RECOVERY FROM POTENTIALLY LETHAL DAMAGE OF HUMAN MELANOMA CELLS AFTER IRRADIATION OF PROTON AND NITROGEN ION BEAM

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It has been noticed the remarkable increase in biological effect for heavy ion irradiation. We examined the effects of various LET irradiations (Table 1) on the damages and their repairs at the cell and DNA levels.

Throughout this work, we used HMV-I cultured cells from human melanoma. This cell line is characterized by its highly radioresistant response to X-rays while RBE of nitrogen ion (N-ion) for cell survival is about 2.2 at 10 percent survival level.

Table 1 Average LET estimations

Radiations	$\bar{L}_D$ (keV/mcm)
180kVp X-rays	3
20MeV Protons	2.8
95MeV N-ions	392

For DNA level study, we examined the production and rejoining (or repair) of DNA single strand breaks (or "alkali labile site") by an alkaline elution technique (Kohn et al., 1976[1]). This technique is sensitive enough to detect the DNA damage induced by small radiation dose. Irradiated cells were trypsinized and filtered onto the 2um pore-size filter and then lysed with 0.2% sarcosyl, 0.02M EDTA, 2M NaCl (pH10.2), which was allowed to flow through the filter by gravity. The filters were eluted in the dark at a pump speed of 0.06ml/m with 0.1M tetrapropylammonium hydroxide and 0.02M EDTA (pH12.1). Eluted fractions were collected and assayed for DNA contents by micro-fluorometric determinations. Decreasing the amount of DNA remained on filter after elution corresponded to increasing the amount of the initial DNA lesions.

For cell level study, recovery from potentially lethal damage (PLDR) was examined using the replating technique of the cells cultured in plateau phase.

The results were as follows:
1) The amount of the initial DNA lesions caused

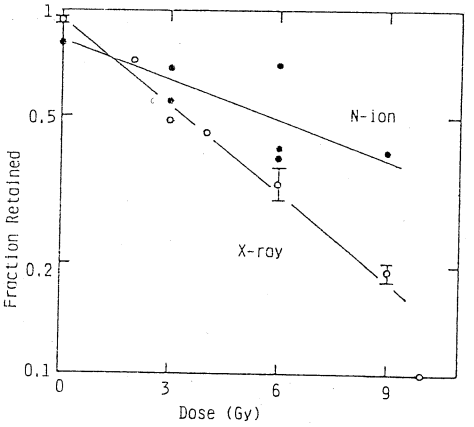

Fig.1 Initial DNA lesions after X-rays and N-ion irradiations.

by N-ion were less than that by X-rays in spite of high RBE value of N-ion (Fig.1).
2) On the other hand, it was shown that the DNA lesions induced by N-ion rejoined more slowly than those by X-rays (Fig. 2).
3) For N-ion, dose dependency of the PLDR was not observed.
4) For both radiations of proton and N-ion, remarkable delay of appearance of the PLDR was observed (Fig. 3).
5) For N-ion, amount of the PLDR was smaller than that for proton and X-rays (Fig. 3).

These results indicate that irradiation of high LET N-ion induces the damages which is difficult to be repaired, and that the appearance of PLDR is expressed by the two different parameters, namely a mean recovery time and a recovery ratio of initial damages.

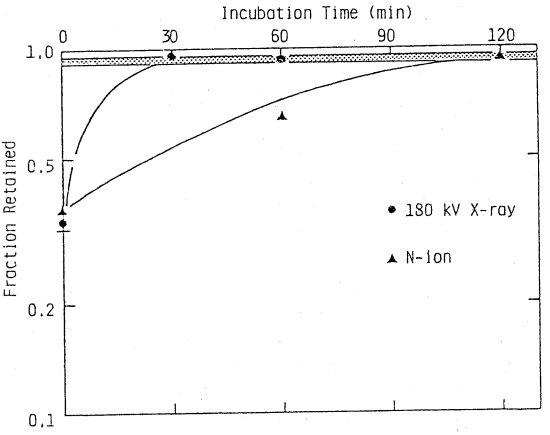

Fig.2 Repair of DNA lesions induced by X-ray (6Gy) and N-ion (9Gy).

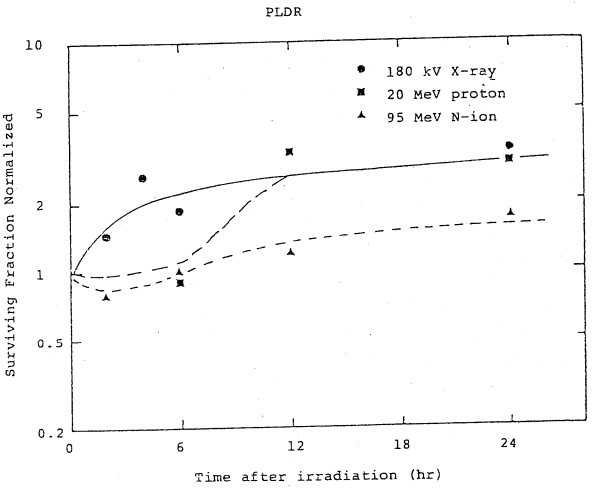

Fig. 3 PLDR of HMV-I cells after X-ray , proton and N-ion irradiations.

Reference
1) Kohn, K.W. et al.: Biochemistry, 15, 4629-4637, 1976