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Optimization of protective effects of melatonin in prevention of induced gamma radiation by chromosomal aberration assay

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Abstract

The chromosome aberration test using cultured mammalian cells is one of the sensitive methods to predict environmental mutagens and/or carcinogens, From our recent survey of 951 chemicals which have been tested for their clastogenicity in cultured mammalian cells such as Chinese hamster fibroblasts or human lymphocytes, it was noted that 47% of them are consistently positive either with or without metabolic activation. Environmental exposures can result in increased mutation rate, and the increased is the mutation rate in the target cells the higher is the cancer risk. The first event in the multistep carcinogenesis (i.e., the initiation) is a mutagenesis requiring cell proliferation in the target cells. Melatonin, a secretory product of the pineal gland in human brain, has been reported to participate in the regulation of a number of important physiological and pathological processes. The importance of the environmental harmful effects in the worldwide increase of cancer incidence is established. The present investigation was carried to find out cellular effects of gamma radiation on blood lymphocytes and radioprotector using cytogenetic biomarker (chromosomal aberration assay). Peripheral blood samples were collected from human volunteers 5-10 min before, and at 1 and 2 h after a single oral dose of 300 mg of melatonin. At each time point: 1 the concentration of melatonin in the serum and in the leukocytes was determined; and 2 the whole blood was exposed in vitro from 2 Gy to 12 Gy of gamma radiation. Immediately after exposure to the radiation, the lymphocytes were examined to determine the extent of primary DNA damage.

Key-Words: Radiation, Biodosimetry, Melatonin, Chromosomal Aberration, Lymphocytes

Introduction

Radiations which have the ability to eject one or more electrons from an atom or molecule are called Ionizing radiations, which are produced naturally as emissions from elements with heavy and/or unstable nuclei. Ionizing radiations are of two types- Electromagnetic radiations and, Particulate radiations [1]. Most experiments with biological systems have included X-rays or γ-rays, two forms of electromagnetic radiations. The electromagnetic radiations travel with a speed of $3x10^8$ m/s, though the frequency and wavelength vary with different types of radiations. Particulate radiations on the other hand include electrons, protons, neutrons, alpha-particles and also heavy charged particles.

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The heavy charged particles consist of nuclei of elements such as carbon, neon, argon or even iron that is positively charged because some or all of their planetary electrons have been stripped from them [2, 3].

They can be useful for radiation therapy only if they can be accelerated to energies of thousands of millions of volts and therefore can be produced only in a few specialized facilities. Based on their mode of action of ionization, radiations can be classified as- Directly ionizing, and indirectly ionizing. Directly ionizing rays, if possess sufficient kinetic energy, disrupt the atomic structure of the absorber through which they pass and directly produce chemical and biological changes. On the other hand indirectly ionizing rays do not cause the chemical and biological damages themselves, but when they are absorbed by material through which they pass and give up their energy to produce fast moving charged particles /free radicals [4]. Direct ionization is

property of particulate radiations which have a higher linear energy transfer (or LET). The linear energy transfer is the amount energy transferred per unit length of the track of the radiation, denoted as keV/µm [5].

