大韓獸醫學會誌(1993) 第33券 第4號
Korean Vet Res (1993) 33(4) : 649~655

Effects of γ-Irradiation from Cobalt-60 on pathogenicity of *Eimeria tenella*

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(Received September 18, 1993)

Cobalt-60 감마선 조사가 *Eimeria tenella*의 병원성에 미치는 영향

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(1993년 9월 18일 접수)

초록 : *E tenella*의 오오시스트에 대한 Cobalt-60 감마선 조사선량과 접종용량을 결정하고 감마선으로 처리한 *E tenella*와 그 계대원충을 닭에 접종하여 병원성을 조사하고자 일련의 시험을 실시하여 다음과 같은 결과를 얻었다. 증체량, 혈변도 및 병변도 등을 비교할 때 Cobalt-60 감마선 조사량은 100 Gy의 수준에서 그리고 계대원충 접종군에서는 10,000개의 오오시스트 수준에서 약간의 병원성이 나타났다. 혈변도, 병변도 및 분변내 오오시스트 배설수는 감마선 조사원충 접종군에서 가장 낮게 나타났고, 1대 및 3대 계대원충 접종군에서는 보통 수준으로 그리고 감마선 조사하지 않은 원충 접종군에서 가장 높았다. 감마선 조사원충 접종군의 증체량이 무감염 대조군을 제외한 다른 시험군보다 높게 나타났다. 1대 및 3대 계대원충 접종군의 증체량이 감마선 조사하지 않은 원충 접종군의 것보다 높게 나타났다. 감마선 조사원충 접종군의 사료요구율이 무감염 대조군을 제외한 다른 시험군보다 낮게 나타났다. 1대 및 3대 계대원충 접종군의 사료요구율이 감마선을 조사하지 않은 원충 접종군보다 낮게 나타났다. 감마선 조사원충 접종군의 항콕시듐지수(190.6)는 1대 및 3대 계대원충 접종군의 항콕시듐지수(142.8과 107.4)보다 높았고, 감마선 조사하지 않은 원충 접종군의 것(87.4)보다는 월등히 높게 나타났다. 그러므로 감마선 조사후 계대수가 증가할수록 병원성이 회복되는 것으로 나타났다. 이는 계대수가 증가할수록 감마선 조사로 인하여 병원성이 약화된 원충의 수는 줄어들고 감마선의 영향을 받지 않은 병원성이 강한 원충의 수가 증가되는 것으로 사료된다.

Key words : Eimeria tenella, Cobalt-60, γ-irradiation, pathogenicity, the 1st and the 3rd progeny, Anticoccidial index.

Introduction

Avian coccidiosis is responsible for substantial losses to the poultry industry in various countries of the world including Korea. After the use of drugs to treat coccidiosis dated from the discovery by Levine(1939) that sulfanilamide would cure coccidiosis in chickens, various anticoccidial drugs were developed and used. Long has indicated that polyether ionophorous antibiotics were mainly used. While those drugs were very effective for the prote-

