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Effects of gamma-irradiation on intracellular proliferation of *Toxoplasma gondii* RH tachyzoites

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Abstract: A quantitative assay was performed on the effects of gamma-irradiation (30-300 Gy) on intracellular proliferation of *Toxoplasma gondii* RH tachyzoites in human leukemic HL-60 cells and murine peritoneal macrophages by means of ^{3}H-uracil uptake assay. Infected non-irradiation group (NI) and uninfected group (incubating only host cells) were prepared. The ^{3}H-uracil uptake by tachyzoites of NI group 12-24 hrs after infection was 2,190-4,787 counts per minute for macrophages and 2,967-8,254 for HL-60 cells, whereas the irradiated tachyzoites revealed only 381-703 (100 Gy) and 218-408 (300 Gy) for macrophages, and 1,911-2,618 (30 Gy), 1,253-1,384 (70 Gy), 1,013-1,090 (100 Gy), and 483-588 (300 Gy) for HL-60 cells. The proliferation inhibition rate was similar in macrophages and HL-60 cells, for example, 89-94% and 80-94% respectively by 300 Gy, 12-24 hrs after infection. It is concluded that RH tachyzoites of *T. gondii* are severely affected by gamma-irradiation in their capability of intracellular proliferation.

Key words: *Toxoplasma gondii*, tachyzoites, HL-60 cells, macrophages, gamma-irradiation, ^{3}H-uracil uptake assay

INTRODUCTION

There have been many reports indicating prevalence of *T. gondii* by demonstration of serum antibodies in human patients and animals in Korea (Soh *et al.*, 1960; Rim *et al.*, 1972; Choi *et al.*, 1987 & 1989). Isolation and characterization of Korean strains of *T. gondii* and other basic studies are needed in Korea.

The effects of radiation especially gamma rays on infectivity as well as survival, development or multiplication of parasites in their hosts have been well documented (International Atomic Energy Agency, 1993). It was also reported that the effects are much variable by different kinds and even different strains of parasites. In the case of *Toxoplasma gondii*, it has been reported that they are highly susceptible to radiation (Kobayashi and Jacobs, 1963; Dubey *et al.*, 1986). However, the effects were variable by different strains of *T. gondii* (Wikerhauser *et al.*, 1991 & 1992) and by tissue cysts or tachyzoites (Kobayashi and Jacobs, 1963). Furthermore, cysts in intact mouse brains required higher doses than isolated ones to render them non-infective (Kobayashi and Jacobs, 1963).

To date, however, most of the previous studies did not quantify the effects of radiation on *T. gondii*, and only morphological observations or bioassays using mouse

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experimental infection were performed. It is well known that *T. gondii* utilize by far greater amount of uracil than mammalian cells, therefore, ^{3}H-uracil can be used to quantitatively measure the degree of intracellular proliferation of *T. gondii* (Pfefferkorn and Pfefferkorn, 1977).

In the present study, we investigated the effects of gamma-irradiation on intracellular proliferation of *T. gondii* tachyzoites in HL-60 cells or macrophages by means of ^{3}H-uracil uptake assay.

MATERIALS AND METHODS

1. Tachyzoites

Toxoplasma gondii tachyzoites (RH strain) were serially passaged in ICR mice every 3 days by intraperitoneal inoculation. They were purified from the peritoneal exudate by percoll gradient (40%, 50%, and 60%) centrifugation at 2,500 rpm for 30 min.

2. Host cells

Resident murine peritoneal cells were harvested from adult BALB/c mice in 10 ml of Hank's balanced salt solution (HBSS). Macrophages were separated by 2 hrs plating of the cell suspension on 96-well plates in complete Earle's minimum essential medium (EMEM) at 37°C 5% CO_2. In order to obtain pure macrophages, the plate was washed with cold HBSS several times. The purified macrophages were maintained in EMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES, pH 7.4. Human promyelocytic leukemia cell line, HL-60 cells, was also maintained in the same media. The host cells were cultured at 37°C in a humidified 95% air and 5% CO_2 atmosphere.

3. Irradiation of *T. gondii* tachyzoites

The source of gamma-ray was Cs-137 irradiator(JL Sheperd and Associates Co.), and *T. gondii* tachyzoites suspended in phosphate-buffered saline (PBS) were irradiated at the rate of 3.80 Gy/min from 40 cm distance. The radiation dose was 30, 70, 100 or 300 Gy. Besides infected non-irradiation (NI) group, uninfected culture of only host cells was prepared.