When gamma radiation passes through the body cells it interacts with the various organelles present in the cell. Each cell has a nucleus. Chromosomes are present in the nucleus of the cell. The interaction of radiation with these chromosomes causes break in these chromosomes. In the event of higher exposure to radiation such breaks may occur in more than one chromosome. During such period there is chance for misrepair, when the broken end of one chromosome may join with a broken end of another, resulting in the formation of a dicentric chromosome (DC). A DC is one that contains two centromeres instead of one normally present in one chromosome [6,7]. The formation of DC is highly specific to ionizing radiation and its frequency serves as a measure of radiation exposure received by a person. Melatonin (or N-aetyl-5-hydroxy tryptophan) is a hormone secreted by the pineal gland in the brain. It is a neurotransmitter or a neurohormone which is derived from serotonin. The small size and high lipophilicity of melatonin molecule readily permits it to diffuse through biomolecules. It helps regulate other hormones and maintains the body's circadian rhythm [8,9,10]. The circadian rhythm is an internal 24-hour "clock" that plays a critical role in when we fall asleep and when we wake up. When it is dark, your body produces more melatonin; when it is light, the production of melatonin rops. Being exposed to bright lights in the evening or too little light during the day can disrupt the body's normal melatonin cycles. For example, jet lag, shift work, and poor vision can disrupt melatonin cycles. Melatonin also helps control the timing and release of female reproductive hormones. It helps determine when a woman starts to menstruate, the frequency and duration of menstrual cycles, and when a woman stops menstruating (menopause). Some researchers also believe that melatonin levels may be related to ageing [11,12,13]. For example, young children have the highest levels of night time melatonin. Researchers believe these levels drop as we age. Some people think lower levels of melatonin may explain why some older adults have sleep problems and tend to go to bed and wake up earlier than when they were younger. However, newer research calls this theory into question. Melatonin is an oxygen scavenger and is found to be highly effective against oxidative damage caused by carcinogens. Preliminary evidence suggests that it may help strengthen the immune system. In mammalian tissues melatonin has the ability to scavenge hydroxyl radicals, peroxyl radicals and concentrate more in the nucleus than the cytosol of the cell [14,15,16].The peripheral lymphocyte population that is mitogenically stimulated is nor- mally non-cycling and resides in the G0 (non-cycling G1) stage of the cell cycle. The chromosome aberrations induced by radiation will consequently be of the chromo- some type, i.e. they involve both chromatids of a chromosome. It is well known that ionizing radiation is an S independent clastogen, unlike UV radiation and chemical mutagens, which are S dependent agents. Therefore, with ionizing radiation, chromo- some and chromatid type aberrations are induced following treatment of G0/G1 and G2/S cells, respectively [17,18]. lymphocytes are a type of White Blood Cells which constitute 20-40% of the body's total WBCs and 99% of the cells in the lymph. Lymphocytes circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, serving thereby a bridge between parts of the immune system [19,20].

Material and Methods

Chromosomal Aberration Assay

Preparation of media

Measure out 5% less distilled water than desired volume of medium using a mixing container that is as close to the final volume as possible.Add powdered medium (RPMI-1640) to distilled water kept at room temperature(15-30°C) and mix with gentle stirring. Do not heat the mixture.Rinse out the inside of the package to remove all traces of the powder.Add 2.4gm of sodium bicarbonate ($NaHCO_2$) per litre of the medium.Dilute to desired volume with distilled water and stir till the powder is dissolved. Do not overmix.Adjust the pH of the medium to 0.2-0.3 units below the desired final working pH using 1N NaOH or 1N HCl. Add slowly with stirring. After the pH has been adjusted, keep the container closed until the medium is filtered.Sterilize the media immediately by membrane (0.22µm) filtration. Note: pH units will usually rise by 0.1-0.3 upon filtration.

Requirements

RPMI-1640 medium, Fetal bovine serum, Phytohaemagglutinin,Whole blood sample, Colcemid, Culture vials, Micropipettes and tips: 1mL, 5mL,Syringe: 1mL, Parafilm, KCl solution, Glass pipettes, Rubber bulb, Glacial acetic acid, Methanol, Pasteur pipettes, Glass slide.

Culture

Take 4ml of sterile RPMI-1640 media in sterile culture vial.Add 0.4ml of Foetal calf serum in it. Add 0.5ml of heparinized whole blood sample to it. Add 0.1ml of PHA (Phytohaemagglutinin- 10µg/ml). Mix well by

thawing and incubate in a CO2 incubator at 37^0Cfor 24hrs. At 24hrs of culturing, add 0.1ml of Colcemid (1.25µg/ml) to the culture and mix well. Incubate for another 28hrs after which processing is done.

