Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 270 (2008) 87–94
CANCER Letters
www.elsevier.com/locate/canlet

Isoflavones and gamma irradiation inhibit cell growth in human salivary gland cells

J. Katz [a,*], E. Blake [a], T.A. Medrano [b], Y. Sun [a], K.T. Shiverick [b]

[a] Department of Oral Maxillofacial Surgery/Diagnostic Sciences, University of Florida, P.O. Box 100414, Gainesville, FL 32610-0414, USA
[b] Department of Pharmacology & Therapeutics, University of Florida, Gainesville, FL, USA

Received 5 February 2008; received in revised form 5 February 2008; accepted 28 April 2008

Abstract

We studied the effects of isoflavones and irradiation on cell cycle in a human salivary gland cell line (HSG). Genistein and a soy isoflavone conjugate (NS) inhibited DNA synthesis. Cells deconjugated the glucoside form of isoflavones in NS to the aglycones genistein and daidzein. NS, genistein and IR increased phosphorylation of p53 and $p21^{CIP1}$ at serine 15 (phos-p53). Irradiation and NS also increased levels of $p21^{CIP1}$. In a cologenic survival assay, cells in log phase growth had high radio-sensitivity with 2 Gy causing a reduction in survival ($SF_2 = 0.45$). *Conclusion.* Isoflavones and radiation may interact to sensitize cancer cells to radiation.

Keywords: Genistein; Soy isoflavones; Vitamin E succinate; HSG cells; Oral cancer; Radiotherapy

1. Introduction

Within the adult population of the United States, oral carcinoma has been reported to be one of the most common oral mucosal lesions, with approximately 21,000 new cases diagnosed annually [1]. Because the overall success rate for treatment of head and neck cancer is about 50%, research is needed for new protocols, including combination treatments, in an effort to decrease the mortality [1]. The specific treatment of choice for a lesion depends on many tumor variables such as histology, size, location, invasion into adjacent structures, and duration of symptoms. Radiation therapy for malignant lesions in the oral cavity is usually indicated when the lesion is radiosensitive, advanced, or deeply invasive and cannot be approached surgically. Combination surgical and radiotherapeutic treatment often provides optimal treatment [1]. Recent improvements in radiation therapy take into account the need for radiation precision, the need to decrease the incidence of tumor underdose, or normal tissue overdose [2]. Utilization of an external beam and implantation of radioactive pellets (brachytherapy) have made this minimally invasive treatment popular among cancer patients [3]. However, at effective doses this modality frequently still results in dosage-related radio-toxicity, including inflammation of nonmalignant adjacent tissues, with severe impacts on patient health [3,4].

* Corresponding author. Tel.: +1 352 2736685; fax: +1 352 8470588.
E-mail address: jkatz@dental.ufl.edu (J. Katz).

0304-3835/$ - see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2008.04.051

Since complications of radiation therapy largely depend on the dose administered, the ability of some antioxidants to radiosensitize cancer cells is of significant importance in the prevention of oral radiation complications [5]. Extensive research has been carried out in experimental animals to demonstrate the anticancer activity of retinoids, carotenoids and tocopherol on oral cancer and oral precancerous leukoplakia. The antioxidants Vitamin C, E, and beta-carotene were found to reduce DNA damage of normal human lymphocytes both before and after gamma irradiation [6]. Vitamin E (VE) has been shown to selectively cause inhibition of growth and decreased mitotic accumulation in human tumor cells, both alone and synergistically with gamma irradiation [7]. It was shown that Vitamin E enhanced the effect of gamma irradiation on murine neuroblastoma cells *in vitro,* while having no effect on normal mouse fibroblasts [8]. The ability of VE to protect normal cells from gamma irradiation-induced DNA damage has been documented in several published reports [9,10]. The soy isoflavone (ISF) genistein, was shown to enhance radio-sensitivity in human esophageal cancer cell lines by suppressing radiation-induced kinase pathways, with different pathways affected depending on the p53 status of the cells [5]. Genistein is known for its multiple cellular effects such as the ability to protect normal cells from DNA damage, while causing cell cycle arrest at the G2/M phase in breast, gastric, and prostate cancer cell lines [11–13]. These studies show promise for the effects of antioxidants to protect normal cells, while enhancing radiation effects in the cancer cells.

Studies are clearly needed to elucidate the effects of genistein, Vitamin E, and a soy isoflavone concentrate (NS) on cancer cells as single agents and in combination with radiotherapy. Thus a better understanding of the relationship between antioxidants and radiotherapy may potentially have a positive clinical impact on the prognosis and quality of life of patients. Our studies with a human salivary gland (HSG) cell line have found that low concentrations of ISF and genistein have growth inhibitory effects characterized by cell cycle arrest. This study investigated the effects of VE and ISF dietary supplements on the cytotoxicity of anti-neoplastic radiotherapy treatments. The hypothesis is that the treatment of oral cancer cell lines with low concentrations of ISF and VE both alone and with irradiation may sensitize cancer cells *in vitro* to radiation. The possible mechanisms for this assumption are being investigated.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), dimethyl sulfoxide (DMSO) and Vitamin E succinate (VES) were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was from Hyclone Laboratories (Logan UT). L-Glutamine, penicillin–streptomycin and trypsin–EDTA were from Gibco-BRL/Life Laboratories (Gaithersburg, MD). Genistein was from Indofine Chemical Co. (Hillsborough, NJ). NovaSoy® is a soy phytochemical concentrate and commercially available dietary supplement provided by Archer Daniels Midland Company (Decatur, IL). This ISF product is prepared through an ethanol extraction process and contains 49% isoflavones by weight. NovaSoy® approximates the natural composition of isoflavones in soybeans with a low content of soy protein (8.5%) and fat (0.42%).

2.2. Cell culture

The human salivary gland (HSG) cell line was established from an irradiated human submandibular gland. HSG cells exhibit the multipotential differentiative nature of intercalated duct cells, which is believed to be responsible for the development and replenishment of acini and thus to function as the salivary stem cells. The HSG cell line has been used as an *in vitro* model for study of the influence of pharmacologic and chemoprevention agents on salivary glands, as well as a model for study of irradiation-induced damage in salivary glands [14]. HSG cells were maintained in DMEM supplemented with 1 g/L glucose, 0.11 g/L Na pyruvate, 0.015 g/L phenol red, 3.7 g/L sodium bicarbonate, 2 mM L-glutamine, 10,000 μ/L penicillin, 10,000 μg/L streptomycin and 10% fetal bovine serum.

Treatments: genistein, NS and VES. Stock solutions of genistein, NS isoflavones, and VES were prepared in DMSO and added to cultures in complete medium with a final DMSO concentration of 0.1%. *Gamma irradiation.* HSG cells plated in 100 mm dishes were allowed to attach, then irradiated with a single dose of 0, 2, 4, or 6 Gy of gamma irradiation. After 24 h, cell lysates were prepared and proteins analyzed by Western immunoblot blot performed as described below. For some experiments, the cells were incubated 3 h in the presence of 50 μM VES or 75 μg/ml NS, irradiated with a single dose of 0 or 4 Gy, incubated an additional 24 h, and cell lysates prepared.

2.3. [^{3}H]Thymidine incorporation assay

Cells in 24-well culture plates were incubated with varying concentrations of genistein, NS isoflavones, VES, or nicotine. After 48 h, cells were pulsed with