Part 2. Biotechnology

UDC 602:576.535:547.261>279.52

DIMETHYL SULFOXIDE AS THE STIMULANT ANTIGEN PRODUCING ACTIVITY OF THE CELL CULTURE FLK-BLV

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Summary. The article observes a capability of the dimethyl sulfoxide (DMSO) use to stimulate the leukemia virus expression and antigen producing activity. Adhesive and proliferative activities of stimulated cell culture FLK-BLV were assessed at each passage of cell growth. Antigen producing activity was determined after the adaptation period in the 5–10 passages by RID. The addition of 1.0% dimethyl sulfoxide (DMSO) positively effects on the cells monolayer within 10 passages of the experiment. The activity of the experimental antigen produced on nutrient medium with the addition of the DMSO was higher 1.75 times than in control group.

Keywords: leukosis, cattle, cells culture, radial immunodiffusion, leukemia virus

Introduction. In modern conditions two serological methods for the identification of the animals infected leukemia virus have been developed: a radial immunodiffusion (RID) and the enzyme-linked immunosorbent assay (ELISA) (OIE, 2008; State Committee of Veterinary Medicine of Ukraine, 2007). Last method used in diagnostic practice in most countries because of high specificity and simplicity. The RID is characterized by light sensitivity threshold. It is causes the registration of seroconversion process in animals infected leukemia virus 2–3 weeks later compared to the productivity of using ELISA (OIE, 2017; Hammar and Gilljam, 1990). In relation to this, the urgent issue is the improvement of RID test systems, towards lowering the roles above named disadvantage regarding the sensitivity threshold (Gilljam, Siridewa and Hammar, 1994; Hammar, Eriksson et al., 1989). This can be achieved by optimizing the culture medium toward expression of viral mass in order to leukemic antigen activity increase (Hammar, Merza et al., 1989). An analysis of literature indicates the culture medium optimization may be provided by introduction to some of biological and chemical ingredients (De Giuseppe et al., 2004; Dyakonov, 2009).

We use dimethyl sulfoxide (DMSO) in our studies as a method of expression leukemia virus. Dimethyl sulfoxide is a chemical substance which is used in pharmacology. DMSO affects the cell metabolism; it has fibrinolytic activity. Dimethyl sulfoxide is characterized by sorbent properties; it increases throughput membranes for lipopolysaccharide. The drug does not effect on proliferation of cell culture FLK-BLV. DMSO facilitates the transition of cells on monolayer cultures from non-productive in the productive state. It increases replication of leukemia virus in vitro and synthesis glycoprotein antigen. If dimethyl sulfoxide is added to the growth medium for the culture long-term cells FLK-BLV, it will continue to product age of formed monolayer to eight and more days [9].

Materials and methods. The long-term cell culture FLK-BLV was grown by conventional methods with nutrient medium. We formed 5 groups — 4 experimental and 1 control. DMSO was added into the culture medium of research groups in a ratio under 0.5, 1.0, 1.5, 2.0, 3.0%. Nutritional culture medium included 45% Eagle medium, 45% 199 medium, 10% native bovine serum. P*assage* culture *cells* served as the fulfillment of a monolayer.

Adhesive and proliferative activities of stimulated cell culture were assessed at each passage of the cell growth. The effect of the stimulant on the morphology of the cell culture, cells vitality, and speed of monolayer performing were evaluated every day by microscopy of fixed and stained cells FLK-BLV.

The activity of stimulated cell culture was evaluated with control group (monolayer cultured without stimulation). The cell culture FLK-BLV morphology of experimental and control samples was studied. We pay attention to the cell ability to actively proliferate, length of productive state cell monolayer, shape and size of the cells as the fulfillment of a monolayer, the presence of vacuoles and inclusions.

Antigen producing activity of stimulated cultures after the adaptation period was determined in the 5–10 passages. To make this work, leukemic antigens for RID from accumulated culture fluids of experimental and control groups were produced. The resulting culture fluids in each group were tested for sterility by bacteriological methods. The fluid was twice defrosted, and than lit by low-speed centrifugation followed by concentration to 1/100 of the original volume by forced dialysis against PEG 6000.

