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Enhanced apoptosis of squamous cell carcinoma cells by interleukin-2-activated cytotoxic lymphocytes combined with radiation and anticancer drugs

T. Yamamoto *, K. Yoneda, E. Ueta, S. Doi, T. Osaki

Department of Oral Surgery, Kochi Medical School Kohasu, Oko-cho, Nankoku-city, Kochi 783-8505, Japan

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Abstract

Induction of potent apoptosis is required in cancer therapy. We examined the combination effect of interleukin-2-activated lymphocytes (LAK cells) and anticancer drugs or gamma (γ)-rays on the induction of apoptosis in an established oral squamous cell carcinoma cell line (OSC-3 cells). By pretreatment of OSC-3 cells with ^{137}Cs (5 Gy), 5-fluorouracil (5-FU) (0.5 μg/ml) or *cis*-dichlorodiammine-platinum (CDDP) (5 μg/ml), the activation of bid and caspase-3 by LAK cells was strongly increased and associated with an enhanced degradation of poly-(ADP-ribose) polymerase (PARP) and/or nuclear mitotic apparatus protein (NuMA) and the increased fragmentation of DNA. The LAK cell–enhanced caspase-3 activity in the pretreated OSC-3 cells was decreased to approximately 70% and 40% of the control by the addition of Z-AAD-CMK (a granzyme B inhibitor) and neutralising monoclonal antibody to Fas antigen (αFas-IgG), respectively. The combined treatment-induced DNA fragmentation was suppressed by approximately 20% and 30% of the control by the addition of Z-AAD-CMK and αFas-IgG, respectively, in the co-culture system. While Ac-DEVD-CHO (a caspase-3 inhibitor) suppressed the DNA fragmentation levels to approximately half and this was similar to the amount of suppression that was obtained by the addition of both αFas-IgG and Z-AAD-CMK. In addition, LAK cell-activated bid may have increased the intracellular reactive oxygen intermediates (ROI) level and induced a decrease of mitochondrial membrane potential. These influences by LAK cells were enhanced when OSC-3 cells were pretreated with each anticancer drug or ^{137}Cs. Furthermore, the increase of ROI by LAK cells was suppressed by αFas-IgG and Z-AAD-CMK to approximately half the level of the control. These results indicate that anticancer drugs and γ-rays prime squamous cell carcinoma cells to be susceptible to apoptosis by LAK cells, that LAK cell-induced apoptosis largely depends on the activation of caspase-3 by the Fas/Fas-ligand signal and granzyme B, and that LAK cells induce ROI in the target cells, which is largely mediated by Fas and granzyme B.

Keywords: Apoptosis; Caspase; Granzyme B; Fas; LAK cells; Reactive oxygen intermediates; Squamous cell carcinoma cells

1. Introduction

Induction of tumour cell differentiation and apoptosis have gradually become targets in cancer therapy [1,2]. The all-*trans*-retinoic acid has been shown to have a substantial ability to induce differentiation in acute promyelocytic leukaemias, and the therapeutic effects of other agents capable of inducing differentiation such as the interferons, 13-*cis*-retinoic acid, 22-oxa-1α, 25-dihydroxyvitamin D_3 and vesnarinone have also been reported in solid tumours including breast cancers, colon cancers, neuroblastomas and salivary gland tumours [3–7]. Therapies capable of inducing differentiation and apoptosis lead to the preservation of the function of the organs, and such function-preserving therapy is recommended in oral carcinomas [8]. However, little data concerning such therapy have been reported in squamous cell carcinomas [9,10].

Differentiation and apoptosis are induced not only by the above differentiation-inducing agents, but also by chemotherapeutic drugs and gamma (γ)-rays [9–14]. In addition, cytotoxic lymphocytes such as major histocompatibility complex class I-restricted cytotoxic T lymphocytes, tumour-infiltrating lymphocytes and lymphokine-activated killer (LAK) cells can induce tumour cell differentiation and apoptosis, although the signal

* Corresponding author. Tel.: +8-888-80-2423; fax: +8-888-80-2424.

E-mail address: yamamott@kochi-ms.ac.jp (T. Yamamoto).

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pathways involved differ somewhat from each other [15–19]. As is well known, induction of differentiation requires a G_1-arrest of the cells, but apoptosis can be induced in all cell cycle phases [20–22]. Therefore, therapy that induces apoptosis may be more effective in the treatment of malignant tumours than drugs that induce differentiation.

