

Inactivation of nano- FMDV type O/IRN/1/2007 particles infectivity using gamma irradiation

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Abstract

Foot and Mouth Disease Virus (FMDV) is a vesicular and contagious disease of cloven-hoofed animals. This study is aimed to inactivate FMD Virus type O/2007/IRN particles by gamma irradiation with unaltered antigenicity. FMD Virus type O/2007/IRN was propagated on BHK21 cells. The virus titration was calculated by TCID₅₀ method. A ⁶⁰Co-gamma cell instrument Nordian, model 220 with dose rate 4.8 Gy/sec and activity of 20469 Ci was used for the inactivation of the frozen FMD virus samples. Safety test was done by cell culture method, as well as antigenicity of irradiated and non-irradiated virus samples were evaluated by Complement Fixation Test. Irradiated and non-irradiated FMDV particles were concentrated by centrifugation and studied under electron microscopy. The virus titration decreased gradually by increasing of gamma ray doses, according to the Dose/Survival curve for irradiated samples. The D₁₀ value factor (dose of gamma ray which can decrease 90% of virus population) and the optimum dose of gamma ray for FMDV type O/2007/IRN inactivation and unaltered antigenicity was obtained 5-5.5 kGy and 45-50 kGy, respectively. The irradiated inactivated FMD virus with unaltered antigenicity can be used as candidate radio-vaccine with superior safety.

Keywords: Foot and Mouth Disease Virus, Gamma irradiation, Inactivation,

Introduction

Foot and Mouth Disease Virus (FMDV) causes a highly contagious vesicular disease in cloven hoofed animals, in particular swine, cattle, sheep, goats, and deer. Severe economic loss was evidenced during the FMD epidemic in the United Kingdom in 2001. The relevant causative agent is Foot-and-Mouth Disease virus (FMDV), with 27-30 nm in diameter and is an Aphovirus of the Picornaviridae family, which there are seven serotypes. Each virion consists of a single stranded positive sense RNA genome, enclosed within an icosahedral capsid, comprising 60 copies of structural proteins (VP1-4). In addition to these structural proteins, the viral genome encodes seven non-structural proteins (Bergamin *et al.*, 2007).

In countries where disease eradication has not been achieved, vaccination plays a crucial role in its control. Although chemical inactivated virus vaccines effectively prevent FMD, their use is accompanied by dangerous chemical residues potency problems (Beck *et al.*, 1987; Doel *et al.*, 2003; Suttmoller *et al.*, 2003; Mingxiao *et al.*, 2007). Chemical inactivation using Binary ethylenimine takes 24-48 hours, while time of inactivation by ionizing radiation decrease a few minutes. Therefore, the advantages of gamma radiation for virus inactivation were decreasing of inactivation time and antigenic changes, without chemical residues. Foot-and-mouth disease outbreaks may lead to explosive epidemics with a major financial impact in agriculture worldwide. For immunity against FMDV, a high level of neutralizing antibodies correlates with protection against challenge infection (Van *et al.*, 1969; Suttmoller *et al.*, 1980; Mccullough *et al.*, 1992; Doel *et al.*, 2003; Bergamin *et*

al., 2007). The aim of this study is impart complete inactivation of FMV type O/IRN/1/2007 using ⁶⁰Co-gamma irradiation without chemical residues, in order to prepare irradiated vaccine in the future study.

Materials and methods

Virus multiplication

FMD virus type O/IRN/1/2007 was propagated in BHK21 (NCBI code: C107, which purchased from Pasteur institute of Iran) suspension culture, by Earl's modified Eagle's medium (EMEM) and 0.5% bovine serum at 37 °C incubator for 18 h (Motamedi Sedeh, 2007). The cytopathic effect (CPE) was observed on BHK21 cells as lyses and suspended detached cells by viability cell count method (Tolnai 1975). The virus suspensions were centrifuged at 1000 ×g for 15 min at 4°C, the supernatants was stored at -70 °C (Barteling *et al.*, 1991; Donn *et al.*, 1995; Feng *et al.*, 2003).

Virus titration

The virus particles per ml which can make CPE at 50% inoculated cells was called Tissue Culture Infection Dose₅₀ /ml (TCID₅₀ /ml) and calculated by Read and Munch method (Reed & Muench, 1938).

