experiment, on the one hand, androgenization of females (daily administration of testosterone proprionate in a dose of 10 mg/kg for 10 days) was accompanied by an increase in the velocity of N-demethylation of aminopyrine from 22 ± 1.2 to 39 ± 3.3 nmoles·min^{-1}·g^{-1} ($p < 0.001$); on the other hand, in the case of estrogenization of males (5 injections of estradiol dipropionate in a dose of 5 μg/kg every other day) this parameter fell to 23.0 ± 1.8 nmoles·min^{-1}·g^{-1} ($p < 0.001$). Stimulation of enzyme activity of hepatic microsomes by testosterone and its inhibition by estrogens have been reported in the literature [7, 9].

Since sex differences in the rate of metabolism of the type I substrate which we used (hexobarbital, aminopyrine) are due to a difference in cytochrome P450 binding with them [12], and since proteins of microsomal membranes are the most powerful ethimizole acceptors [2], it can be postulated that the effect of the drug on the microsomal enzyme system which is determined by sexual dimorphism, is manifested at this level.

LITERATURE CITED

1. Yu. S. Borodkin, Antiphenes (Experimental and Clinical Data) [in Russian], Moscow (1966).
2. Yu. S. Borodkin, L. M. Belyaevskaya, O. G. Kulikova, et al., Byull. Éksp. Biol. Med., No. 10, 53 (1983).
3. I. S. Zavodskaya, É. A. Migas, and V. V. Bul'on, Byull. Éksp. Biol. Med., No. 3, 54 (1975).
4. I. S. Zavodskaya, E. V. Moreva, and N. A. Novikova, The Effect of Neurotropic Substances on Neurogenic Heart Damage [in Russian], Moscow (1977).
5. A. F. Leshchinskii, T. A. Zolotareva, O. T. Karpenchuk, and Z. I. Zuza, Farmakol. Toksikol., No. 3, 265 (1979).
6. A. F. Leshchinskii, V. L. Konovalenko, and Z. I. Zuza, Vopr. Kurortol., No. 2, 9 (1985).
7. P. V. Sergeev, N. N. Vedernikova, A. I. Maiskii, et al., Byull. Éksp. Biol. Med., No. 9, 43 (1975).
8. J. A. Castro, F. E. Green, P. Gigon, et al., Biochem. Pharmacol., 19, 2461 (1970).
9. A. H. Conney and J. J. Burnes, Acta Pharmacol. (Copenhagen), 19, 31 (1962).
10. R. Kato, Xenobiotica, 7, 25 (1977).
11. L. Lombrozo and C. Mitoma, Biochem. Pharmacol., 19, 2317 (1970).
12. D. M. Moor, Foreign Compound Metabolism in Mammals, Vol. 2, London (1972), pp. 398-411.

RETINOIC ACID MODIFIED CELL CULTURE FOR REPRODUCING ENTEROPATHOGENIC VIRUSES

V. N. Tatskaya, V. I. Nozdrin, and V. G. Kvachev

UDC 578.835.11.083.22

KEY WORDS: retinoic acid; cell culture; enteropathogenic viruses; infectivity titer

Among the various aspects of the biological action of retinoic acid (RA), namely its vitamin A, immunomodulating, and growth-stimulating actions [2, 4, 5], it is the ability of RA to exert a differentiating action on tissue cell cultures that has attracted particular attention.

It was suggested previously that the water-soluble form of RA can be used to increase the sensitivity of embryonic cells in vitro to viruses capable of reproducing only in mature differentiated cells [3]. The experimental verification of this phenomenon and its use in biotechnology has presented new opportunities for the study of cell biology in culture and for increasing the productivity of cultures during reproduction of different viruses.

The aim of this investigation was to study the efficacy of culture of enteropathogenic viruses in RA-modified cell culture.

EXPERIMENTAL METHOD

Reproduction of enteropathogenic porcine viruses was studied in a transplantable culture of hog kidney cells (HK), treated beforehand with RA. The growing cell culture was treated

Ukrainian Veterinary Research Institute, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. V. Kovanov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 93-95, July, 1989. Original article submitted December 26, 1988.

0007-4888/89/0007-1015$12.50

TABLE 1. Effect of Duration of Incubation of Cell Culture with RA on Yield of Virus

Expt. No.	Duration of incubation, h	Infectivity titer of TGE Virus, Strain Purdew-115, log $TCD_{50/1.0}$
1	6	5,5±0,61
2	9	6,0±0,63
3	12	6,5±0,69
4	15	6,78±0,71
5	18	7,48±0,72
6	21	7,24±0,75
7	24	7,23±0,71
8	27	6,0±0,62
9	30	6,0±0,63

TABLE 2. Effect of RA Concentration in Growth Medium in Yield of Virus

Expt. No.	RA concentration in growth medium, %	Infectivity titer of TGE Virus, Strain Purdew-115, log $TCD_{50/1.0}$
1	0,005	5,23±0,49
2	0,001	6,55±0,69
3	0,003	7,78±0,67
4	0,005	7,24±0,75
5	0,01	5,83±0,61
6	Control	5,5±0,61

