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Effects of adrenochrome monoguanylhydrazone methanesulfonate given before or after irradiation on the induction of chromosome aberrations

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放射線誘発染色体異常からみたアドレノクローム（AMM）の防護及び回復効果

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放射線防護機構は分子及び個体レベルで多くの報告がなされているが，細胞レベルでの研究は少い．最近アドレノクローム（AMM）がヒトに適用され，その実用的有効性が報告された．この機構を細胞学的に検討した．腫瘍細胞の染色体切断及び娘染色体切断を指標とすると，照射前投与では著明な各切断の減少が認められ，DRFは1.26を示した．照射後投与では異常頻度の減少はないが，染色体及び娘染色体切断の頻度は減少し，回復効果が認められた．

Kobayashi and Kawamura (1965) showed that chromosome aberrations in tumor cells after sublethal irradiation could be restored by the treatment with cysteamine (MEA).

The radioprotective effects of adrenochrome monoguanylhydrazone methanesulfonate (AMM) have been studied in mice and human subjects (Sugahara and Tanaka 1968, Tanaka and Sugahara 1970, Yamashita *et al.* 1971). However, the mechanism of its effect has not been examined at the cellular level.

In the present investigation chromosome aberrations in ascites tumor cells were used as indicators of radiation injury and the protective and restorative effects of AMM treatment given before or after irradiation were demonstrated.

Materials and Methods

Ehrlich carcinoma ascites tumor cells were used as materials. The tumor cells were grown in 60-day

old, male Swiss albino mice obtained from an inbred colony in the author's laboratory. AMM was injected into mice intraperitoneally on the 6th day after the transplantation of ascites tumor cells. It was injected at a dose of 5 mg/kg 15 min before or 5 hr after whole-body irradiation of the mice. Mice were irradiated with 500 R from a Toshiba therapeutic X-ray machine operated at 200 kvp and 20 mA at a dose rate of 148 R/min as measured by a Victoreen Radocon dosimeter.

Chromosome preparations were made by a modification of the method of Kobayashi and Kawamura (1965) and breaks in chromosomes and chromatids were scored in each experiment. Values were calculated from the results on about 50 to 100 metaphasic cells examined 24 hour after irradiation. The values recorded were the aberration rate per cell, chromatid breaks per cell, chromosome breaks per cell, aberration rate per chromosome and aberration rate/cell/R.

Results

Results are shown in Table 1. The chromosome number was 72 in the stem cells of this tumor. In control samples, 6% of the metaphasic cells had chromatid breaks but no chromosome breaks were observed. The aberration rate per chromosome was 8.3×10^{-4}.

Table 1. Chromosome aberrations in tumor cells from mice treated with AMM before or after irradiation

	Control(OR)	500R	AMM— 500R	500R—AMM
Chromosome aberration (%)	6	48	38	48
Aberration rate/cell	0.06	2.48	2.10	1.88
Chromatid breaks/cell	0.06	0.68	0.46	0.42
Chromosome breaks/cell	—	1.82	1.64	1.46
Aberration rate/chromosome	8.3×10^{-4}	3.3×10^{-2}	2.9×10^{-2}	2.7×10^{-2}
Aberration rate/cell/R ($\times10^{-3}$)	—	4.8	4.2	3.8

After irradiation with 500 R without AMM treatment 48% of the cells showed chromosome aberrations with average of 0.68 chromatid breaks and 1.82 chromosome breaks per cell. The aberration rate per cell was 2.48. The aberration rate per chromosome was 3.3×10^{-2} and the aberration rate/cell/R was 4.8×10^{-3}.

In samples from animals treated with AMM before irradiation 38% of the cells showed chromosome aberrations, with averages of 0.46 chromatid breaks and 1.64 chromosome breaks per cell. The aberration rate per cell was 2.10 and the aberration rate per chromosome was 2.9×10^{-2}. The aberration rate/cell/R was 4.2×10^{-3}.

In cells from mice treated with AMM after irradiation 48% of them had chromosome aberration with 0.42 chromatid breaks and 1.46 chromosome breaks per cell. The aberration rate per cell was 1.88 and the aberration rate per chromosome was 2.7×10^{-2}. The aberration rate/cell/R was 3.8×10^{-3}.

Discussion

Many chemicals, such as cysteamine, AET and serotonin, have been reported to protect chromosomes

from aberrations induced by irradiation and/or to induce elimination of these aberrations (Maisin and Moutschen 1960, Curtis *et al.* 1964, Kobayashi and Kawamura 1965). However, there have been few reports of cytological analyses on the mechanism of action of these chemicals after irradiation.

The present work showed that when injected before irradiation AMM caused marked decrease in the frequency of chromosome and chromatid breaks per cell induced by irradiation. From the results dose reduction factor is estimated roughly to be 1.26, which is lower than the value of 1.32 of Sugahara and Tanaka (1968) based on the $LD_{50/30}$ in mice. However, the value calculated from chromatid breaks per cell was 1.48.

Tanaka and Sugahara (1970) concluded from studies on human lymphocytes that if cell proliferation to supplement lymphopenia induced by radiation starts after the second week of radiotherapy, the proliferating cells are protected more effectively by AMM than cells in G_0. The present results on tumor cells showed that the frequency of chromosome breaks induced by irradiation was protected more effectivelly by AMM than that of chromatid breaks. This suggests that cells in G_1 may be more protected by this chemical than cells in S or G_2. These results on the effectiveness of AMM in protection against irradiation agree well with those of Tanaka and Sugahara (1970).

On administration 5 hour after irradiation with 500 R, AMM had no effect on the frequency of chromosome aberrations induced by irradiation. However, it reduced chromatid breaks from 0.68 (irradiation only) to 0.42 per cell and chromosome breaks from 1.82 to 1.46 per cell. Five hour after irradiation, DNA synthesis and mitoses of this tumor were not observed. The mitotic cells of 24 hours' samples after irradiation included mainly S cells with some of G_2 and G_1 cells at the irradiation (Kobayashi 1968). Further work on the timing of administration and preparation will have to be done on a similar cell system.

Summary

The radioprotective and restorative effects of adrenochrome monoguanylhydrazone methanesulfonate (AMM) were studied using chromosome aberration as an indicator of radiation injury. This chemical had a protective effect against breaks in chromosomes and chromatids induced by radiation. It also had a slight stimulatory effect on elimination of chromosome aberrations after irradiation.

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