Gamma radiation of FMD

A Nordian, Model 220 gamma cell instrument was used at a dose rate of 4.8 Gy/sec and activity of 20469 Ci to irradiate FMDV RNA and thus inactivate virus infectivity. Doses of 10, 15, 20, 25, 30, 35, 40 and 45 and 50 kGy were used to samples kept frozen on dry ice and 3 samples were irradiated at each dose. Also duration of the virus samples radiation were 17.36 min, 34.72 min, 69.44 min, 86.80 min, 104.16 min, 121.52 min, 138.88 min, 156.25 min and 173.61 respectively (Lombardo,

1990; Smolko, 2005). There was one negative control sample at the same condition of each experience.

Safety test and antigenicity evaluation

All of the irradiated virus samples were inoculated on IBRS2 cells (obtained from Razi of Vaccine and Serum Research Institute of Iran), and the virus titration was obtained by TCID₅₀ method. The irradiated virus samples were inoculated on IBRS2 cells by Earl's modified Eagle's medium (EMEM) at 37 °C incubator with 5% CO₂ for 18 h, then it sub cultured on another IBRS2 cells for four times. Antigenicity of irradiated and non-irradiated FMDV samples were tested by Complement Fixation test (CF test) (Frescura *et al.*, 1973).

Concentration and evaluation of FMDV type O particles using electron microscopy (E.M.)

Irradiated and non-irradiated FMDV type O/IRN/1/2007 particles were clarified by centrifugation at 500 x g for 10 min at 4°C and ultra-centrifuged at 112400 x g (Beckman, Model L2-65 B) for 2 h at 4°C, and the pellet was resuspended in the 1.0 ml PBS. The irradiated and non-irradiated FMDV particles were evaluated by electron microscopy, a formvar-coated grid was floated on 10 µl virus suspension and negatively stained (with 2% phosphor-tungstic acid solution) and examined using a Philips - EM 208S electron microscopy at 100 KV.

Statistical analysis

Statistical analysis was done by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. Significance was defined at P value < 0.05.

Result

The titration of FMD virus type O/2007/IRN by TCID₅₀ was obtained 10^{8.5}/ml. The virus titration of non-irradiated and irradiated FMDV samples by different doses of gamma ray (0, 10, 15, 20, 25, 30, 35, 40, 45 and 50 kGy)

Fig. 1. Dose/Survival curve for irradiated FMDV type O/2007/IRN

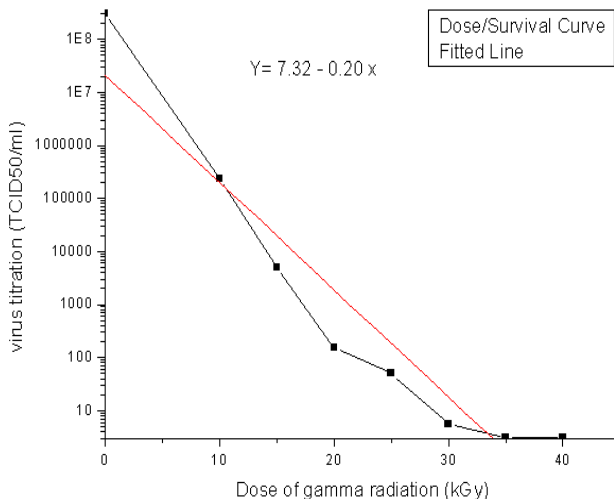


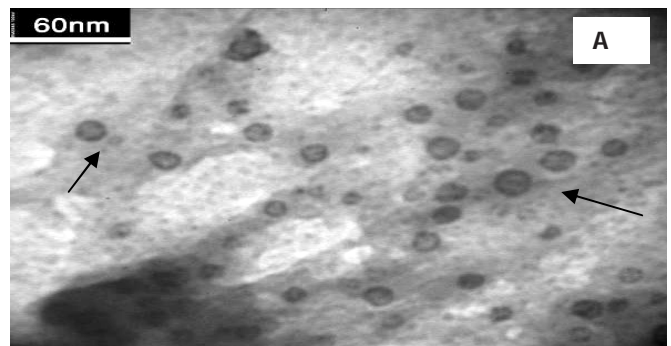
Table 2. Complement Fixation Test for irradiated and non-irradiated FMD type O/IRN/1/2007 samples as antigenicity

