Short communication

Removal of Viral Pathogens from Calf Serum by Gamma Irradiation Techniques

Mohammadi*§, A., Mirchamcy, H., Shafyi, A.

§: Amo@rvsri.com

Human Viral Disease Vaccines Research & Production Dept., Razi Vaccine & Serum Research Institute, P.O.Box 11365-1558, Tehran, Iran

Received 26 Dec 2001; accepted 15 Feb 2002

Summary

To study the effect of gamma rays on viral pathogens of animal sera, samples of three virus preparations which belonging to different families were exposed in the frozen state to cobalt-60. Each samples was tested for reduction in virus titer. The rate of inactivation for viruses tested indicated that gamma radiation (^{60}cobalt) proves to be an effective method for inactivation of some viruses which presence in animal sera.

Key words: gamma irradiation, virus, calf serum, inactivation

Introduction

In the absence of optimized serum-free culture system it is often necessary to supplement the cell culture media with animal sera. Animal sera often harbor adventitious viruses such as Bovine viral diarrhea (BVD), mucosal disease (MD) and infectious bovine rhinotracheits (IBR). Which can not be totally removed even by most vigorous aseptic filling and manufacturing procedures.

The presence of such viruses not only interferes with the study being carried out (Calfat *et al* 1976) but also with the production of biologically active products, raising significant regulatory concerns in the manufacture of therapeutic human or animal products (QBP 1997a). However, because animal sera are complex and undefined mixtures of many biologically active compounds such as nutrients, vitamins, growth factors and attachment factors, many of the treatments used to

eliminate adventitious viruses also have a negative impact on the growth-promoting properties of sera (Giard 1987). Several approaches to inactivate viruses from biological have been developed and studied. These include the use of physical separation or partition technique (QBP 1997b), chemical disinfectants (Spire *et al* 1984, 1985) and the use of heat, gamma radiation or UV light (House *et al* 1990, Spire *et al* 1985). Gamma irradiation has been found to be an effective way to inactivate both animal viruses and mycoplasmal agent (Spire *et al* 1984, Sullivan *et al* 1974, Thomas *et al* 1981).

We have chosen an irradiation protocol for calf serum, which allows for optimal viral killing. This is accomplished without significantly reducing the ability of the serum to promote cell growth of a culture applications.

Materials and Methods

Preparation of calf serum. Three different batches of calf serum were used. The bloods were collected from low age calf (below 6 month) from a slaughterhouse (Ziaran, Tehran province). After centrifugation at 4500rpm at room temperature and isolation of clot, primary filtration was made by 0.2-0.45μm Millipore filter and last filtration with 0.2μm size.

Gamma Irradiation of calf serum. Gamma irradiation of sera was carried out at Atomic Energy Organization (AEO) of Iran using a cobalt-60 source (a patent document). The calf serum used in this study was irradiated as final product in 1000ml polyethylen glycol (PEG) bottles. Sera were irradiated at 15, 20, 25, 35 and 45KGY doses.

Virus. The viruses used in this study were chosen to reflect different physiochemical characteristics including IBR (*Herpesviridae*, ds-DNA, enveloped, 100nm), BVD (*Flaviviridae*, ss-RNA, enveloped, 30-60nm) and BR-3 (*Reoviridae*, ds-RNA, enveloped, 75-80nm) viruses. All of the viruses were passaged in R.B.K (Razi Bovine Kidney cell line) cell cultures. Each virus material with known titer was spiked 1:10 (V/V) to different sterilized serum sample.

Irradiation. The material (virus+serum) frozen quickly and irradiated at irradiation protocol proprietary of AEO, Gamma-Ray unit. Following irradiation the residual viral titers were determined by an independent commercial laboratory using a plaque assay system.

Virus titration. All of the viruses were passaged in R.B.K cell cultures. Cell sheets showing advanced cytopothogenic effect were frozen and thawed three times and the virus was harvested. The Harvested was clarified by centrifugation at 2000rpm for 10min at 4°C. The titers of harvested virus were determined by using a plaque-forming unit assay system.

Biochemical analysis. A complete biochemical analysis of the irradiated and control sera was performed by an independent testing laboratory (Bahar, Tehran) and included quantitative analysis of serum proteins, enzyme and hormone levels.

Biochemical performance testing. For evaluating the effect of gamma irradiation on the ability of serum to support cell growth the following cell lines were used: Vero (African green monkey cells), MRC-5 (Human diploid fibroblast) and CHEF (Chicken-embryo fibroblast). The biological tests were long-term growth assays.