TABLE 3. Accumulation of Enteropathogenic Porcine Viruses in a Cell Culture Modified by RA

Titration	Infectivity titer of viruses in culture			
	vaccine strain of enterovirus	control	TGE virus	control
1	8,5	7,5	6,83	5,5
2	9,0	7,66	7,24	5,0
3	9,5	7,5	7,17	5,33
4	9,0	7,23	7,23	5,48
5	9,0	7,23	7,0	5,83

with a 0.5% solution of the water-soluble form of RA in a final concentration of 0.001-0.005%. The RA was obtained from the laboratory of chemistry of polyenic compounds (Head, Profressor G. I. Samokhvalov), "Vitaminy" Research and Production Combine, Ministry of the Medical Industry of the USSR.

A transplantable culture of HK cells was grown in 50-ml flasks on a 5% blood digest with 10% bovine serum at 37°C. RA was added to the growth medium 12-24 h before infection of the cell culture, and the flask and its contents were incubated for growth to continue. No RA was added to the control cell cultures.

The cell culture was infected with a vaccine strain of serogroup 6 of porcine enterovirus, according to Derbyshire's classification [1], in a dose of 0.5×10^7 TCD_{50} and with reference strain Purdew-115 of transmissible gastroenteritis virus (TGE) of swine, in a dose of $0.5 \times 10^{5.5}$ TCD_{50}.

After absorption of the corresponding virus for 1 h at 37°C maintenance medium, which was a 5% blood digest without serum, was added to the flasks. The culture inoculated with the porcine enterovirus was incubated for a further 18-24 h at 37°C, whereas that inoculated with TGE virus was incubated for a further 48-72 h. The cultures were kept under observation until the cytopathic action of the virus appeared, when the virus-containing fluid was collected and the yield of the virus in the experimental and control samples was compared relative to infectivity titers.

EXPERIMENTAL RESULTS

Determination of the duration of incubation of the cell culture with RA showed that the highest yields of virus were obtained when the cell culture was treated 12-24 h before infection, the optimal incubation time being 18-20 h (Table 1). Incubation of the cell culture with RA for less than 12 h did not allow sufficient modification of the cells and growth of the infectious virus, but incubation for over 24 had no significant effect on yield.

The infectivity titer of TGE virus, strain Purdew-115, in the control was 5.5 log $TCD_{50/1.0}$.

The data in Table 2 show how the yield of TGE virus depended on the RA concentration in growth medium. The increase in sensitivity of the cell culture took place when the RA concentration in the culture was between 0.001 and 0.005%. A lower concentration did not achieve the required effect on the cells in culture, but a higher concentration led to a reduced yield of the virus due to partial degeneration of the cells as a result of the toxic action of RA in a concentration of 0.01% and a consequent decrease in the population of sensitive, virus-productive cells. The optimal RA concentration in the growth medium was 0.003%.

Culture of the enteropathogenic porcine viruses in a transplantable HK cell culture (Table 3), using the optimal duration of treatment and RA concentration, yielded the vaccine strain of the porcine enterovirus in a titer of (8.33-9.5) log $TCD_{50/1.0}$ ($M_1 = 9.0 \pm 0.95$). In a cell culture without preliminary incubation with RA (control) the infectivity titer of this virus did not exceed (7.23-8.0) log $TCD_{50/1.0}$ ($M_2 = 7.42 \pm 0.81$). Thus the yield of the vaccine strain of the porcine enterovirus was 1.58 log higher in the cell culture modified by RA.

Accumulation of TGE virus in the modified culture was 1.68 log higher, reaching (6.83-7.24) log $TCD_{50/1.0}$ ($M_3 = 7.10 \pm 0.98$) compared with (5.0-5.8) log $TCD_{50/1.0}$ ($M_4 = 5.42 \pm 0.88$) in the control.

Statistical analysis of the data [2] showed that the differences between the mean values M_1, M_2, M_3, and M_4 are statistically significant ($p < 0.05$).

Comparison of the results of the experiments on culture of enteropathogenic viruses in a transplantable culture of NK cells and on virus production in a culture of HK cells showed that preliminary incubation of the cell cultures with RA 12-24 h before infection with virus or with pathological material increases the sensitivity of the treated culture and leads to an increased yield of the cultured viruses.

LITERATURE CITED

1. Yu. I. Afanas'ev, V. I. Nozdrin, and O. I. Mikhailov, Usp. Sovrem. Biol., 95, No. 3, 358 (1983).
2. V. V. Gorodilova and M. N. Boeva, Immunobiology of Tumor Growth [in Russian], Moscow (1983).
3. V. I. Nozdrin, V. G. Kvachev, and T. I. Galenko, Byull. Éksp. Biol. Med., No. 6, 705 (1987).
4. J. B. Derbyshire, Diseases of Swine, Ames, Iowa (1981), Vol. 42.
5. R. Femina and J. S. Hayward, J. Virol., 58, No. 2, 434 (1986).