Irradiation Dose(kGy)	Dilution of FMDV type O/2007/IRN (10 ^{8.5} TCID ₅₀ /ml)				
	1	1/2	1/4	1/8	1/16
0	4	4	4	Tr	0
5	4	4	4	Tr	0
10	4	4	4	Tr	0
30	4	4	1	Tr	0
40	4	4	1	Tr	0
45	4	4	0.5	Tr	0
50	4	4	0.5	Tr	0

were 10^{8.5}, 10^{5.36}, 10^{3.57}, 10^{2.19}, 10^{1.37}, 10^{0.75}, 10^{0.5}, 10^{0.5}, 10^{0.5} and 10^{0.5}, respectively (Table 1). The Fig. 1 shows Dose/survival curve of irradiated samples, which is drawn by Origin software. According to the Table 1 and Fig. 1, D₁₀ value factor (dose of gamma ray which can decrease 90% virus population) was obtained 5- 5.5 kGy and the virus titration was decreased gradually by increasing of gamma ray doses. According to ANOVA- one way method there are significant differences in different doses of gamma radiation and virus titration (P < 0.05). The optimum dose of gamma ray for FMD Virus inactivation with virus titration 10^{8.5} TCID₅₀ /ml was obtained 45 -50 kGy, the antigenicity remained essentially unchanged as measured by complement

Fig.2. Electron microscopy evaluation of nano- FMDV type O/IRN/1/2007 particles,

(A) non-irradiated viral particles



(B) irradiated viral particles



Table 1. Virus titration for irradiated and non-irradiated FMD type O/IRN/1/2007 as TCID₅₀/ml

Dose (kGy)	0	10	15	20	25	30	35	40	45	50
TCID ₅₀ /ml	10 ^{8.5}	10 ^{5.36}	10 ^{3.57}	10 ^{2.194}	10 ^{1.375}	10 ^{0.75}	10 ^{0.5}	10 ^{0.5}	10 ^{0.5}	10 ^{0.5}

fixation. The results of safety test for irradiated samples with gamma ray doses of 45 and 50 kGy were suitable since not showed CPE after four times blind culture.

The results of CF test were showed in Table 2. The results of CF test for irradiated and non-irradiated samples showed fortunately the antigenicity of irradiated *FMD virus* from 10-50 kGy was not changed.

The concentration of FMDV type O/IRN/1/2007 antigen particles were measured 3.9 mg/ml and 0.4 mg/ml for non-irradiated and irradiated viral particles, respectively by drop (ND-1000). According to Fig. 2, evaluation of non-irradiated and irradiated FMDV type O/IRN/1/2007 particles using E.M. was shown degradation of viral nucleic acid.

Discussion

FMD inactivated vaccines play a key role in control campaigns and eradication of FMD (Doel *et al.*, 2003). But vaccine produced from the tissue culture virus is associated with the risk of virus release during vaccine production and with the risk of improper inactivation of the virus leading to vaccine related outbreaks (Barteling *et al.*, 1991; Doel *et al.*, 2003). Therefore, it is profitable to develop novel vaccines to reduce these elements of risk.

Known methods of virus inactivation are based on the chemical action of some substance such as acetylenimine, binary ethylenimine (BEI), betapropiolactone, formaldehyde, etc. In such a process the viral suspension should be kept at room or higher temperatures for 24-48 h. Under these conditions, physical and chemical agents act to degrade the virus antigenic proteins. On the contrary with ionizing radiation at low temperatures, the treatment doses not cause such degradation which allowing the study of different viral functions (Lombardo & Smolko, 1990; Doel *et al.*, 2003).

Irradiated inactivated viruses have been reported to retain most of their antigenicity. Lombardo and Smolko (2005) from Argentina studied for the production of some inactivated vaccines by ionizing irradiation of some viruses such as: FMD virus and Herpes Simplex virus (Smolko & Lombardo, 2005). Motamedi *et al.* (2008) showed gamma radiation to inactivate FMDV type A87/IRN without any change in antigenicity. They also used the inactivated virus as radio-vaccine to induce the immune response on guinea pigs, it showed three dilutions of the radio-vaccine (1:1, 1:2 and 1:4) can immunize the guinea pigs as well as the normal vaccine. In this study the optimum dose of gamma ray for inactivation of frozen FMD Virus type O/IRN/1/2007 particles, without any change in antigenicity was obtained using 45-50 kGy. Therefore the irradiated inactivated FMD virus with unaltered antigenicity can be used as candidate radio-vaccine with superior safety in the future study. The E.M evaluation of the non-irradiated and irradiated FMDV particles can also confirmed the mechanism of nucleic acid degradation by gamma radiation.

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