Cell growth in multiple passage long-term assays. A panel of continuous cell lines, diploid cell strain and primary chicken embryo fibroblast was selected to determine whether gamma irradiated serum could support sequential sub-culturing. Cells were inoculated into $175cm^2$ Roux bottles at densities of 1×10^5–1.5×10^5 cells/flask (depending upon cell line) containing DMEM+%5 calf serum at final concentration. The flasks were incubated at 37°C and were harvested every 4-7 days (depending on cell line) counted and sub-cultured for a total of three passages. All of the cells grew equally well in the irradiated or control sera of special note is the fact that cell morphology was consistent with that of the control culture for each of the cell lines studied. Vero cell and MRC-5 cell passaged twice weekly and weekly, respectively and CHEF cell (primary culture) produced weekly.

HPLC. The effect of gamma irradiation on quality of serum also tested by high performance liquid chromatography (HPLC). The fetal calf serum (Gibco) was used as standard.

Results and Discussion

Dose dependent decline in viral titer. The viruses show a dose dependent decline in titter under conditions of gamma irradiation (Table 1). All the viruses showed a significant decline at 25, 35 and 45 KGY. But the turbidity of serum under doses 35 and 45 was very much and makes trouble in color of media so 25KGY chose as best condition for irradiation.

Table 1. *The effect of increasing doses of gamma radiation on the viral titer*

Radiation dose (KGY)	Infectious bovine rhinotracheities*	Bovine viral diarrhea*
Control	1×10^{8}	2.5×10^{7}
15	5×10^{6}	3×10^{6}
20	3×10^{5}	2.2×10^{5}
25	2.3×10^{0}	1×10^{0}
35	≤0.5	≤0.5
45	≤0.5	≤0.5
Reduction factor	≥6.7	≥6.78

*: plaque assay

Biochemical profile of irradiated bovine calf serum. Bovine calf serum is a complex mixture of many molecules. All these components are responsible for the growth promoting properties of the sera. No changes were observed in physiochemical parameters such as pH or osmolarity. In addition gamma irradiation did not alter endotoxin levels, hormone levels or the appearance of bovine calf serum.

Cell growth in multiple passage long-term assays. For approximately 2 years no differences in growth of cell lines have been shown in the irradiated sera in comparison with non-irradiated control sera (Table 2).

HPLC. The effect of gamma irradiation on quality of calf serum was evaluated by HPLC (Fig 1a, b). As shown, for irradiated serum (b) HPLC diagram completely changed and close to fetal calf serum. Gibco calf serum used as standard.

Sterilization in biopharmaceutical processing known as the important problem in good manufacturing process. Filtration is one of the important processes for removing of contaminating virus and bacteria from biological products. The pore size of these filters defined between 0.2-0.45μm, but in 1998 Mortality and Morbidity

Table 2. *The effect of increasing doses of gamma radiation on the ability of serum to support the long-term growth of cell lines in culture*

Cell line	Radiation dose (KGY)	Growth (%of control)
Vero	15	97±3
	20	99±2
	25	95±3
	30	95±4
	35	91±2
	40	88±5
MRC-5	15	99±3
	20	100±2
	25	99±3
	30	96±4
	35	98±3
	40	98±4

Average±SEM of p1 and p3 for three different calf serum batches

Weekly Repoet (MMWR) wrote that *Ralstonia picketti* (a very small bacterium) might not have been completely retained in the filtration process. On the other hand animal sera as a necessary supplement of culture media often contain harbor adventitious viruses (Rossi *et al* 1984, Yanagi *et al* 1996) which can not be totally removed even by most rigorous aseptic procedures and raising significant concern in the biological product (Revised CGMP 1996, QBP 1997a). Several approaches to inactivate viruses from biological have been developed and studied. These include the use of physical separation (QBP 1997b), chemical disinfectants (Spire *et al* 1984, Dichtelmuller *et al* 1993) and the use of heat or UV (House *et al* 1990, Spire *et al* 1985) and ultrafiltration. However, because of animal sera in cell culture contain many biologically active compounds such as nutrient, vitamins and attachment factor many of the treatment used to eliminate pathogens also have a negative impact on the growth-promoting properties of sera (Giard 1987). As our study has shown use of gamma irradiation significantly reduces the titter of adventitious virus without any destroying affect on growth promoting properties of the sera. This method is safe and scalable which is accepted by the Food and Drug Administration (FDA) and European Pharmacopea (E.U) (Commission of European Communities 1992).

At present the serum which is used for production of human viral vaccines in Razi Institute irradiated by ^{60}cobalt at 25KGY after prefiltration and filtration (0.2μm pore size).