Processing

Transfer the culture into a centrifuge tube and spin at 1000-1200rpm for 8 mins to get firm pellet with clear supernatant. Aspirate the supernatant using a suction line. If the supernatant is not clear, centrifuge again for a few more minutes. Also check for the imbalance of the rotor. While aspirating, ensure that the buffy-coat present on the RBC layer is not disturbed. Disturb the pellet by finger tapping and mix thoroughly. Resuspend the cells in KCl solution (0.075M) for 20 mins (vortex while adding KCl solution). Spin at 900rpm for 8 mins and discard the supernatant by suction and thoroughly mix the pellet by gentle tapping. Cells should be treated delicately after hypotonic treatment. Increased speed of centrifugation may break the cells. Fix the cells using a freshly prepared chilled mixture of 1:3 proportion of glacial acetic acid and methanol for 6 mins. Centrifuge at 800rpm. Add about 8ml of fixative drop-by-drop while gently vortexing to prevent the cell button becoming a clump and resuspend the pellet. Allow the cell suspension to stand at $0\ ^0$ C for about 5mins in the first wash. For this purpose keep the fixative in the freezer for at least half an hour, so that the fixative is chilled. Repeat the step twice. Improper mixing during first fixation may lead to a black colored precipitate. In case if such precipitate is found, mix the pellet in fixative solution and transfer into a new centrifuge tube does not contain any cells. Cells can be stored in fixative/methanol for slide preparation till next day or few months. To store the suspension, after two fixative washes, resuspend the cells in about 10ml of fixative and store at $0\text{-}4^0$ C.To prepare metaphase spreads on the slides, centrifuge the cell suspension at 800rpm and discard the supernatant. Resuspend the cell pellet in about 0.5ml of fixative. Flush-mix the suspension gently for several times using a plastic Pasteur pipette. Draw about 0.2ml of the mixture in the Pasteur pipette, hold a chilled slide at an angle of 45 degrees and drop the mixture in the pipette from a height. This helps in bursting the cells and to get a uniform spread of the cells. Put the slides immediately onto a hot-plate pre-heated to upto $45\text{-}50^0$ C and blow to dry the slides. Staining of the slides can be done using 5% Giemsa solution (2.5ml of Giemsa stain in 10ml of Sorenson's buffer and 40ml distilled water) for light microscopy or acridine orange (10µg/ml in Phosphate buffered saline) for fluorescence microscope.

Results and Discussion

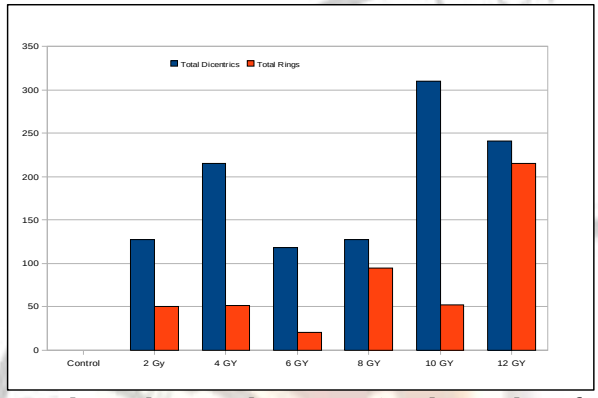
The chromosomal aberration assay was performed on whole blood samples with radiation doses of 2Gy, 4Gy, 6Gy, 8Gy, and 10Gy . The cultured blood samples were treated with calyculin ½ an hour prior to cell harvesting or processing. The result obtained on scoring the samples are as given below: (in table, graphs and figures)

Dicentrics: There were no dicentric formation in the 0 Gy or control sample, indicating a normalcy of the cell. In sample irradiated with a dose of 2 Gy, there were 128 dicentrics, showing some chromosomal damage. There were 215 in 4 Gy sample, 118 dicentrics in 6 Gy, 128 dicentrics in 8 Gy, 310 dicentrics in 10 Gy and 241 dicentrics in 12 Gy. **Rings**: There were no rings in the control sample. In sample irradiated with a dose of 2 Gy showed 50 rings in 290 cells, 51 rings in 205 cells in 4 Gy, 20 rings in 55 cells in 6 Gy, 95 rings in 119 cells in 8 Gy, 52 rings in 105 cells in 10 Gy and 215 rings in 211 cells in 12 Gy. **Fragments:** No fragments were ob served in control sample. In sample irradiated with a dose of 2 Gy, there were 126 fragments, showing some chromosomal damage. There were 170 fragments in 4 Gy sample, 166 fragments in 6 Gy, 121 fragments in 8 Gy, 300 fragments in 10 Gy and 237 fragments in 12 Gy. **Dicentric yield**: Yield of dicentrics for control sample was zero. The sample irradiated with a dose of 2 Gy gave a dicentric of 0.441 and that for 4 Gy gave 1.04. The dicentric yields for the rest of the sample were 2.14, 1.09, 2.95 and 1.14 for the radiation doses 6 Gy, 8 Gy, 10 Gy and 12 Gy respectively. **Ring yield**: Ring yield for control sample was zero. The yield of rings for sample irradiated with a dose of 2 Gy was 0.1724, for 4 Gy it was 0.24, for 6 Gy it was 0.36. The ring yields for the rest of the samples are 0.75, 0.495 and 1.01 respectively for the radiations 8 Gy, 10 Gy and 12 Gy. **Fragment yield**: The fragment yield for the control sample was zero. The fragment yield for the sample irradiated with a dose of 2 Gy was 0.434, that for 4 Gy was 0.82. The fragment yields for the rest of the sample are 1.01, 2.85 and 1.12 respectively for the radiation doses 8 Gy, 10 Gy and 12 Gy.

