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COMBINED TREATMENT EFFECTS OF IRRADIATION AND SN-38, AN ACTIVE METABOLITE OF CAMPTOTHECIN DERIVATIVE IRINOTECAN, ON THE HUMAN TUMOR CELL LINE PECA 4197

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Abstract The *in vitro* combined effects of irradiation and SN-38, an active metabolite of irinotecan, on the human squamous cell carcinoma cell line PECA 4197 derived from a head and neck carcinoma were examined. Exponentially growing cell cultures were pretreated with SN-38 for 90 min, in the concentration of 0.005 and 0.5 μg/ml, respectively, and then irradiated. As a control, cells without pretreatment were also irradiated. The cell survival rate was determined using a conventional clonogenic survival assay. The enhanced effects of radiation-induced cell killing, depending upon the concentration of SN-38, were presented with a decrease of shoulder part or extrapolation number (n) of survival curve, corrected for the cytotoxicity of SN-38 alone. The value of D_0 was unchanged after SN-38 pretreatment. These results suggested that the pretreatment with SN-38 inhibited potentially lethal damage repair in the cell line, leading to the potentiation of irradiation effects.

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Key words : SN-38 ; irinotecan ; irradiation ; inhibition of PLDR ; clonogenic survival assay.

ヒト腫瘍培養細胞株 PECA 4197 に対する放射線および SN-38（カンプトテシン誘導体イリノテカン活性代謝物）併用効果

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抄録　頭頸部癌由来のヒト扁平上皮癌培養細胞株 PECA 4197 に対する，放射線照射とイリノテカン活性代謝物 SN-38 の *in vitro* 併用効果について検討した．指数増殖期の細胞を SN-38 (0.005および 0.5 μg/ml) で90分間前処理したのち放射線照射を加え，コロニー法によって細胞生存率を求めた．対照として，前処理なしの細胞で同様に放射線照射を加え，細胞生存率を求めた．この結果，SN-38 の前処理は，濃度依存性に放射線殺細胞効果を増強し，これは生存率曲線の肩あるいは外挿値(n)の減少を伴っていた．D_0 の値は，前処理の有無で変化を認めなかった．SN-38 による前処理は，細胞の放射線による潜在的致死損傷からの回復を阻害し，これによって放射線増感効果を示すと考えられた．

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Introduction

Irinotecan {7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin} is a new semisynthesized derivative of camptothecin, which interferes with mammalian DNA topoisomerase I and possesses anti-tumor activity[1]. It has been postulated that the cell killing action of camptothecin and its derivatives is due to the collision of drug-stabilized cleavable complexes with moving replication forks, leading to replication arrest and conversion of cleavable complexes into DNA strand breaks[2~5]. Although several metabolites are known to be produced when irinotecan is administered to patients, SN-38 (7-ethyl-10-hydroxy-camptotecin) is regarded as a major one, having a strong cytotoxic activity 100- to 1,000-fold greater than that of the parent compound irinotecan, and its intracellular role appears determinant for irinotecan activity[1, 6].

Recent clinical studies[7~10] have demonstrated a promising anti-tumor activity and tolerable toxicities of irinotecan, although camptothecin itself had to be withdrawn from clinical trials because of its unpredictable and excessive toxicity[11]. Irinotecan has shown favorable treatment results in various types of tumors, therefore, for clinical purposes, the combined treatment of irinotecan and irradiation should be taken into account for multidisciplinary treatment, especially for head and neck carcinomas. Thus, in the present study, we examined *in vitro* the combined treatment effects of irradiation and SN-38, an active metabolite of irinotecan, using a human tumor cell line derived from a head and neck squamous cell carcinoma.

Materials and Methods

Cell line and agent preparation

An established tumor cell line PECA 4197 derived from a human squamous cell carcinoma of gingiva[12] was used. The line was grown as monolayers in Eagle's minimum essential medium (Gibco) supplemented with 20% fetal calf serum (Gibco) and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. All experiments were carried out during exponential growth phase of the cells, and 5×10^5 cells were incubated in 25 cm^2 flasks for 24 h before treatment.

SN-38 was kindly provided by Daiichi Pharmaceutical Co., LTD (Tokyo, Japan) and prepared as a series of stock solutions with various, appropriate concentrations (1 to 100 μg/ml) in dimethylsulfoxide (DMSO ; Serva), stored at −20°C and thawed immediately before use.