The final product, which was subjected to test a specificity and activity by RID with using a positive and negative control diagnostic serum, was concentrated as antigen. The leukemic antigen was resuspended in *phosphate* buffered *saline*, pH (7.0–7.2).

Results. The fact of the addition dimethyl sulfoxide to the nutrient medium at the first passage not produced a negative effect on cell cultures has been established. Cells are well attached to the glass. During the first day culture monolayer formed on 95–100% compared to control groups. The formation of monolayer in control groups was late for 1.5–2 days. Monolayer's destruction was observed for 2–3 days before. The formation of cell culture FLK-BLV monolayer influenced DMSO at 5th passage shown in Table 1.

Table 1 – The formation and preservation of cell culture FLK-BLV monolayers of experimental and control samples at the 5th passage

Group name	Attaching and preservation of monolayer cells FLK-BLV, %							Multiplicity reseeding cells at passages
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	
Control	–	65	100	100	85	70	40	1:1
D1 (0.5%)	90	100	100	100	100	100	100	1:4
D2 (1.0%)	85	100	100	100	100	100	100	1:4
D3 (1.5%)	95	100	100	100	100	100	90	1:3(1:4)
D4 (2.0%)	90	100	100	100	100	100	100	1:3
D5 (3.0%)	–	60	80	85	70	50	35	–

The table 1 shows, that adding 3.0% DMSO to the nutrient medium has a toxic effect on cells culture FLK-BLV. This negative impact has been observed on the first day after passage. Culture medium with the addition of DMSO in other experimental concentrations from the first day of passage had positive effect on the cell cultures. The cells were well attached to the walls of culture dishes. During the first day monolayer formed on 95–100% compared to control group, where the forming , process was longer than 1.5–2 days, and cells destruction was observed for 2–3 days before. All research groups (except D5) at the seventh day of cultivation maintained monolayer, and had a high potential for subsequent reproduction.

DMSO (group D1, D2 and D3) has a positive effect on the cell monolayer for 5 experiment passages. Dimethyl sulfoxide continued to age cells functional activity, as evidenced by typical for this culture elongated cells without inclusions and vacuoles, integrity and availability of monolayer growth areas. High functional activity of cells persisted throughout the passage, in contrast to the control samples. Control cells were active only on cell division the first 2–3 days after passage. In the following terms cultivation cells grew old, and we observed inclusions and vacuoles in the cytoplasm. The formed zone of layers led to the rapid destruction of monolayer.

Adding to the nutrient medium 1.0% and 1.5% DMSO significantly effect on the cell adhesion to the substrate throughout of the experimental cultivation (10 passages). By the end of the 1st day of each passage transparent monolayer of the typical cell culture FLK-BLV was formed. The monolayer formation was observed in the control groups at the end of 2nd day. For groups of cell culture FLK-BLV (D2, D3), where the dispersion of Versen solution was insufficient, we used a mixture of Versen 0.02% (90%) and trypsin solution 0.25% (10%).

The proliferative activity of cell culture FLK-BLV under the influence of dimethyl sulfoxide evaluated on the fourth day of 1st, 5th and 10th passages cultivation. Results of experiments are shown in Table 2.

The Table 2 shows the introduction to the nutrient medium 1.0% and 1.5% DMSO positively influence the process of cell division. Evaluation results of proliferation with DMSO stimulation statistically higher than those of control samples.

Table 2 – Evaluation cell culture FLK-BLV proliferative activity

Group	The growth of cells on the fourth day of cultivation, %		
	1st passage	5th passage	10th passage
D1	18	19	18
D2	19	23	21
D3	18	23	19
D4	14	–	–
D5	9	–	–
Control	13	15	17

The experience for comparative determination of antigen producing activity of experimental and control fluids at 5th and 10th passages was carried out. After a double defrosting the control fluid was concentrated by forced dialysis method against PEG 6000 to 1/10 of the original volume. Total experiments with DMSO 5 experimental and 1 control antigens were received. Control antigens subjected to testing for specificity and activity by RID with using a positive and negative control diagnostic serum. The result of the tests is shown in Table 3.

