EFFECT OF FOLIC ACID AND METHOTREXATE ON REPRODUCTION OF RAUSCHER LEUKEMIA VIRUS

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Folic acid was found to stimulate and its structural analog methotrexate was found to inhibit the reproduction of an oncogenic RNA-containing virus in tissue culture.

KEY WORDS: Rauscher virus; leukemia; folic acid; methotrexate; RNA synthesis and virus reproduction.

The reproduction of the oncornaviruses is closely linked with DNA synthesis of the host cell and the continuous functioning of the cellular enzyme systems. Work in Bird's laboratory, for instance, has shown that the reproduction of one member of this group of viruses – the virus of avian myeloblastosis – is highly dependent on the system of folate coenzymes [2]. An important coenzyme function of folic acid derivatives is their participation in the biosynthesis of thymidylic acid.

It was therefore decided to study the effect of methotrexate, a structural analog of folic acid preventing thymidylic acid synthesis by blocking the intracellular enzyme dihydrofolate reductase [3], on the reproduction of an RNA-containing oncogenic virus.

EXPERIMENTAL METHOD

Rauscher leukemia virus was used as the experimental model. A culture of BALB/c mouse embryonic cells after growth for 48 h was infected by exposure for 60 min with a 0.5% splenic extract from leukemic mice, after which the cells were suspended in nutrient medium (20% bovine serum, 80% medium No. 199, and 100 i.u./ml penicillin) in a concentration of 500,000 cells/ml, and samples of 10 ml were poured into 100-ml flasks. After 3 days the cells were transfered to fresh flasks with the same seeding dose. On the 6th day of secondary growth, when the cell concentration was 1,500,000/ml, the culture fluid was tested for its content of infectious virus. Folic acid in a concentration of 100 μg/ml or methotrexate (Lederle, USA) in a concentration of 5 μg/ml was added 1 h after the beginning of contact between virus and cells, after which the preparations were added at each change of the medium. In some experiments the methotrexate was added 72 h after conjugation of the virus with the cells. Cultures grown without addition of the preparations acted as the control; the folic acid concentration in this case was 0.01 μg/ml on account of the medium No. 199. To test the infectious activity of the culture fluid it was injected intramuscularly in doses of 0.025 and 0.5 ml into BALB/c mice aged 3-4 weeks. The animals remained under observation for 4 months, during which the number of those dying from leukemia was counted.

EXPERIMENTAL RESULTS

The results of the tests of the culture fluid for infectious activity are given in Table 1. They show that reducing the dose of culture fluid by 20 times (from 0.5 to 0.025 ml) of the control cultures reduced the number of mice dying from leukemia from 65 to 3.7%; on infection with culture material with a folic acid

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TABLE 1. Reproduction of Rauscher Leukemia Virus in Mouse Embryonic Cells Depending on Concentration of Folic Acid and Methotrexate

Preparation	Concentration (in μg/ml)	Addition after adsorption of virus	Vol. of culture fluid injected (in ml)	No. of mice		Mortality (in %)	P
				infected	dying		
Folic acid	0,01	Initially	0,5	60	39	65,0	—
			0,025	54	2	3,7	—
	100,0	After 1 h	0,5	60	60	100,0	<0,01
			0,025	58	33	56,9	<0,01
Methotrexate	5,0	» 1 h	0,5	60	0	0,0	<0,01
		» 72 h	0,5	57	52	91,2	<0,01

Note. Results of 3 parallel experiments are pooled in the table. The level of significance (P) and differences between responses in the experimental and control series were calculated with the aid of the χ^2 criterion.

concentration in the medium of 100 μg/ml, the mortality among the experimental animals was 100 and 56.9% respectively. The culture fluid from cultures to which methotrexate was added 1 h after adsorption of the virus did not cause death of the animals from leukemia. If culture fluid from cultures in which methotrexate was present 72 h after contact between virus and the cells was injected into mice, 91.2% of the infected animals died.

The results indicate that the reproduction of Rauscher virus depends on the concentration of folic acid and on the presence of a folic acid deficiency in cells infected by this virus. Increasing the folic acid concentration in the nutrient medium to 100 μg/ml led to a considerable (almost 20-fold) increase in the infective activity of the culture fluid. Methotrexate evidently potentiates the folic acid deficiency and leads to inhibition of thymidylic acid biosynthesis. In previous experiments with avian myeloblastosis virus stimulation of thymidylate biosynthesis was demonstrated in infected cells [4]. The data now obtained, indicating differences in the effects of early and late addition of methotrexate, are in good agreement with observations on the action of abnormal nucleosides [1], the inhibitory effect of which is manifested only during the first few hours after infection of the cells and is due to dependence of the reproduction of RNA-oncogenic viruses on DNA synthesis of the host cell. Meanwhile it can be postulated that a more efficient isoenzyme of dihydrofolate reductase with lower affinity for methotrexate than its precursor functions in mouse embryonic cells 72 h after infection with Rauscher leukemia virus.

LITERATURE CITED

1. J. P. Bader, Virology, 26, 253 (1965).
2. G. S. Beaudreau, C. Becker, T. Stim, et al., J. Nat. Cancer Inst., 4, 167 (1960).
3. J. R. Bertino, Cancer Res., 23, 1286 (1963).
4. D. K. Karapetyan, N. F. Pravdina, V. A. Parnes, et al., Acta Virol., 12, 187 (1968).