Clonogenic survival assay

On the first series of experiments, the cells were treated with SN-38 alone in the desired final concentrations for 90 and 180 min, respectively. The cells were then washed twice with agent-free medium and immediately trypsinized, counted, and plated in appropriate numbers for colony formation into 19.6 cm^2 tissue culture dishes (Becton-Dickinson, USA). Eight to nine days later, colonies were fixed with 96% methanol, stained with 1% crystal violet and counted to assess the clonogenic survival. Colonies containing more than 50 cells were counted. Two independent experiments, each performed in triplicate, were carried out.

In the second series of experiments, cells were pretreated with SN-38 for 90 min in the final concentration of 0.005 and 0.5 μg/ml, respectively. Then they were washed twice with agent-free medium, and irradiated using an X-ray machine (Stabilipan, Siemens, Germany ; 240 kV, 15 mA, 0.5 mm Cu filter, and a dose rate of 1 Gy/min) with doses of 1, 2, 4, 6 and 8 Gy, applied at room temperature and room air. Control cells received sham irradiation. To obtain a survival curve treated with irradiation alone, the cells without SN-38 pretreatment were also irradiated with doses of 1, 2, 3, 4, 6 and 8 Gy. Two and four independent experiments, each performed in triplicate, were carried out for irradiation with and without SN-38 pretreatment, respectively. The enhancement ratio (ER)[13] of combined effects of irradiation with SN-38 pretreatment was determined from the radiation doses required to reduce the surviving fraction to 0.1, of which values were corrected for cytotoxicity predicted from SN-38 treatment alone.

Results

Clonogenic survival curves of cells treated with SN-38 alone are presented in Figure 1. Cytotoxicity appeared with low concentration (0.005-0.2 μg/ml), and it reached a maximum over a dose range of 0.5 μg/ml. An increased cytotoxicity was observed in

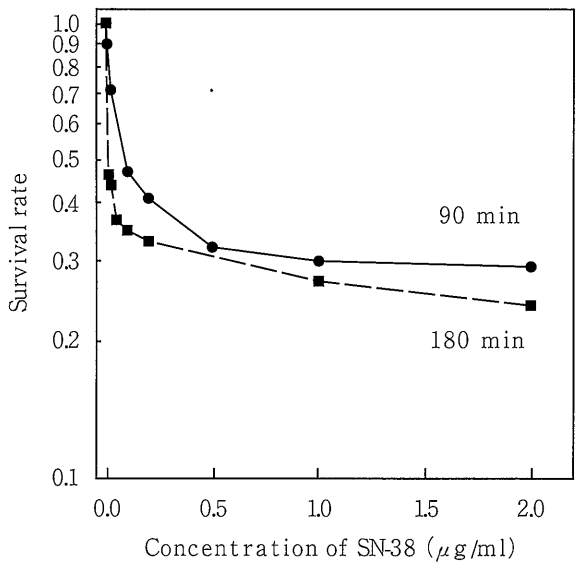

Fig. 1 Clonogenic survival curves of cells treated with SN-38 for 90 min (●) and 180 min (■). Symbols represent the mean of two independent experiments, performed in triplicate, respectively.

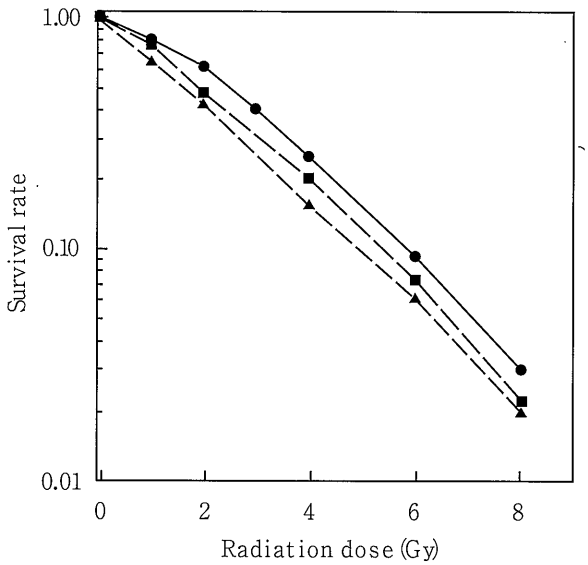

Fig. 2 Clonogenic survival curves of cells treated with irradiation alone (●) and irradiation pretreated with SN-38 (■ : 0.005 μg/ml, 90 min ; ▲ : 0.5 μg/ml, 90 min). Symbols represent the mean of two and four independent experiments, with and without pretreatment, respectively, performed in triplicate. The survival rates were corrected for cytotoxicity predicted from SN-38 treatment alone.

cells treated with SN-38 for 180 min compared with those treated for 90 min.