ction of avian coccidiosis, some problems were recognized in the use of the anticoccidial drugs.[6] One of those is the emergency of drug resistance strains according to continuous use or misuse of the drugs. The emergency of drug resistance strains causes the development of new drugs and the rotation program of some drugs. In other hand, as the anticoccidial drug feed additives were ascended expenditure of poultry products, the drugs or antibiotics would be retained as residue in poultry product and these residual drugs may have a demerit influence to the final consumer the human being. Therefore, the enhancement of the regulations for the anticoccidial drugs should be strengthen gradually. For safety and economic control of avian coccidiosis, we were concerned about the development of vaccine.[14] Some scientists studied on the pathogenicity of attenuated *Eimeria* spp. for the immunogen of avian coccidiosis. In recent. development of avian coccidial vaccine is accompanied two methods ; the method of genetic engineering technology and avirulent coccidial oocysts. The method of genetic engineering technology was accompanied with recombinant DNA technique by *Escherichia coli*. The immunogen produced from recombinant DNA technique was not pathogenic, but it was estimated at non-effect. The other was the use of the avirulent coccidial oocysts with precocious line and the application of γ-irradiation. Many scientists studied on the pathogenicity of precocious line for immunogen of avian coccidiosis.[2,4,5,7,8,11,12,15,17,18] This method is required of time and labor to make and preserve of passaged oocysts and possibility of interspecific contamination. Fitzerald initiated the method of use of γ-irradiated oocysts with bovine coccidia, such as *E bovis*.[3] Singh and Gill also repoted to the pathogenic effect of γ-irradiation ot *E necatrix*.[13] To investigate the pathogenicity and immunogenicity of coccidia, they only used with γ-irradiated oocysts. The use of γ-irradiated oocysts was not satisfied because of the problems to preserve immunogenicity and mass production. In this examination, for making avirulent coccidia, we treated *E tenella* by γ-irradiation and passaged it in SPF chicken. To make use of passaged oocyst to coccidial imunogen, its pathogenicity was investigated.

Materials and Methods

Eimeria tenella : A stock of *E tenella* provided from the Protozoology Laboratory of Animal parasitology Institute of the USDA was fed to the SPF chickens for the propagation. The oocysts were preserved in 2% potassium dichromate solution to be sporulated and preserved in a refreezerator(2~5℃) until used. It was used to investigate the pathogenicity for the γ-irradiated dose of ^{60}Co and the numbers of inoculum of the γ-irradiated *E tenella* and its progeny by comparison of body weight gains, feed efficacy, blood in fece, lesion scores of anticoccidial indices.

Experimental animals : Four hundred and twenty SPF chickens of the same numbers of sex of white colored layer Lohmann were used as the experimental animals. They were reared 12 chickens per group on cage. One hundred and eighty 7 week-old chickens were used to determine proper irradiation and immune doses. Two hundres and forty 4 week-old chickens were used to investigate the pathogenicity of γ-irradiated *E tenella* and its progeny.

Experimental feed. : Experimental feed is manufactured for early broiler without anticoccidial feed additives at Kon-Kuk feed manufacture Co. in Kon-Kuk University and its composition followed the commercial chicken production manual(Notth. 1984). Feed and water were administered at liberty and were measured.

Dose of γ-irradiated oocysts of *E tenella* and Determination of γ-irradiated dose : Sporulated oocysts of *E tenella* were irradiated with γ-ray from ^{60}Co of 950 curi in Korean Atomic laboratory. 100 Gy was irradiated in 57cm, 200 Gy, 41cm, 300 Gy, 32cm, 600 Gy, 23cm during 3 times, respectively. Twelve 7 week-Old chickens per each group were immunized with 1×10^5 oocysts by 100, 200, 300 and 600 Gy γ-irradiated from ^{60}Co. One group of 12 chickens was not inoculated control group. Two chickens of each group were slaughtered to examine lesion score at the 1st week after immunization. Body weight gain of each group was investigated at the 2nd week after immunization. Blood in feces, lesion score and excreted oocysts per gram in feces were investigated at the 1st week after immunization.

Determination of immune dose. : Twelve 7 week-old chickens per each group were immunized with 1×10^2, 1×10^3, 1×10^4, and 1×10^5 oocysts of the 1st and the 3rd passaged *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively. One group of 12 chickens was control

group. Two chickens of each group were slaughtered to examine lesion score at the 1st week after immunization. The body weight gain of each group was investigated at the 2nd week after immunization. Blood in feces, lesion score and excreted oocysts per gram in feces were investigated as the same as previous methods.