4. ^{3}H-uracil uptake assay

The amount of incorporation of ^{3}H-uracil into acid precipitable material was measured by a filtration procedure (McLeod and Remington, 1977). Briefly, 100 μl of host cells (2×10^6 cells/ml), murine peritoneal macrophages or HL-60 cells, in 96-well plates were incubated with 100 μl of irradiated tachyzoites (5×10^5 cells/ml) for 12 hrs, 24 hrs, 36 hrs or 48 hrs. Ten μl of ^{3}H-uracil (1 μCi/ml) was added to each well, and incubated for 2 hrs. The cells were harvested onto a membrane filter using a multiple channel cell harvester (Skatron, USA) and ^{3}H-uracil uptake was measured by β-liquid scintillation counter (Beckman, USA).

The amount of ^{3}H-uracil uptake by the tachyzoites of each group was expressed as β-ray count per minute (CPM) deducting the background level of uninfected cultures. The effect of radiation was also expressed by percentage (%) inhibition of parasite proliferation compared with NI group. The data (Table 1) represent the mean and standard deviation of values derived from three independent experiments. In each experiment triplicate wells were prepared.

RESULTS

The average ^{3}H-uracil uptake by macrophages of NI group was 2,190 CPM 12 hrs after the infection with *T. gondii* tachyzoites (Table 1). Contrarily, the average CPM of 100 Gy and 300 Gy irradiation groups was only 381 and 218 respectively, which represented 83% and 90% inhibition of parasite proliferation (Fig. 1). At 24 hrs after infection, the uracil uptake of NI group increased to 4,789 CPM (Table 1). But the CPM of irradiation groups with 100 Gy and 300 Gy was 703 and 408, representing 85% or 92% inhibition of proliferation, respectively (Fig. 1). At 36 hrs, the CPM of NI group decreased, due to rupture of macrophages, to 1,724, and that of 100 Gy and 300 Gy groups was 368 and 188, respectively (Table 1). The inhibition rate at 36 hrs was 79% and 89%, respectively. At 48 hrs, the CPM of NI group further decreased to 795, and that of 100 Gy

Table 1. ^{3}H-uracil uptake by *T. gondii* tachyzoites in murine peritoneal macrophages and HL-60 cells

Host cells	Incubation time (hr)	3H-uracill uptake (CPM: count per minute)[a]				
		Non-irraditated *T. gondii*	Irradiated *T. gondii* with 30 Gy	70 Gy	100 Gy	300 Gy
macrophage	12	2,190 ± 322	ND[b]	ND	381 ± 78	218 ± 8
	24	4,789 ± 973	ND	ND	703 ± 108	408 ± 47
	36	1,724 ± 192	ND	ND	368 ± 44	188 ± 20
	48	795 ± 45	ND	ND	120 ± 19	45 ± 8
HL-60	12	2,967 ± 571	1,911 ± 140	1,253 ± 272	1,090 ± 141	588 ± 84
	24	8,254 ± 1,561	2,618 ± 140	1,384 ± 13	1,013 ± 64	483 ± 127
	36	3,638 ± 169	1,804 ± 488	1,049 ± 575	293 ± 38	353 ± 257
	48	2,560 ± 646	835 ± 61	469 ± 237	259 ± 36	272 ± 19

[a]Values represent the mean of triplicate wells with standard deviation in a representative experiment. Experiments were repeated 3-4 times with similar results.
[b]ND, Not done

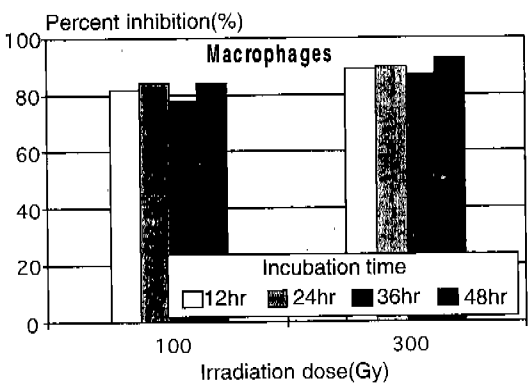

Fig. 1. Inhibition of intracellular proliferation of *T. gondii* RH tachyzoites in murine peritoneal macrophages by gamma-irradiation. Percent (%) inhibition represents the extent of inhibition setting the uracil uptake (CPM) of non-irradiated (NI) group as 100%.