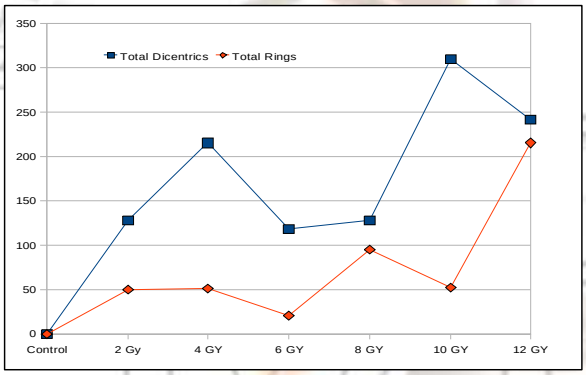
Table 1: Result of chromosomal aberration assay

Sample	Total Cells	Total Dicentrics	Dicentric yield	Total Rings	Yield of Rings
Control	100	0	0	0	0
2 Gy	290	128	0.44	50	0.17
4 Gy	205	215	1.04	51	0.24
6 Gy	55	118	2.14	20	0.36

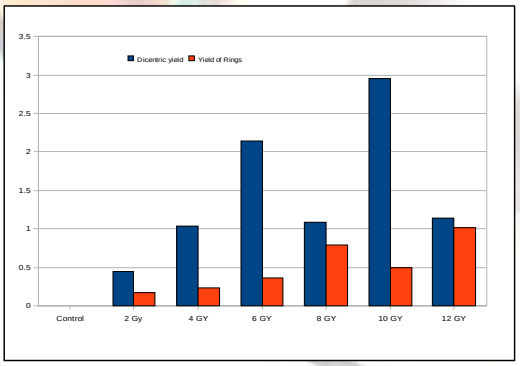
8 Gy	119	128	1.09	95	0.79
10 Gy	105	310	2.95	52	0.5
12 Gy	211	241	1.14	215	1.01



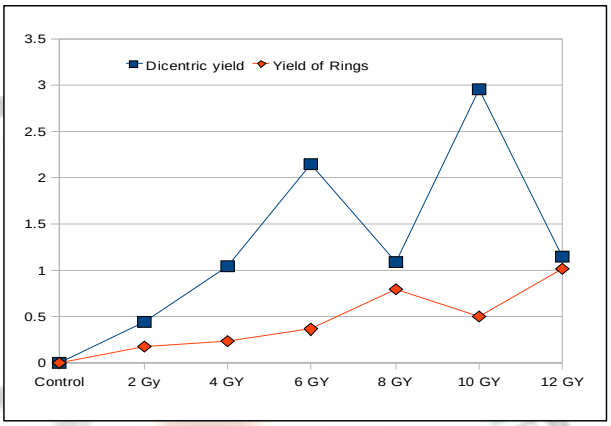
Graph 1: A bar-graph representing the number of dicentrics and rings at given doses



Graph 2: A line-graph representing the number of dicentrics and rings at given doses



Graph 3: A bar graph representing the yields of dicentrics and rings at the different doses



Graph 4: A line-graph representing the yields of dicentrics and rings at the different dose

Conclusion

In chromosomal aberration assay, upto 6 Gy the values of dicentric and ring yields increased gradually. This was followed by a dip at 8 Gy, a steep rise at 10 Gy and a second dip at 12 Gy in the values of dicentric yield. On the other hand, the values for ring yield increases gradually with radiation, though there is slight drop at 10 Gy. Radiations usually cause a delay in mitosis by an average value of 1hr/Gy. This happens because the cell goes through damage repair processes. Hence the conventional dicentric assay does not provide an accurate dose response estimate in the case of accidental exposure to ionizing radiations above 6 Gy due to radiation induced mitotic delay and poor mitotic index.

