Radiaion Induced Damage of Cryptosporidium parvum Measured by Apoptosis and Phosphorylated Histon H2AX

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Cryptosporidium parvum is one of the strongest radioresistant organism ever known. The mechanism of radioresistance of C. parvum has not been evaluated yet. We observed radiation induced damage by annexin V labeling and γ-H2AX detection in C. parvum. C. parvum was irradiated with Co-60 with various doses (1, 5, 10 and 25 kGy) for 2 h. For the detection of DNA repair with time sequence, experiments were performed at 6, 12, 24, 48, 72 and 96 h after γ-irradiation with 10 kGy. FITC labeled annexin V and propidium iodide (PI) were used for apoptosis study. γ-H2AX was detected using rabbit anti-γ-H2AX and FITC labeled goat anti-rabbit IgG and counterstained with PI. Quantification was performed with flow cytometry. After 1 kGy to 25 kGy irradiation, 80-67% of oocysts were still alive respectively. Early apoptosis occurred in 1 to 5 KGy irradiated oocysts were the same level with non-irradiated control oocysts (3%), but the proportion increased to 5-6% with increase of irradiation doses (10 kGy and 25 kGy). The number of apoptotic cells increased fastly between 6 h and 12 h after 10 kGy irradiation and maintained the same level until 96 h. γ-H2AX phosphorylation also increased from 18 to 36% in non-irradiated group and 25 kGy group respectively. γ-H2AX phosphorylation also increased with time passage after irradiation, but it decreased to the level of non-irradiated oocysts after 96 h. We found that apoptosis after irradiation was not main cause of cell death of C. parvum although it was induced in some amount. DNA double strand break was induced as dose dependant manner by irradiation in C. parvum and it was suggested that DNA damage repair occurred at 96 h after 10 kGy irradiation.

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