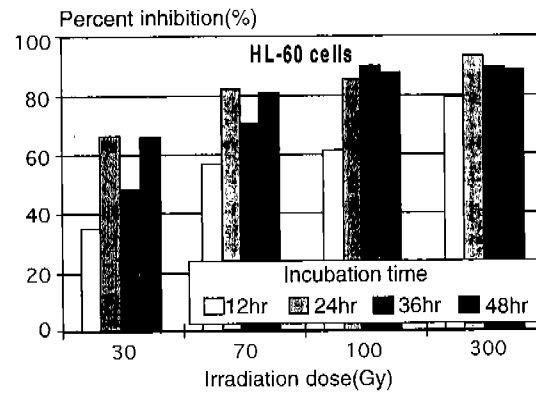

Fig. 2. Inhibition of intracellular proliferation of *T. gondii* RH tachyzoites in human leukemic HL-60 cells by gamma-irradiation. Percent (%) inhibition represents the extent of inhibition setting the uracil uptake (CPM) of non-irradiated (NI) group as 100%.

and 300 Gy groups was only 120 and 45, respectively. At this time, the inhibition rate was 85% and 94%, respectively. The degree of inhibition was not so much different by the duration of incubation, but highly dependent upon the dose of radiation.

In HL-60 cells, at 12 hrs after infection, the average value of ^{3}H-uracil uptake by tachyzoites of *T. gondii* in NI group was 2,967 CPM, a little higher than that in macrophages (Table 1). The irradiation groups with 30 Gy, 70 Gy, 100 Gy or 300 Gy showed 1,911, 1,253, 1,090 or 588 CPM, for which the inhibition rate of intracellular proliferation was 36%, 58%, 63% or 80%, respectively (Fig. 2). At 24 hrs after incubation, the irradiation groups showed 2,618, 1,384, 1,013 or 483 CPM of uracil uptake, for which the inhibition rate was 68%, 83%, 88% or 94%, respectively (Fig. 2). At 36 hrs, the CPM of NI group decreased, due to rupture of HL-60 cells, to 3,638, and that of 30 Gy, 70 Gy, 100 Gy and 300 Gy groups was 1,804, 1,049, 293 and 352, respectively (Table 1). The inhibition rate at 36 hrs was 50%, 71%,

92% and 90%, respectively. At 48 hrs, the CPM of NI group further decreased to 2,560, and that of 30 Gy, 70 Gy, 100 Gy and 300 Gy groups was only 835, 469, 259 and 272, respectively. At this time, the inhibition rate was 67%, 82%, 90% and 89%, respectively (Fig. 2). Also in HL-60 cells, the degree of inhibition of parasite proliferation was not so much different by the duration of incubation, but dependent upon the dose of radiation.

DISCUSSION

The application of radiation such as gamma rays to control infectivity of parasites such as *T. gondii* has received much attention. It has been demonstrated that radiations from various sources have strong effects to kill or attenuate *T. gondii* tissue cysts (Kobayashi and Jacobs, 1963; Dubey *et al.*, 1986; Wikerhauser *et al.*, 1991 & 1992; Song *et al.*, 1993; Dubey and Thayer, 1994), or tachyzoites (Lund *et al.*, 1961; Kobayashi and Jacobs, 1963; Grimwood, 1980). Most of those studies, however, were based on morphological observations or mouse *in vivo* bioassays. Quantitative assays on parasite proliferation in cell culture have never been performed.

The results of the present study showed that the RH tachyzoites of *T. gondii* are severely affected in their capability of proliferation in the two kinds of host cells used; murine peritoneal macrophages and human promyelocytic leukemia (HL-60) cells. The effect was radiation dose-dependent and 300 Gy was needed to obtain about 90% inhibition of intracellular proliferation in both kinds of host cells.

The use of two kinds of host cells in this study was in consideration that macrophages are well-known phagocytic cells whereas HL-60 cells are not, and they may be different in the susceptibility to infection with *T. gondii* tachyzoites. These cells are also different in the adherence to the bottom of the culture flasks; macrophages are adherent and HL-60 cells are not, which may also affect their susceptibility. Considering the amount of uracil uptake in this study, HL-60 cells appeared to be more susceptible than macrophages. However, the effects of radiation, especially in terms of the percent inhibition of tachyzoite proliferation, were very similar in these two kinds of host cells.