Degrees of the pathogenicity of experimental *Eimeria tenella*. Twelve 4 week-old chickens per each group were immunized with 1×10^4 oocysts of non-passage, the 1st and the 3rd passaged *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively. One group of 12 chickens was immunized with non-iradiated *E tenella* group and the other was control groups. This trial was accompanied by 4 replications. The items of investigation were as follow ; Blood in feces. The blood in feces of each group was investigated from the 4th to the 8th day after immunization. Degree of blood in feces was divided with 4 grades ; those were normal, mild, moderate, and severe. Lesion score. The lesion score of each group was investigated according to the method suggested by Conway at the 7th day after immunization.[1]

Packed cell volume of erythrocytes(PCV) : The packed cell volume of each group was investigated at the 7th day after immunization.

Number of excreted oocysts in feces : The excreted oocysts per gram in feces each group was examined by new MacMaster's egg counting chamber from the 5th to the 11th day after immunization.

Body weight gain : The body weight gains and its rates of each group were investigated at the 1st and the 2nd week after immunization.

Feed conversion ration: The feed conversion ratio of each group was investigated at the 1st and the 2nd week after immunization.

Anticoccidial index : Anticoccidial index was investigated at the 1st week after immunization to evaluate the pathogenicity of γ-irradiated *E tenella* and its progeny. The method of calculation of anticoccidial index was as the same as that of Sunoda, such as follows ; Anticoccidial index = (Survival rate + rate of relative body weight gain)−(oocysts index+lesion score index).[16]

Statistical analysis : The results of body weight gain, feed conversion ratio, lesion score, packed cell volume of erythrocytes and anticoccidial index were analyzed by Tukey's studentized range test.

Results

Pathogenicity of *E tenella* treated with various doses of γ-ray from ^{60}Co. : The pathogenicity of the group immunized with 100 Gy γ-irradiated *E tenella* was more severe than those of the other groups by comparison of

Table 1. The Pathogenicity of *E tenella* irradiated with various doses of γ-ray form ^{60}Co

Group	Body weight gain(g)	Blood in feces	Lesion score	OPG
	2 weeks	1 week	1 week	1 week
I	248.0	−	0.00	0
II	215.0	+	1.00	13,000
III	213.5	+	0.50	2.400
IV	233.0	−	0.00	600
V	242.0	−	0.00	<200

group I : control group with not immunized, group II, III, IV and V : experimental groups immunized with 1×10^5 of oocysts irradiated with 100, 200, 300 and 600 Gy γ-ray from ^{60}Co, respectively, OPG : oocysts per gram of feces, − : normal, + : mild.

Table 2. The pathogenicity of the 1st progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co

Group	Number of oocysts (immunized)	Body weight gain(g)	Blood in feces	Lesion score
		2 weeks	1 week	1 week
I	−	165.0	−	0.00
II	1×10^2	146.5	−	0.50
III	1×10^3	145.0	−	1.00
IV	1×10^4	130.5	+	2.00
V	1×10^5	116.5	++	2.50

group I : control group with not immunized, gourp II, III, IV and V : experimental groups immunized with the 1st progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively, − : normal, + : mild, ++ : moderate.

Table 3. The pathogenicity of the 3rd progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co

Group	Number of oocysts (immunized)	Body weight gain(g)	Blood in feces	Lesion score
		2 weeks	1 week	1 week
I	−	172.0	−	0.00
II	1×10^2	142.0	−	0.50
III	1×10^3	133.5	+	1.50
IV	1×10^4	125.5	++	2.50
V	1×10^5	109.0	+++	3.00

group I : control group with not immunized, group II, III, IV and V : experimental groups immunized with the 3rd progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively, − : normal, + : mild, ++ : moderate, +++ : severe.

Table 4. The pathogenicity after immunization with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Blood in feces					Lesion score	PCV
	4	5	6	7	8(days)	(M±SD)	(M±SD)
C_0	−	−	−	−	−	0.0 ± 0.0^a	29.5 ± 0.1^a
C_1	+	+++	++	−	−	3.4 ± 0.5^c	24.2 ± 0.4^c
P_0	−	−	−	−	−	0.0 ± 0.0^a	27.9 ± 1.1^{ab}
P_1	−	+	+	−	−	2.0 ± 0.0^b	27.0 ± 0.4^b
P_3	−	++	+	−	−	2.5 ± 0.5^b	26.9 ± 0.5^b

C_0 : control group with not immunized, C_1 : control group immunized with not irradiated oocysts, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treament group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts, − : normal, + : mild, ++ : moderate, +++ : severs. a, b, and c values with different superscripts differ significantly($p<0.05$).