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References

1. Walter Fenech, M., and Morley, A. 1985. Measurement of micronuclei Lymphocytes. Mutation Research.147: 29-36.
2. Balakrishnan, S., Shisath, K., Bhat, N. and Anjaria K. 2010. Biodosimetry for high dose accidental exposures by drug induced premature chromosome condensation (PCC) assay. Mutation Research, 699 (1-2): 11-16. Radiological Physics & Advisory Dept., BARC, Trombay, India.
3. Reiter, R. J. 1980. The pineal gland and its hormones in the control of reproduction in mammals. Endocr. Rev., 1: 109-131.
4. Reiter, R. J. 1991. Pineal melatonin: cell biology of its synthesis and of its physiological interactions, Endocr. Rev., 12: 151–180.

5. Balakrishnan, S. and Bhatt, B.1989 Studies of Radiation Induced Micronuclei-A Rapid method for screening suspected over exposed individuals. Bull. Radiat. Prot., 12: 86-90 .
6. Balakrishnan, S., Rao, B. S. And Bhatt, B.1993, Radiation induced chromosome aberration yields following an accidentalnon-uniform exposure. Radiat. Prot. Dosimetry, 50: 45-49.
7. Chaube R C., Bhilwade H N., Rajesh R. and Bannur. 2001. Gamma Ray Induced DNA damage in Human & Mouse Leucocytes Measure using SCGE-Pro.: Soft. For image analysis and Data Processing for comet Assay. Mutat. Res., 419: 187-197.
8. Barlow, W. L. R., Reiter R. J., Abe, M., Pablos M., Menendez, P. A., Chen, L. D. and Poeggeler, B. 1995. Melatonin stimulates brain glutathione peroxide activity. Neurochem. Int., 26: 497-502.
9. Gilad, E., Cuzzocrea, S., Zingarelli, B., Salzman, A. L. and Szaba, C. 1997. Melatonin is a scavenger of peroxynitrite. Life Sci., 60: 169-174.
10. Loyd, L. 1996. "Retrospective dosimetry by chromosomal analysis", The Radiological Consequences of the Chernobyl Accident. Rep. Eur., 16: 965-973.
11. Vyas, R. C., Darroudi, F. and Natarajan, A. T. 1991 Radiation-induced chromosomal breakage and rejoining in interphase-metaphase chromosomes of human lymphocytes. Mutat. Res., 249: 29-35.
12. Durante, M., Furusawa, Y. and Gotoh, E. 1998.A simple method for simultaneous inter- phase-metaphase chromosome analysis in biodosimetry. Int. J. Radiat. Biol., 74: 457-462.
13. Countryman, P. I. and Heddle, J. A. 1976. The production of micronuclei from chromo- some aberrations in irradiated cultures of human lymphocytes. Mutat. Res.,41: 321-331.
14. Bairati, I., Meyer, F., Gelinas, M., Fortin, A., Nabid, A., Brochet, F., Mercier, J. P., Tetu, B., Harel, F., Abdous, B., Vigneault, E., Vass, S., Del, V. P. and Roy, J. 2005. Randomized trial of antioxidant vitamins to prevent acute adverse effects of radiation therapy in head and neck cancer patients. Journal of Clinical Oncology, 23: 5805-5813.
15. Blumberg, A. L., Nelson, D. F., Gramkowski, M., Glover, D., Glick, J. H, Juhas, J. M. and Kligerman, M. M. 1982. Clinical trials of WR-2721 with radiation therapy. International Journal of Radiation Oncology Biology Physics, 8: 561-563.
16. Eifel, P. J. 1988. Decreased bone growth arrest in growing rats with multiple radiation fractions per day. International Journal of Radiation Oncology Biology Physics, 15: 141-145.
17. Seabra, M. L., Bignotto, M., Pinto, L. R., Tufik, S. 2000. Randomized, double-blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. Journal of Pineal Research, 29: 193-200.
18. Alheit, H., Baumann, M., Thames, H. D., Geyer, P., Kumpf, R., Herrmann, T. 1998. Fractionation effect on radiation-induced growth retardation of tibia in rabbits and rats. Acta Oncologica, 372: 151-158.
19. Anwar, M. M., Moustafa, M. A. 2001. The effect of melatonin on eye lens of rats exposed to ultraviolet irradiation. Comparative Biochemistry and Physiology Part C: Toxicology and Pharma-cology 129: 57-63.
20. Ishidate, M. and Odashima, S.1977. Chromosome tests with 134 compounds on Chinese hamster cells in vitro: a screening for chemical carcinogens. Mutat. Res. 48: 337-354.

