# §21. Study on Biological Effects of Tritium at Animal Level

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Since it was a final year of this study, the meeting for the total conclusion was held. Furthermore, the outline about the effects of low dose tritium on living body was published[1)2)3)]. Therefore, to prepare the data for biological effects of low dose tritium, the present report focused on, i) analysis of biological effects of tritium by using genetically engineered animals, ii) analysis of the mechanism of DNA damage response, and iii) analysis of the biological effects of tritium at the cellular level. Followings are summary of the results for 3 years.

i) Analysis of biological effects of tritium by using genetically engineered animals

We have established a novel experimental system that can examine the biological effects of low dose (rate) tritium radiation at animal level. We used two strains of transgenic mice, the gpt-delta mice[4)] and Rev1 mice[5)]. The gpt-delta mice carry a mutation reporter gene, and Rev1 mice are over-expressing Rev1. We also used the p53 (a tumor suppressor gene) knockout mice[6)], in order to clarify the importance of the regulation of DNA damage checkpoint in prevention of teratogenic effects by low dose tritium exposure. Tritiated water (HTO) was administrated to the gpt-delta mice at low dose rate, and the mutation frequency at several tissues are analyzed. With the total dose of 3 Gy, the mutant frequency was elevated approximately 2-fold above control level, and it remained at a similar level up to 6 Gy. These results indicate that gpt delta mouse could be a good model animal to study genotoxicity (induction of mutation) of tritium.

ii) Analysis of the mechanism of DNA damage response

Understanding the molecular mechanism of cellular DNA damage responses is another important point of view to understand the biological risk of low dose (rate) radiation. If the mechanisms are fully clarified, we believe that one can simulate the biological responses to low dose tritium radiation.

Activation of the DNA-dependent protein kinase (DNA-PK) and AKT signaling pathway was found in cellular response to X-ray exposure. Therefore, we tested whether the pathway is also activated by HTO exposure. As well as X-rays, exposure of HepG2 and HeLa cells to HTO activated AKT, which was shown by phosphorylated-AKT at Serine 473 residue. The activation of DNA-PK was also observed in these cells by X-ray irradiation but not by exposure to HTO[7)](Fig.1). These suggest the existence of difference in cellular response between X-rays and HTO.

iii) Analysis of the biological effects of tritium at the cellular level

We previously found that the human-X-carrying hamster cell system appeared to be able to detect a wide range of mutation spectrum, even if those mutations affect the expression of important human genes for cell survival. The system showed about 100-fold sensitivity compared to the conventional system that uses endogenous *Hprt* gene. By using the cell system, we are testing the mutation induction by HTO exposure at low dose rate. Our results suggested that the reverse dose rate effect does not apply in the case of mutation induction by HTO and that exposure to low level of HTO (0.1 Gy with 12mGy/day) could merely enhance the spontaneous mutagenesis[8)](Fig.2).

We have demonstrated that the recognition of primary-dose and adaptive response could be mediated by a feedback signal pathway which involves protein kinase C (PKC), p38 mitogen activated protein kinase (p38/MAPK), and phospholipase C (PLC). We are doing experiments to clarify the effect of PKC knockdown by siRNA on radio-adaptive response. By the experiments, we may verify the importance of PKC pathway for expression of radio-adaptive responses. In addition, we found that an adaptive response was induced by pre-treatment of the cells with low concentration of tritium compound such as $^{3}$H-thymidine for a few days[9)].

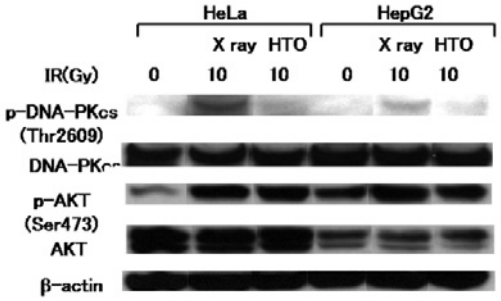


Fig. 1. Activation of the AKT signaling pathway by HTO.

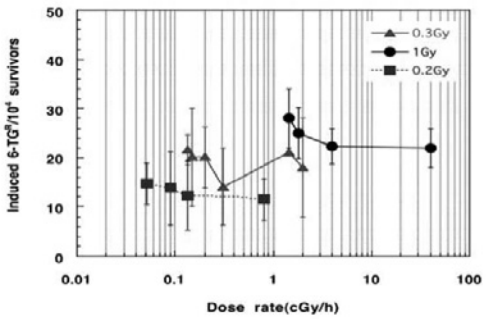


Fig. 2. Dose-rate dependency of mutation frequency induced by exposure to HTO.

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