviral effect of IFN. Conceivably this partial reversal was effected through an increase in the fluidity of the plasma membrane of the IFN-treated cells, but we do not have direct evidence for this interpretation.

The antiretroviral effect of IFN persists for a long time after removal of IFN from the cell culture [9]. It appears that procaine can hasten the dissipation of the antiretroviral state once IFN is removed, but it fails to exert its effect in the presence of continued IFN action.

Recent results from ours and other laboratories indicate that the pathways leading to IFN-mediated inhibition of retrovirus production and those responsible for IFN-mediated inhibition of replication of exogenously infecting cytopathic viruses, overlap only partially. Both kinds of antiviral actions seem to rely on similar IFN-receptor interactions and seem to need continued cellular gene expression after IFN-treatment. However, several cell lines have been described where one kind of antiviral action can be demonstrated in total or partial absence of the other, indicating different underlying mechanisms for the two actions [6,9,20]. Recently Pottathil et al. [14] suggested that an early step in IFN action may be an enhanced synthesis of prostaglandin which is needed for the anti-VSV action of IFN since inhibitors of prostaglandin synthesis partially reversed the effect of IFN. We wanted to know whether the same would hold true for the antiretroviral action of IFN.

Oxyphenylbutazone (OPB) is a potent inhibitor of cyclooxygenase, a key enzyme in

prostaglandin biosynthesis. Pottathil et al. [14] observed that, at a concentration of 10 μM, OPB partially reverses the IFN-mediated inhibition of VSV replication in L929 cells. We tested the effect of increasing doses of OPB on MuLV production by IFN-treated and untreated NIH/MOL, C cells. The results are shown in Table 2. At a concentration of 10 μM, OPB did not affect MuLV production by these cells. Higher doses of OPB inhibited MuLV production in a dose-dependent manner. This inhibition was most probably a direct consequence of inhibition of protein synthesis by these higher doses of OPB [16]. No dose of OPB however reversed the IFN-mediated inhibition of MuLV production.

We then tested the effect of 10 μM OPB on IFN-mediated inhibition of VSV replication in NIH/MOL, C cells. We also included L929 cells in this experiment to be able to directly compare our results with those of Pottathil et al. [14]. The results are shown in Table 3. VSV replication was inhibited about 1000-fold in NIH/MOL, C cells and 10 μM OPB did not decrease the degree of this inhibition. Surprisingly we also did not observe an alleviating effect of OPB on IFN-mediated inhibition of VSV multiplication in L929 cells. If anything, OPB treatment enhanced the IFN-mediated inhibition of VSV replication in L929 cells.

These experiments indicate that OPB does not affect the classical antiviral and the antiretroviral effects of IFN. We observed that other inhibitors of prostaglandin biosynthesis such as indomethacin and aspirin were also without any effect on the antiretroviral action of IFN. It appears therefore that prostaglandin synthesis is not required for this action of IFN, although it may be required for other actions of IFN such as increasing the intracellular cyclic GMP concentration [25]. Although a part of our results contradicts the observations by Pottathil et al. [14], it is in line with the very recent observations by Tovey et al. [24] and Sarkar and Gupta [16]. The latter two groups also failed to demonstrate any effect of these cycloxygenase inhibitors on IFN-mediated inhibition of VSV replication.

TABLE 2

Effect of oxyphenylbutazone on IFN-mediated inhibition of MuLV production[a]

Dose of OPB (μM)	MuLV production (cpm/ml × 10^{-3})		Percent inhibition ($\frac{A-B}{A} \times 100$)
	OPB (A)	OPB + IFN (B)	
0	321.8	26.0	92
10	325.0	25.4	92
65	244.9	19.6	92
160	216.6	18.4	91
325	114.6	17.3	85
485	92.9	4.4	96

[a] Different doses of OPB (a gift from Ciba-Geigy Corp., Summit, NJ) and 500 U/ml of IFN were added to the culture medium. After 11 h the medium was replaced by fresh medium containing the same additives. At 24 h, the medium was collected and the amount of MuLV released was measured by reverse transcriptase activity (see Table 1).

TABLE 3

Effect of oxyphenylbutazone on IFN-mediated inhibition of VSV replication[a]

Cells	IFN (500 U/ml)	OPB (10 μM)	VSV Yield (pfu/ml)	Inhibition ($\Delta\log_{10}$ pfu/ml)
NIH/MOL, C	-	-	9.0×10^6	
	+	-	1.6×10^4	2.8
	-	+	6.4×10^6	
	+	+	8.8×10^3	2.9
L929	-	-	4.3×10^7	
	+	-	2.1×10^4	3.3
	-	+	3.7×10^7	
	+	+	1.7×10^3	4.3

[a] The indicated treatments were given for 24 h. Then cells were infected with VSV at a m.o.i. of 1 and incubated overnight. VSV yields were measured by plaque assay on L929 cells [20].

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