Table 5. The number of oocysts per gram in feces after immunization with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Shedding of oocysts(OPG×1000)					
	6	7	8	9	10	11(days)
C_1	0.0	61.1^{bc}	59.5^b	36.9^b	16.5^b	8.6^b
	±0.0	±48.9	±24.1	±17.0	±6.6	±3.6
P_0	0.0	0.2^a	2.4^a	1.2^a	0.3^a	0.1^a
	±0.0	±0.1	±0.7	±0.4	±0.2	±0.1
P_1	0.0	14.2^{ab}	23.7^a	10.1^a	12.3^b	3.4^a
	±0.0	±5.3	±13.5	±2.5	±5.9	±2.6
P_3	0.0	115.0^c	52.7^b	32.8^b	15.2^b	18.2^c
	±0.0	±84.9	±35.5	±15.2	±8.3	±5.3

C_1 : immunized control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts.
a b and c values with different superscripts differ significantly($p<0.05$).

body weight gains, blood in feces, lesion scores, and excreted oocysts per gram in feces(Table 1).

Pathogenicity of the 1st progeny of *E tenella* irradiated with 100 Gy-ray from ^{60}Co : The pathogenicity of group immunized with 1×10^2 oocysts of the 1st passaged *E tenella* was milder than those of the other groups by comparison of body weight gains, blood in feces and lesion scores. The more the numbers of immunized oocysts increased, the more the pathogenicity of them got severe(Table 2).

Pathogenicity of the 3rd progeny of *E tenella* irradiated with 100 Gy of γ-ray from ^{60}Co : The pathogenicity of group immunized with 1×10^2 oocysts of the 3rd passaged *E tenella* was milder than those of the other groups by comparison of body weight gains, blood in feces and lesion scores(Table 3).

Pathogenicity of 100 Gy γ-irradiated *E tenella* and its progeny. : In the preliminary trials, the number of non-irradiated, γ-irradiated *E tenella* and its progeny were estimated 1×10^4 oocysts by comparison of pathogenicity. S-

Table 6. The body weight gain and the feed conversion ratio after immunization with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Body weight gain(g)		Feed conversion rate	
	1 week	2 weeks	1 week	2 weeks
C_0	112.5±11.2[a]	230.8±17.4[a]	2.06±0.17[a]	2.21±0.16[a]
	(100.0±0.0)*	(100.0±0.0)		
C_1	68.1±5.1[d]	176.2±9.6[c]	4.08±0.21[d]	3.14±0.15[d]
	(61.1±9.2)	(76.8±8.2)		
P_0	101.7±15.9[ab]	209.6±17.0[ab]	2.63±0.35[b]	2.45±0.17[ab]
	(90.7±13.3)	(90.9±4.7)		
P_1	86.6±7.4[bc]	195.1±8.1[bc]	3.52±0.29[c]	2.66±0.09[bc]
	(77.7±11.5)	(84.9±7.4)		
P_3	76.8±5.0[cd]	189.1±6.0[bc]	4.01±0.16[d]	2.84±0.84[c]
	(68.7±6.6)	(82.2±4.3)		

C_0 : control group with not immunized and not challlenged, C_1 : immunized control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts. ( )* : rate of relative body weight gains=body weight gain of treatment group/body weight gain of control group.
a, b, c and d values with different superscripts differ significantly($p<0.05$).

