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Antisickling potential of bat interferon

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Sickle cell disease is a severe hereditary disorder which affects millions of people. The disease has its highest incidence in Africa (Dean and Schechter, 1978). Several attempts to inhibit sickling of human erythrocytes containing hemoglobins have been made (Nalbandian, 1971; Cerami and Manning, 1971; Kraus and Kraus, 1971; Sofowora and Isaccs, 1971). It has been established that there is a decrease in sialic acid content in the erythrocyte of sickle cells (Riggs and Ingrams, 1971). In addition, it has recently been observed that there is an elevated cell-surface sialic acid phenotype and increased glycosphingolipid (GSL) synthesis in interferon-treated L929 cells (Yogeeswaran et al., 1982 and 1983). This observation raised the possibility that interferon, being a glycoprotein, could contribute its sialic acid residue to the erythrocyte membranes of sickle cells.

Blood from sickle cell patients was obtained from the University of Ife Teaching Hospital Complex, Nigeria. Bat interferon was obtained by inducing bat primary tissue cultures with synthetic polynucleotide (poly A: poly Br^5U). The interferon obtained was purified to a specific activity of 2.1×10^5 U/mg protein. Human lymphoblastoid interferon (α-interferon) was purchased from Sigma Chemicals Company, U.S.A.

Blood samples from sickle cell patients were incubated in vitro under anerobic conditions with two-fold dilutions of human and bat interferon. In a typical case the following procedure was adopted (Adesanya and Sofowora, 1983):

Blood (0.5 ml) from each of the HbSS patients and 0.5 ml of growth medium were mixed in test tubes covered with 3.0 ml of liquid paraffin. The mixture was incubated in an incubator at 37°C for 10 min. At the end of the incubation, 1.0 ml of the prepared interferon samples was added under the paraffin layer, bringing down the final concentration in each test tube to half. The mixture was further incubated for 3 h before the cells were finally fixed by rapid dilution of the incubated mixture with 3.0 ml of 5% buffered formalin. The experiment was repeated using growth medium as control. Freshly prepared slides were inspected. For the establishment of antisickling activity the final incubated concentration for interferon from human and bat were 1250 U/ml and 1750 U/ml, respectively. A significant reduction of the populations of sickled cells was observed (Table 1; Fig. 1). One very good advantage interferon has over other known antisickling agents like urea and cyanate is that interferon is reasonably non-toxic to cells. There is therefore the possibility of in-

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TABLE 1

Dose-range of antisickling activity (reversal) for bat interferon and human interferon

Dilution (two-fold)	Bat interferon		Human interferon	
	IFN concentration (U/ml)	Cells sickled (%)	IFN concentration (U/ml)	Cells sickled (%)
0	7000	5 ± 1	5000	2 ± 1
1 : 2	3500	5 ± 1	2500	2 ± 1
1 : 4	1750	5 ± 1	1250	2 ± 1
1 : 8	875	7 ± 1	625	5 ± 2
1 : 16	438.5	15 ± 2	312.5	10 ± 3
1 : 32	218.8	20 ± 2	156.3	15 ± 4
1 : 64	109.4	28 ± 3	78.1	25 ± 4
1 : 128	54.7	48 ± 3	39.1	54 ± 3
1 : 256	27.3	65 ± 3	19.5	60 ± 2
1 : 512	13.7	75 ± 2	9.8	70 ± 1
Control	0.0	100 ± 1	0.0	100 ± 1

The experiments were repeated 4 times from which S.D. were calculated.

creasing the dose. This modifying effect of interferon on sickle cells is a new discovery. The mechanism of action of interferon as regards its antisickling behavior is yet to be elucidated, although several biochemical alterations have been described in cells treated with interferon (Yogeeswaran et al., 1983). It is possible that the mechanism cuts across species specificity.

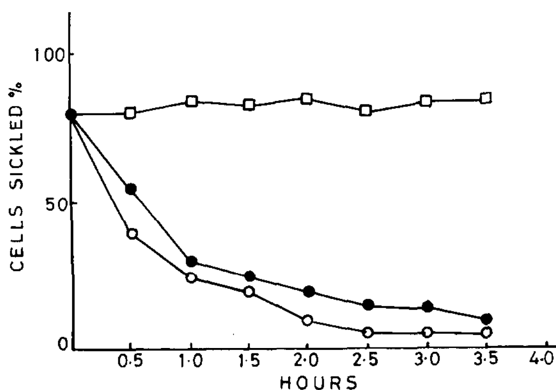

Fig. 1. Establishment of antisickling activity (reversal) using human and bat interferon. □——□, Control; ●——●, bat interferon; ○——○, human interferon. Final concentration was fixed at 1250 U/ml for human interferon and 1750 U/ml for bat interferon.

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