Apoptosis is characterised by marked morphological changes such as membrane blebbing, chromatin condensation, nuclear breakdown and internucleosomal DNA fragmentation [2]. Caspases, cysteinyl aspartate-specific proteases, play an important role in signalling the induction of apoptosis [23–25]. The caspases are activated through two signal transduction pathways [26–28]. One of the pathways starts from the Fas-associated death domain (FADD) which is activated by the binding of Fas with Fas-ligand (Fas-L). This leads to the assembly of a signal transduction complex that results in activation of the caspase cascade [29]. In this cascade, FADD-associated caspase-8 is activated first resulting in cleavage of the downstream caspases and finally, in activation of caspase-3. The other pathway starts in the mitochondria [30,31]. Anticancer agents such as bleomycin, *cis*-dichlorodiammineplatinum (CDDP), 5-fluorouracil (5-FU) and γ-rays induce reactive oxygen intermediates (ROI) in the internal mitochondrial membrane and these ROI decrease the potential of the external mitochondrial membrane, which leads to release of cytochrome c [32–35].

Chemotherapeutic drugs and γ-rays appear to induce tumour cell apoptosis via the release of cytochrome c, while cytotoxic lymphocytes induce apoptosis by the Fas/Fas-L system and perforin/granzyme B. Therefore, strong apoptotic signals are expected when tumour cells are treated with cytotoxic lymphocytes and the apoptosis-inducing anticancer drugs or γ-rays. Frost and colleagues have reported that prostate carcinoma cell lines are sensitised for lymphocyte-mediated apoptosis via the Fas/Fas-L pathway by their pretreatment with anticancer drugs [17]. However, co-operation of anticancer agents and γ-rays with lymphocytes has not been investigated in squamous cell carcinoma cells. In the present study, we investigated whether LAK cell-induced apoptosis of squamous cell carcinoma cells is augmented by pretreatment with chemotherapeutic drugs such as 5-FU, CDDP and γ-rays.

2. Materials and methods

2.1. Cell line

An OSC-3 cell line was established from an oral squamous cell carcinoma and cultured in Dulbecco's modified Eagle's essential medium (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 2 mM L-glutamine and antibiotics. Daudi cells, derived from Burkitt's lymphoma, were grown in suspension in Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharmaceutical Co., Ltd) supplemented with 10% (v/v) heat inactivated FBS, 2 mM L-glutamine and antibiotics. OSC-3 cells can be transplanted into nu/nu mice and the expressed *TP53* gene is mutated at codon 176, Cys to Phe.

2.2. Reagents

The cytotoxic anti-Fas monoclonal antibody (MAb) (αFas-IgM, clone CH-11) and neutralising MAb of Fas (αFas-IgG, clone ZB4) were purchased from Medical & Biological Laboratories Co., Ltd (Nagoya, Japan). Caspase-3 inhibitor, Ac-DEVD-CHO and granzyme B inhibitor, Z-AAD-CMK were obtained from Calbiochem (Cambridge, MA, USA).

2.3. LAK cell induction

Peripheral blood mononuclear cells (PBMC) were isolated from heparinised peripheral blood of healthy donors by the Ficoll-Paque (Pharmacia Fine Chemicals, NJ, USA) gradient method. The obtained PBMC were washed twice with phosphate-buffered saline (PBS), resuspended in RPMI1640 medium containing interleukin-2 (IL-2, 75 U/ml, Shionogi Pharmaceutical Co. Osaka, Japan) and 10% (v/v) heat-inactivated human AB serum, and cultured for at least 7 days at 37°C in plastic flasks. The mean cytotoxic activities of the induced LAK cells against Daudi cells were 50–75% at an effector cell:target cell (E:T) ratio of 20:1.

2.4. Cell growth inhibition assay

OSC-3 cells were exposed to the indicated doses of γ-rays using a ^{137}Cs source and cultured in medium for 48 h at 37°C or were cultured in medium containing the indicated doses of 5-FU (Sigma-Aldrich Chemicals, Tokyo, Japan) or CDDP (Sigma) for 48 h at 37°C. The cells were then washed and cultured with or without LAK cells at an E:T ratio of 10:1 for 48 h. After the cultivation, viable OSC-3 cells were counted by excluding non-viable cells which were positively stained with trypan blue. In the trypan blue exclusion, squamous OSC-3 cells were morphologically distinguished from LAK cells which were round and small. Per cent inhibition of cell proliferation was calculated using the following formula: % of growth inhibition = $[1-(Nt-No)/(Ns-No)]\times 100$, where No is the number of seeded OSC-3 cells, Ns is the number of OSC-3 cells cultured without LAK cells for 48 h, and Nt is the number of OSC-3 cells cultured with or without LAK cells for 48 h after each pretreatment which is shown in the figure legends.