EFFECT OF TRANSPLACENTAL EXPOSURE TO COBALAMINE COENZYMES AND FOLIC ACID ON CARCINOGENIC ACTIVITY OF N-NITROSO-N-ETHYLUREA IN MOUSE KIDNEY ORGAN CULTURES

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One important aspect of the modifying effect of cobalamine (Cbl) and folic coenzymes on chemical carcinogenesis is their transplacental action. Recent investigations have shown that hormones, vitamins, and certain polypeptide growth factors are able to modify the carcinogenic transplacental effect of N-nitrosoethylurea [2, 8]. Cbl and folate coenzymes have not been characterized as endogenous modifiers of chemical carcinogens, in the case of transplacental exposure. In the development of our research into the modifying role of Cbl and folate coenzymes as endogenous regulators of proliferation in carcinogenesis, a comparative analysis was made of their transplacental action on activity of N-nitrosoalkylurea.

For this purpose we studied the transplacental action of adenosyl- and methylcobalamine (Ado- and MeCbl), and also of folic acid (formyltetrahydrofolate – f-THF) on the carcinogenic effect of N-nitrosoethylurea (NEU) in organ cultures of embryonic mouse kidneys (OCEK).

EXPERIMENTAL METHOD

The Cbl-coenzymes and f-THF (calcium folate) used in the work were synthesized at the Vitaminy Research and Production Combine, Ministry of the Medical Industry of the USSR. In three series of experiments, AdoCbl, MeCbl, and f-THF were given in isolation or simultaneously with NEU to female DBA/2 mice on the 14th through the 20th days of pregnancy, in accordance with a schedule described by the writers previously (Fig 1) [3]. The kidney explants were cultured for 23 days [1] and investigated by the standard histoautoradiographic method. To assess the transplacental action of AdoCbl, MeCbl, and f-THF, and also of NEU from the mouse OCEK, the frequency of hyperplastic changes was determined, by isolating areas of diffuse hyperplasia of the epithelium of the convoluted tubules and foci of proliferation of atypical epithelium, and also cystlike structures with proliferating epithelium. In all these structures the number of epithelial cells synthesizing DNA was analyzed. Meanwhile the frequency of destructive changes was determined in the kidney explants. Altogether we studied 723 kidney explants from mouse fetuses. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Injection of MeCbl and f-THF into mice in the prenatal period had a marked growth-stimulating effect in OCEK of the mice and significantly increased the frequency of preneoplastic changes, due to the transplacental action of NEU. Thus in the majority of kidney explants, together with uneven diffuse hyperplasia of the epithelium of the convoluted tubules, foci of proliferation of atypical epithelium, characteristic of the early stages of transplacental carcinogenesis, were observed. The frequency of these foci of proliferation in the kidney explants in the case of simultaneous transplacental exposure to MeCbl with NEU was 3.3 times greater than when the carcinogen was given alone (Table 1). A similar modifying action on induction of hyperplastic changes in the renal epithelium by NEU was exerted by injection of f-THF into the mice in the prenatal period.

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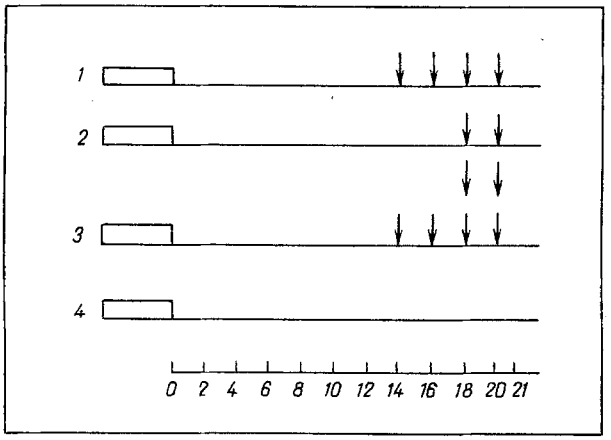

Fig. 1. Schedule of experiment: transplacental action of AdoCbl, MeCbl, and f-THF on carcinogenic activity of NEU in mouse OCEK 1-4) Groups of mice; 0-21) days of pregnancy. Mice of group 1 received (arrow) AdoCbl or MeCbl alone (intramuscularly, 2.5 mg/kg), or f-THF alone (intramuscularly, 25 mg/kg) on 14th, 16th, 18th, and 20th days of pregnancy; mice of group 2 received injection of NEU alone (intraperitoneally, on 18th and 20th days of pregnancy, 30 mg/kg); mice of group 3 received a combination of AdoCbl and MeCbl, and also of f-THF with NEU according to the same schedule as when given alone; mice of group 4 — control (21-day-old fetuses of intact mothers).