Whether the inhibition of proliferation by irradiation is in part due to weakened ability of tachyzoites to invade into the host cells remains uncertain. Uracil is normally uptaken by intracellular dividing tachyzoites, therefore, having invaded but not yet dividing tachyzoites can not be measured only by uracil uptake assay. In order to verify this point, morphological studies should be done to observe intracellular tachyzoites within 5 hrs post-infection after which they begin to divide (Nam *et al.*, 1990).

Anyhow, the results of this study agreed well to the general results of three previous authors who worked with tachyzoites of *T. gondii*, although some different findings were noticed. According to Lund *et al.* (1961) who studied the influence of x-irradiation on propagation of *T. gondii* (RH strain) in cell cultures, the doses of 6-30 Gy had absolutely no effect on the penetration of tachyzoites into the cells and subsequent multiplication. But when a maximum of 300 Gy was used the x-irradiated tachyzoites appeared to no longer multiply although abortive multiplications up to no more than 4 parasites per clone were occasionally observed in the cell culture (Lund *et al.*, 1961). On the other hand, complete loss of infectivity to mice was obtained when RH or Beverley tachyzoites were exposed to radiation doses of only 154 Gy or 127 Gy respectively (Kobayashi and Jacobs, 1963). In the present study the percent (%) inhibition of intracellular tachyzoite proliferation by 300 Gy irradiation was not perfect but only about 90-94%. Therefore, our results rather supports and explains well the results of morphological observations made by Lund *et al.* (1961).

Strain difference in the radiosensitivity was reported in *T. gondii* cysts, although the degree of difference was not so remarkable (Wikerhauser *et al.*, 1991 & 1992). They reported that the radiosensitivity of the Yugoslavian strain TG-3 was more resistant to gamma-irradiation (resistant to below 700 Gy) than the American strain ME 49 (resistant to below 400 Gy). On the other hand, no strain difference was recognized by other workers

(Song *et al.*, 1993; Dubey and Thayer, 1994). For example, it was reported that all of the tissue cysts of 95 different isolates or strains were rendered nonviable at 400 Gy radiation (Dubey and Thayer, 1994).

It was also reported that the tachyzoites of *T. gondii* were more sensitive to radiation than the cyst forms, when Beverley strain was used for study (Kobayashi and Jacobs, 1963). It seems to be greatly due to more rapid proliferation of tachyzoites than cyst forms since radiation primarily affects the cells which divide very quickly. However, the difference seems not so significant in practice. In the present study 300 Gy was not sufficient for complete killing of tachyzoites. It is speculated that regardless of strains, cysts or tachyzoites 1 kGy seems to be the sufficient dose to render them non-infective to mice or cell cultures.

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=초록=

톡소포자충 RH tachyzoites에 대한 감마선 조사가 충체의 숙주세포내 증식에 미치는 영향

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톡소포자충(RH strain) tachyzoites를 감마선에 조사하여 인체 백혈병 세포인 HL-60 세포와 정상 마우스의 복강 대식세포에 감염시킨 다음 충체 증식 능력에 어떠한 변화가 오는지 ^{3}H-uracil 흡수시험을 통하여 알아보았다. 감마선 조사량은 30, 70, 100 및 300 Gy로 하였고 tachyzoite 감염은 시켰으나 감마선을 조사하지 않은 비조사군(NI 군)과 tachyzoites를 넣지 않고 숙주세포만을 배양한 비감염군을 각각 두었다. ^{3}H-uracil 흡수시험 결과 12-24시간 후 대식세포의 NI 군은 2,190-4,787(count per minute, 이하 같음), HL-60세포의 NI 군은 2,967-8,254의 높은 흡수량을 보였으나, 감마선 조사군은 대식세포 감염시 381-703(100 Gy 조사군) 및 218-408(300 Gy 조사군), HL-60 세포 감염시 1,911-2,618(30 Gy), 1,253-1,384(70 Gy), 1,013-1,090(100 Gy) 및 483-588(300 Gy)의 흡수량을 각각 보였다. 충체 증식에 대한 억제율은 300 Gy 조사시 12-24시간에 대식세포의 경우 90-92%, HL-60 세포의 경우 80-94%이었다. 이상과 같이 톡소포자충 RH tachyzoites가 감마선 조사 후 세포내 분열증식 능력에 치명적인 영향을 받는다는 것을 uracil 흡수시험법으로 확인하였다.

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