Fig. 1: Metaphase spreads of a normal cell

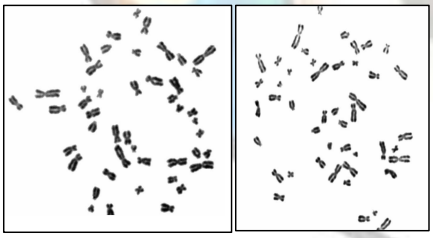
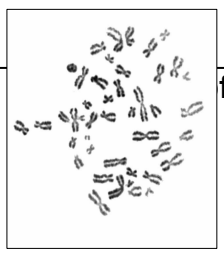
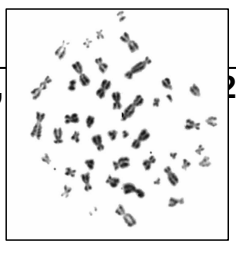
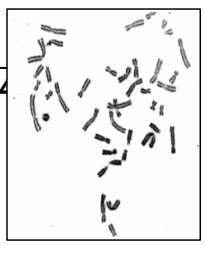


Fig 2: Damages caused by a radiation dose of 2Gy







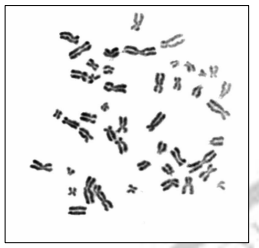
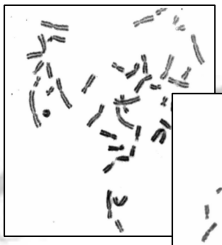



Fig: 3 Damages caused by a radiation dose of 4Gy

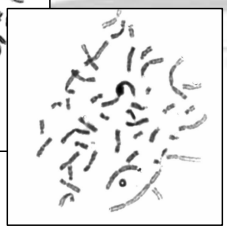
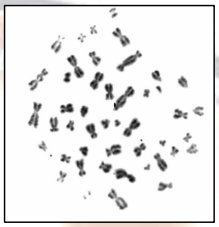
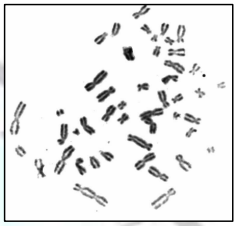
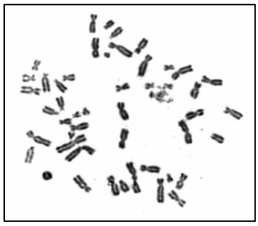





Fig: 4 Damages caused by a radiation dose of 6Gy



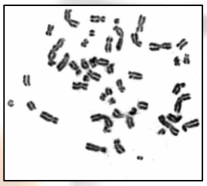
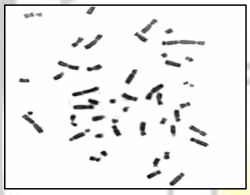



Fig: 5 Damages caused by a radiation dose of 8Gy



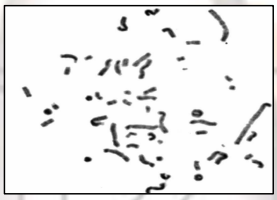
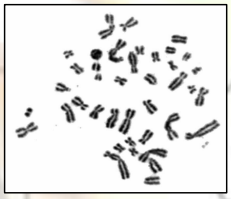



Fig: 6 Damages caused by a radiation dose of 10Gy