Table 7. The anti-coccidial index after immunization with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Gorup	Anti-coccidial index(M±SD)
C_0	200.0± 0.0[a]
C_1	87.4± 4.6[d]
P_0	190.6±13.3[a]
P_1	142.8±25.9[b]
O_3	107.4± 8.9[c]

C_0 : control group with not immunized and not challenged, C_1 : immunized control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st proteny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts.
a, b, c and d values with different superscripts differ significantly($p<0.05$).

o we immunized with 1×10^4 oocysts of 100 Gy of γ-irradiated *E tenella* and its progeny to each bird. The pathogenicity of group immunized with γ-irradiated *E tenella* was mildest of any other groups by comparison of blood in feces, lesion score and packed cell volume of erythrocytes. The pathogenicity of groups immunized with the 1st and the 3rd passaged *E tenella* was milder than that of non-irradiated group(Table 4).

Shedding of oocysts : Shedding of oocysts in group immunized with γ-irradiated non-passaged *E tenella* was smallest of any other groups. Shedding of oocysts in group immunized with the 1st passaged *E tenella* was samller than those of non-irradiated and the 3rd passaged *E tenella* groups(Tagle 5).

Body weight gain and feed conversion ratio : The body weight gain of group immunized with 100 Gy γ-irradiated *E tenella* was the most of any other groups except control group. The body weight gains of groups immunized with the 1st and the 3rd passaged *E tenella* were more than that of non-irradiated group. The feed conversion ratio of group immunized with 100 Gy γ-irradiated *E tenella* was the lowest of any other groups except control group. Feed conversion ratio of groups immunized with the 1st and the 3rd passaged *E tenella* were more than that of non-irradiated *E tenella* group(Table 6).

Anti-coccidial index : The anticoccidial index of group immunized with 100 Gy γ-irradiated *E tenella* was the most of any other groups except control group. Those of groups immunized with the 1st and the 3rd passaged *E tenella* were more than that of non-irradiated *E tenella* group(Table 7).

Discussion

In this experiment, we irradiate γ-ray to coccidial oocysts and passage them to SPF chicken to be weak pathogenicity by use of the proper immunogen for avian coccidiosis. Their pathogenicity was investigated by comparison of body weight gains, blood in feces, lesion scores, number of excreted oocysts in feces and packed cell volume of erythrocysts.

In the experiment of effects of ionizing irradiation from Cobalt-60 on oocysts of *Eimeria bovis*, Fitzgerald reported that the group irradiated with 100 Gy γ-ray from ^{60}Co

was the most pathogenic by comparison of blood in feces and number of excreted oocysts between 100 and 2,000 Gy γ -ray from ^{60}Co[3]. Also, Singh and Gill reported that the groups 100 to 200 Gy γ -ray from ^{60}Co to *E necatrix* were more pathogenic than those of the other γ -irradiation doses.[13] In this experiment, The pathogenicity of the group immunized with 100 Gy γ -irradiated *E tenella* was more severe than those of the other groups by comparison of body weight gains, blood in feces, lesion score, and excreted oocysts per gram in feces. The pathogenicity of groups immunized with 1×10^5 oocysts of the 1st and the 3rd passaged *E tenella* irradiated with 100 Gy γ -ray was more severe than those of the other groups by comparison of body weight gains, blood in feces and lesion score.