These results in Table 3 show that the antigens cultured of cell culture FLK-BLV in nutrient medium with 1% DMSO were most active by RID (1.7 times higher than the control in the 5th passage; 1.75 — in the 10th passage). The antigen activity of experimental culture on the 10th passage was above than in control culture on the 5th passage. It is indicates an increase of functional activity experimental cell culture as the passage.

Table 3 – Antigen activity of experimental and control samples

Group	5th passage		10th passage	
	Antigen activity by RID	High activity of control, times	Antigen activity by RID	High activity of control, times
Control	1:2		1:2	
D1	1:2.2	1.1	1:2.5	1.25
D2	1:3.4	1.7	1:3.5	1.75
D3	1:3.2	1.6	1:3	1.5
D4	1:2.8	1.4	1:2.8	1.4
D5	1:1.6	≤ 0.2	–	–

Conclusions. 1. The addition of 1.0% dimethyl sulfoxide (DMSO) positively effects cells monolayer within 10 passages of the experiment. DMSO prolongs cells functional activity compared to controls.

2. The activity of the experimental antigen against leukemia virus produced on nutrient medium with the addition of the DMSO was higher 1.75 times compared to controls.

3. The antigen production system which use the developed optimized nutrient medium may be recommended for testing under conditions of biological enterprises for enhance antigen-producing activity of cell culture and for using in research.

References

De Giuseppe, A., Feliziani, F., Rutili, D. and De Mia, G.M. (2004) 'Expression of the bovine leukemia virus envelope Glycoprotein (gp51) by recombinant Baculovirus and its use in an enzyme-linked Immunosorbent assay', *Clinical and Vaccine Immunology*, 11(1), pp. 147–151. doi: 10.1128/cdli.11.1.147-151.2004.

Dyakonov, L. P. (ed.) (2009) Animal cells in culture (methods and application in biotechnology) [Zhivotnaya kletka v kul'ture (metody i primenenie v biotekhnologii)]. Moscow: Sputnik+. ISBN 9785997302146. [in Russian].

Gilljam, G., Siridewa, K. and Hammar, L. (1994) 'Purification of simian immunodeficiency virus, SIVMAC251, and of its external envelope glycoprotein, gp148', *Journal of Chromatography A*, 675(1-2), pp. 89–100. doi: 10.1016/0021-9673(94)85263-4.

Hammar, L., Eriksson, S., Malm, K. and Morein, B. (1989) 'Concentration and purification of feline leukaemia virus (FeLV) and its outer envelope protein gp70 by aqueous two-phase systems', *Journal of Virological Methods*, 24(1-2), pp. 91–101. doi: 10.1016/0166-0934(89)90011-6.

Hammar, L. and Gilljam, G. (1990) 'Extraction of HIV-1 in aqueous two-phase systems to obtain a high yield of gp120', *AIDS Research and Human Retroviruses*, 6(12), pp. 1379–1388. doi: 10.1089/aid.1990.6.1379.

Hammar, L., Merza, M., Malm, K., Eriksson, S. and Morein, B. (1989) 'The use of aqueous two-phase systems to concentrate and purify bovine leukemia virus outer envelope protein gp51', *Biotechnology and Applied Biochemistry*, 11(3), pp. 296–306. doi: 10.1111/j.1470-8744.1989.tb00062.x.

OIE (World Organisation for Animal Health) (2008) 'Chapter 2.4.11. Enzootic Bovine Leukosis', in *Manual of diagnostic tests and vaccines for terrestrial animals*. 6th ed. Vol. 2, pp. 729–738. Available at: http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.11_EBL.pdf.

OIE (World Organisation for Animal Health) (2017) *Enzootic bovine leukosis*. Available at: http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countrytimelines (Accessed: 17 January 2017).

State Committee of Veterinary Medicine of Ukraine (2007) Order № 21 from 21.12.2007 'On approval of Guidance on prevention and rehabilitation of cattle from bovine leukemia' [Nakaz № 21 vid 21.12.2007 'Pro zatverdzhennia Instruktsii z profilaktyky ta ozdorovlennia velykoi rohatoi khudoby vid leikozu'], *Official Bulletin of Ukraine [Ofitsiinyi visnyk Ukrainy]*, 4, p. 39, art. 116. Available at: http://zakon.rada.gov.ua/laws/show/z0012-08. [in Ukrainian].