Clonogenic survival curves of cells irradiated with and without SN-38 pretreatment are demonstrated in Figure 2. The pretreatment with SN-38 resulted in a decrease of shoulder part of survival curve depending upon the concentration. The extrapolation number (n) was 2.3 for irradiation alone and was, corrected for the cytotoxicity of SN-38, 1.7 and 1.0 for irradiation with SN-38 pretreatment in the concentration of 0.005 and 0.5 μg/ml, respectively (Table 1). The D_0 values were unchanged (1.9 Gy) with or without SN-38 pretreatment. ER was 1.1 and 1.2 for SN-38 pretreatment in the concentration of 0.005 and 0.5 μg/ml, respectively, indicating the enhanced effects on radiation-induced cell killing.

Discussion

Because recent clinical studies[7~10] have indicated favorable therapeutic results with irinotecan against various types of tumors, it is important to examine the combined anti-tumor activity of irinotecan with irradiation for its further clinical use. This is the case especially for the management of advanced head and neck carcinomas, for which multidisciplinary treatment including radiotherapy and chemotherapy is often effective and needed.

The combined cytotoxic mechanisms of irradiation and camptothecin or its derivatives have been reported[14~18]. Regarding cell cycle effects, Tamura et al.[14] reported that brief exposure of a low dose SN-38 to *in vivo* human lung cancer cell lines induced an initial cell cycle block in the G2/M phase. They concluded the modulation of cell cycle distribution might explain a part of combined cytotoxic effects. In turn, there have been several reports about the inhibition of radiation-induced DNA damage repair with camptothecin or the derivatives. Ng et al.[15] demonstrated that camptothecin inhibited sublethal damage repair (SLDR) but did not so with potentially lethal damage repair (PLDR) in two human melanoma cell lines. Musk and Steel[16] showed the inhibition of SLDR and PLDR with camptothecin in human bladder carcinoma cells. Boothman et al.[17] demonstrated that camptothecin, administered either during or immediately following irradiation, inhibited PLDR resulting in a syner-

Table 1 Parameters of clonogenic survival curve modified by SN-38 pretreatment

SN-38 pretreatment	D_0*	n**	ER***
None	1.9	2.3	—
0.005 μg/ml, 90 min	1.9	1.7	1.1
0.5 μg/ml, 90 min	1.9	1.0	1.2

*The dose (Gy) required to reduce survival to e^{-1} on the exponential part of the survival curve.
**The extrapolation number, corrected for cytotoxicity predicted from SN-38 treatment alone.
***Enhancement ratio of combined effects of irradiation with SN-38 pretreatment.

gistic effects of an extremely resistant human melanoma cell line. Using human colon adenocarcinoma cells grown as spheroids, Omura et al.[18] indicated that exposure to SN-38 following irradiation inhibited PLDR.

In the present study, the survival curve of SN-38 treatment alone, acutely bending with increased agent concentration and then reaching a maximum, suggested an S-phase specific cytotoxiticy as previously reported[19]. When the cells were pretreated with SN-38, the enhanced effects on radiation-induced cell killing with a decreased shoulder part of the curve were presented, depending upon the concentration of SN-38. This indicated that pretreatment with SN-38 inhibited repair of radiation-induced DNA damage, classified as PLDR, and led to the potentiation of irradiation effects in the human head and neck squamous cell carcinoma cell line, being consistent with the above-mentioned reports using other cell lines of different histological types.

As to the pharmacokinetics of SN-38 in treated patients, it was reported that the mean plasma concentration of SN-38 was kept within the range between 0.01 and 0.1 μg/ml for almost one day when 100-350 mg/m^2 of irinotecan is administered to patients in a 30-min intravenous infusion[20]. Thus, taking into account our data, the concentration of SN-38 in tumor tissues of patients treated with standard dose of irinotecan can be expected to be high enough for its own cytotoxic actions and the combined ones with irradiation for a certain period after the administration. Furthermore, it might be possible to optimize the combined schedule with irradiation for obtaining good tumor response, considering the *in vivo* pharmacokinetics of irinotecan and SN-38[1, 11, 13~20].

Although further experiments are necessary for determining the combined effects of irradiation with irinotecan for clinical purpose, our results suggest that the combination is promising for the multidisciplinary management of advanced head and neck squamous cell carcinomas.

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