Thus, with simultaneous exposure to f-THF and NEU the frequency of foci of proliferation of atypical epithelium in the kidney explants was 1.9 times greater than in the case of isolated administration of NEU. The frequency of diffuse hyperplasia of the epithelium of the convoluted tubules, and also the number of cystlike structures with proliferating epithelium in the renal explants were not significantly changed under these circumstances. An increase in sensitivity of the mouse embryonic kidney tissue to the transplacental action of NEU was largely determined by the marked stimulating effect of MeCbl and f-THF on proliferation of epithelial cells. The same factor was evidently responsible also for the improved survival rate of the mouse kidney explants under the influence of MeCbl and f-THF, and the decrease in frequency of the destructive changes as a result of the development of regenerative processes in zones replacing the primary central necrotic foci (Fig. 2). An essential feature distinguishing the transplacental growth-modifying action of MeCbl and f-THF in mouse OCEK also was the development of diffuse hyperplasia in the epithelium of the convoluted tubules (49.5 and 50.0% compared with 3.3% in the control cultures). With marked diffuse hyperplasia the number of proliferating cells in the epithelium of the convoluted tubules was significantly higher (19.4 ± 1.8 and 16.2 ± 1.3% for administration of MeCbl and f-THF respectively) than in the epithelium of tubules with no hyperplastic changes (2.2 ± 0.6%, $p < 0.01$).

By contrast with MeCbl and f-THF, AdoCbl exhibited much weaker growth-stimulating activity in the mouse OCEK and did not potentiate induction of hyperplastic changes by NEU. Following the isolated action of AdoCbl the frequency of diffuse hyperplasia in the epithelium of the convoluted tubules was significantly lower than after injection of MeCbl and f-THF, namely 29.7% (Table 1). Under these circumstances the number of proliferating cells in the epithelium of the convoluted renal tubules was increased but not significantly (8.6 ± 1.4%). Under the influence of AdoCbl with NEU the frequency of diffuse hyperplasia in the epithelium of the convoluted tubules and the number of cystlike structures with proliferating epithelium in the kidney explants were significantly lower than after isolated action of the carcinogen, and also when it was given together with MeCbl. Extratubular proliferation was noted in the kidney explants. In this case mainly sheets of atypical epithelium (23.4%) were found, but the frequency of the characteristic foci of proliferation was reduced to 8.7% (Table 1). On the whole, throughout the period of culture the frequency of foci of proliferation of atypical epithelium in the kidney explants with exposure to the transplacental action of AdoCbl with NEU was 2.3 times lower than when MeCbl was given together with the carcinogen. The results of these experiments thus demonstrated the differences between the character of the modifying transplacental action of AdoCbl and of MeCbl and f-THF on the carcinogenic effect of NEU in mouse OCEK.

TABLE 1. Frequency of Preneoplastic Changes in Epithelium in Mouse OCEK during Transplacental Action of Cobalamine Coenzymes and f-THF with NEU