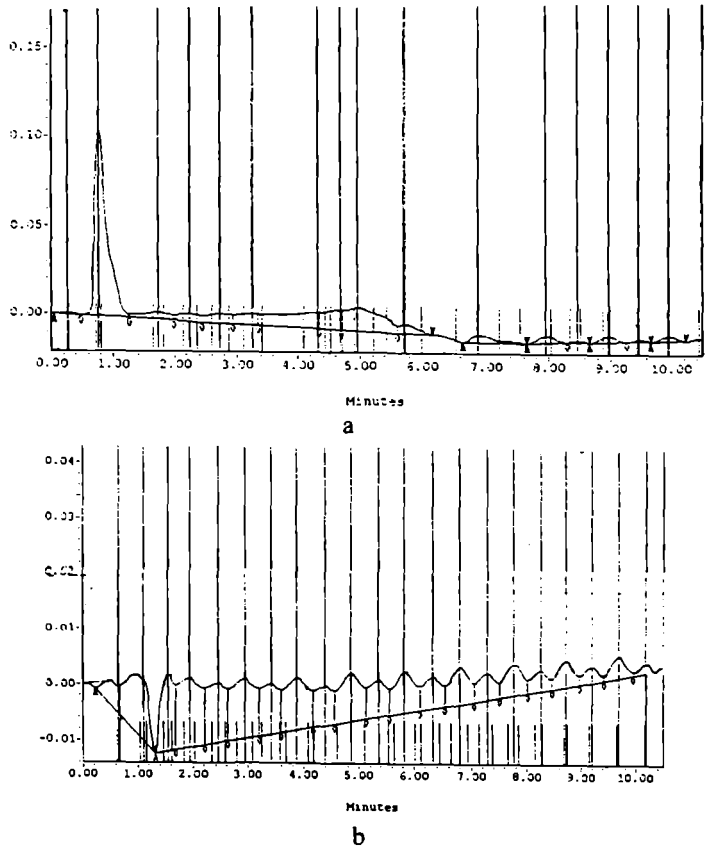

Figure 1. *Comparison between HPLC FIG of irradiated (a) and non-irradiated sera (b)*

References

Commission of European Communities. (1992). 111/8115/89-EN.

Clafat, J. Hageman, P.C. (1976). Virus–like particles in bovine sera for tissue culture. *Journal of General Virology* 33:151-154.

Dichtelmuller, H., Rudnik, D., Breuer, B. and Ganshirt, K.H. (1993). Validation of virus inactivation and removal for the manufacturing procedure of two immunogloblins and a 5% serum protein solution treated with b-propiolactone. *Biologicals* 21:259-268.

Giard, D.J. (1987). Routine heat inactivation of serum reduces its capacity to promote cell attachment. *In Vitro Cellular and Developmental Biology* 23:691-697.

House, C., House, J.A. and Yedloutschnig, R.J. (1990). Inactivation of viral agents in bovine serum by gamma irradiation. *Canadian Journal of Microbiology* 36:737-740.

Quality of Biotechnological Products. (1997a). Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. ICH/Q5H.

Quality of Biotechnological Products. (1997b). Viral titer reduction demonstrated by sequential challenge of a tangential flow membrane filtration system and a direct flow pleated filter cartridge. *Journal of Pharmaceutical Science and Technology* 51:208-212.

Rossi, C.R., Bridgman, C.R. and Kiessl, G.K. (1980). Viral contamination of bovine fetal lung cultures and bovine fetal serum. *American Journal of Veterinary Research* 41:1680-1681.

Revised CGMP Guideline on Medicinal Products Derived from Human Blood and Plasma (1996). CPMP/269.

Spire, B., Barre-sinoussi, F., Montagnier, L. *et al.* (1984). Inactivation of lymphadenopathy associated virus by chemical disinfectants. *Lancet* ii:899-901.

Spire, B., Barre-Sinoussi, F., Dormont, D. and Montagnier, L. (1985). Inactivation of lymphadenopathy–associated virus by heat, gamma rays and ultraviolet light. *Lancet* January 26:188-189.

Sullivan, R., Fassolitis, A.C., Larkin, E.P., Read, R.B. and Peeler, J.T. (1971). Inactivation of thirty viruses by gamma radiation. *Applied Microbiology* 22:61-65.

Thomas, F.C., Davies, A.G., Dulac, G.C., Willis, N.G., Papp-Vid, G. and Girard, A. (1981). Gamma ray inactivation of some animal viruses. *Canadian Journal of Comparative Medicine* 45:397-399.

Yanagi, M., Bukh, J., Emerson, S.U. and Purcell, R.H. (1996). Contamination of commercially available fetal bovine sera with bovine viral diarrhea virus genomes: Implicatios for the study of hepatitis C virus in cell cultures. *Journal of Infectious Diseases* 174:1324-1327.