Long and Johnson reported that the more the number of immunized oocysts increased, the more the pathogenicity got severe, in the experiment of *Eimeria* of American chickens[7] : Characteristics of 6 attenuated strains produced by selection of precocious development. They reported that the lesion scores(0.2 ~ 2.2) in the groups inoculated with the precocious line of *E tenella* were milder than those(1.2 ~ 3.7) of in the groups inoculated with parent strain. They also reported that the body weight gains(309 ~ 332g) in the groups inoculated with the precocious line were higher than those(118 ~ 317g) in the groups inoculated with the parent strain in the $1\times10^3\sim25\times10^4$ oocysts of inoculum. Kawaguchi et. al. reported that bloody dirarrhea appeared between the 3rd and the 7th day, especially most severe at the 5th day and oocysts excreted maximun at the 6th day after inoculation with the precocious line of *E tenella*[5]. They also reported that the relative body weight gains(81.5 ~ 111.9%) in the groups inoculated with the precocious line were higher than those(46.3 ~ 103.7%) in the groups inoculated with the parent strain in the $5\times10\sim5\times10^4$ oocysts of inoculum. McDonald et. al. reported that the lesion scores(0.15 ~ 0.65) in the groups inoculated with the precocious line of *E tenella* were milder than those(1.57 ~ 1.96) of in the groups inoculated with parent strain. They reported that the body weight gains(136 ~ 187g) in the groups inoculated with the precocious line of *E tenella* were higher than those(107 ~ 143g) in the groups inoculated with the parent strain.[8] They also reported that the survival rate(0 ~ 20%) in the groups inoculated with the parent strain of *E tenella* were higher than those(0 ~ 3.3%) in the groups inoculated with the precocious line in the $5\times10^3\sim5\times10^4$ oocysts of inoculum. Therefore, they indicated that the precocious line of *Eimeria* spp. was less pathogenic than the parent strains.

In these trials of pathogenicity between γ -irradiated *E tenella* and its progeny, the pathogenicity of group immunized with γ -irradiated *E tenella* was mildest of any other groups by comparison of blood in feces, lesion scores, packed cell volume and excreted oocysts in feces. The pathogenicity of groups immunized with the 1st and the 3rd passaged *E tenella* was milder than that of non-irradiated *E tenella* group. The body weight gain and the feed conversion ratios of group immunized with γ -irradiated *E tenella* were the most of any other groups except control group. The body weight gains and the feed conversion ration of groups immunized with the 1st and the 3rd passaged *E tenella* were more than those of non-irradiated *E tenella* group.

To compare pathogenicity between γ -irradiated *E tenella* and its progeny, we introduced anticoccidial index to evaluate anticoccidial drugs. The anticoccidial index of group immunized with γ -irradiated *E tenella* was the most of any other groups except control group. Those of groups immunized with the 1st and the 3rd passaged *E tenella* were more than that of non-irradiated *E tenella* group.

It was thought that the pathogenicity of γ -irradiated *E tenella* would be recovered according to increase the number of generation passaged in chicken.

Summary

A series of experiments on the effects of γ -irradiation was performed to reveal the pathogenicity of γ -irradiated oocysts of *E tenella* from Cobalt-60 and its progeny. The SPF chickens were inoculated with differnt doses of radiation and inoculum. The level of 100 Gy γ -irradiation from ^{60}Co and the level of inoculum with 1×10^4 oocysts were recognized more pathogenic than those of the other groups by comparison of body weight gains, blood in feces and lesion scores. The signs of blood in feces, lesion score and the number of excreted oocysts in the feces were revealed as the lowest in the group of the γ -irradiated oocysts, the average in the group of the 1st and the 3rd progeny, and the highest in the group of

non-irradiated oocysts of *E tenella*. The body weight gain of the group immunized with γ-irradiated oocysts of *E tenella* was higher than those of the non-irradiated, the 1st and 3rd progeny groups. The body weight gain of the groups immunized with the 1st and the 3rd progeny of *E tenella* were higher than that of the non-irradiated group. The feed conversion ration of the group immunized with γ-irradiated oocysts of *E tenella* was lower than those of the non-irradiated, the 1st and the 3rd progeny groups. The feed conversion ratios of the group immunized with the 1st and 3rd progeny of γ-irradiated oocysts were lower than that of the group infected with non-irradiated *E tenella*. The anticoccidial index(ACI 190.6) in the chickens immunized with the γ-irradiated oocysts of *E tenella* and those(ACI 142.8 and 107.4) of the 1st and the 3rd progeny groups were higher than that (ACT 87.4) of the group infected with non-irradiated *E tenella*. It was thought that the pathogenicity of γ-irradiated *E tenella* would be recovered according to increase the number of generation passaged in chicken.

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