Substance	Total dose, mg/kg	Number of kidney explants	Frequency of hyperplastic changes in renal epithelium		
			diffuse hyperplasia of convoluted tubules	foci of proliferation	cyst-like structures
AdoCbl	10	74	22/29,7 p_1 0,001	—	2/2,7
MeCbl	10	104	51/49,5 p_1 0,001 p_2 0,01	—	14/13,6 p_1 0,01 p_2 0,01
f-THF	100	102	36/50,0 p_1 0,001 p_2 0,01	—	6/5,8
NEU	60	79	43/54,4 p_1 0,001	17/21,5	11/13,9 p_1 0,01
AdoCbl + NEU	10+60	115	33/28,7 p_1 0,001 p_3 0,001	37/32,2* p_3 0,05*	3/2,6 p_3 0,02
MeCbl + NEU	10+60	57	33/57,9 p_1 0,001	41/71,9 p_3 0,001	8/14,0 p_1 0,01
f-THF + NEU	100+60	72	36/50,0 p_1 0,001 p_4 0,001	30/41,6 p_3 0,01 p_4 0,01	1/5,5 p_4 0,02
Control		121	4/3,3	—	3/2,5

Legend. Numerator gives absolute number, denominator percent. p_1) Significance compared with control, p_2) compared with transplacental action of MeCbl, p_3) compared with transplacental action of NEU; p_4) compared with transplacental action of MeCbl + NEU. *) 23.4% — Sheets of atypical epithelium and 8.7% — foci of proliferation; ** (for p) 32.2% — extratubular proliferation.

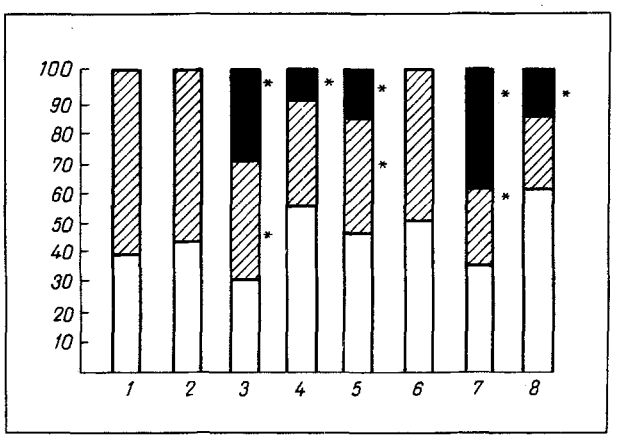

Fig. 2. Frequency of destructive changes in mouse OCEK during transplacental exposure to AdoCbl, MeCbl, and f-THF with NEU. Unshaded part of column represents kidney explants with no changes, obliquely shaded part — explants with central necrosis, and black parts — with zones of restoration of central necrotic areas. Ordinate: frequency of primary central necrotic foci in kidney explants (in %); abscissa: 1) control cultures, 2-4) transplacental exposure to AdoCbl, MeCbl, and f-THF respectively, 5) transplacental exposure to NEU, 6-8) transplacental exposure to AdoCbl, MeCbl, and f-THF with NEU respectively.

The view is held that stimulation of proliferation or synchronization of cells in target tissues increases their sensitivity to the action of chemical carcinogens [9]. Data on the sensitivity of resting and proliferating cells to the action of NEU are contradictory [5, 6]. In our investigations the modifying transplacental effect of MeCbl and f-THF on the carcinogenic activity of NEU was due to an increase in the number of S-phase cells in the epithelium of the convoluted tubules of the mouse kidneys, which are evidently the cells that are most sensitive to the action of the carcinogen. During proliferation and growth of embryon-

ic tissue cells, a coupled action of Cbl and folic acid compounds is observed. The cobalamine-dependent methionine-synthetase reaction is the main stage of their interaction in proliferating cells with the formation of active forms of folate. The cellular level of methionine synthetase, for which MeCbl is the coenzyme, largely determines the intensity of proliferation and growth of embryonic tissues [7, 10]. The results confirm the stimulating action of MeCbl and f-THF in embryonic tissue cell proliferation as well as their modifying effect on the carcinogenic activity of NEU. In the embryonic period, Cbl-coenzymes are synthesized in the fetal liver and kidneys [4]. The final form of their accumulation in the cells, namely AdoCbl, a coenzyme of methylmalonyl-CoA-mutase, is connected with the energy metabolism of the cells and differs in its growth-stimulating activity from MeCbl and f-THF. The positive modifying effect of AdoCbl on the transplacental carcinogenic effect of NEU, which the present investigation revealed, is of great significance for the prevention of prenatally induced tumors.

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