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ORIGINAL ARTICLE

Effect of curcumin and irradiation in PE/CA-PJ15 oral squamous cell carcinoma

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Abstract

Objective. An *in vitro* study was made to evaluate the effect of curcumin and irradiation upon oral squamous cell carcinoma. ***Materials and method.*** Curcumin was administered at doses of 3, 3.75, 4.50 and 5.25 μM in PE/CA-PJ15 oral squamous cell carcinoma cultures irradiated with different doses (1, 2.5 and 5 Gy), followed by evaluation of the effects upon cell viability after 24, 48 and 72 h, based on the MTT colorimetric test. ***Results.*** The application of curcumin to the PECA/PJ15 tumor cells during 24, 48 and 72 h of incubation without irradiation exerted an inhibitor effect upon cell viability. The curcumin concentration at which the inhibition of cell viability proved maximum was 5.25 μM, with statistically significant differences for 24 h ($p = 0.002$), 48 h ($p < 0.001$) and 72 h of incubation ($p < 0.001$). In contrast, the combination of curcumin and irradiation exerted a synergic effect—the greatest effects in relation to cell viability being recorded with a curcumin concentration of 3.75 μM and 5 Gy of irradiation, in the studied cell line. ***Conclusions.*** Curcumin increases cytotoxic activity in the PE/CA PJ15 cell line, while the combination of curcumin and irradiation exerts a synergic effect.

Key Words: *Curcumin, squamous cell carcinoma, irradiation, in vitro cell line*

Introduction

Head and neck cancer accounts for ~5% of all malignancies. More than 500 000 new cases are diagnosed worldwide each year, 100 000 in Europe alone. More than 90% of all head and neck cancers are of squamous cell origin. The minority of patients presenting with early-stage disease are subjected to surgery and in some cases additional radiotherapy is provided [1,2]. Nearly 80% of these patients are cured. Chemotherapy added to locoregional treatment has been shown to prolong survival in patients with non-metastatic squamous carcinoma of the head and neck [1–3]. However, despite significant advances in the use of surgery, chemotherapy and irradiation for the treatment of head and neck cancer, there has been little improvement in the prognosis of the disease during the past 30 years and the outlook for the patients remains poor [1–4].

The search for new molecules with anti-cancer potential based on natural products remains an important priority [5]. Curcumin (diferuloylmethane), a polyphenol, is one of the main components of the Indian curry spice turmeric and is known to be a powerful antioxidant with strong anti-inflammatory properties [6]. Recently, curcumin has also been shown to possess potent anti-neoplastic activity against a number of tumors including prostate, breast, colon and oral cancer [6–14]. (To date, the exact mechanism or mechanisms underlying the anti-neoplastic activity of curcumin has not been established. However, curcumin has been shown to affect numerous signaling pathways and curcumin-induced cytotoxicity has been suggested to vary among different cell types [7,14]). Studies of plant extracts and phytochemicals as modifiers of irradiation effects constitute a new area of research. In effect, humans consume a variety of phytochemicals that afford protection from irradiation exposure. In this context, it is necessary to assess the protective action of such commonly used phytochemicals and exploit their possible application in cancer irradiation therapy as an alternative source of non-toxic radioprotectors.

Radiosensitization has been extensively studied with various chemotherapy drugs (cisplatin, 5-fluorouracyl, taxol) prior to irradiation or as concomitant treatment

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for patients with head and neck malignancies. However, the side effects of these drugs preclude their routine use in all irradiated patients and thus chemotherapy-irradiation protocols are currently used only as a part of clinical trials for organ preservation or for patients with non-resectable cancers [1,15].

The present study explores the *in vitro* effects of curcumin and irradiation upon oral squamous cell carcinoma.

Materials and methods

Cell line

Use was made of the PECA-PJ15 human oral squamous carcinoma cell line (European Collection of Cell Cultures) cultured in Iscove's modified Dulbecco's modified Eagle's medium (DMEM) (IMDM) supplemented with 10% fetal calf serum (FCS), 1% penicillin and 1% streptomycin (full medium) at 37°C, in an atmosphere of 95% oxygen and 5% CO_2. The medium (IMDM), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), dimethylsulfoxide (DMSO) and curcumin were obtained from Sigma Co. (Spain).

Drug preparation

Curcumin was dissolved in 0.5% DMSO, with 1 mg/ml of curcumin being used as stock solution. The working solution was diluted with sterile distilled water. All manipulations with curcumin were performed under subdued light, the dose range being 3, 3.75, 4.5 and 5.25 μM of curcumin.

Irradiation of the cells was performed using a linear accelerator (Yxlon Smart, Krautkrämer-Forster Española, S.A.). The machine was calibrated for the field size of interest using both special small ionization chambers and thermo-luminescence dosimetry. Single irradiation doses of 1, 2.5 and 5 Gy were administered. The cells were irradiated in 96-microwell plates.

Cell viability test (MTT)

Use was made of the technique described by Carmichael et al. [16,17], adapted to our culture conditions for the quantification of cell viability. The cell were cultured at a density of 5×10^3 cells/well in 96-microwell plates, after which curcumin was added at different concentrations (3, 3.75, 4.75 and 5.25 μM) 15 min after plate irradiation (1, 2.5 and 5 Gy).

At different timepoints after the start of treatment (24, 48 and 72 h), the medium was eliminated and the cells were incubated with MTT (Sigma Chemical Co., Spain) (1 mg/ml) during 4 h, after which the non-metabolized MTT was discarded and 100 μl of DMSO was added to each well. We measured the absorbance in each well with an enzyme-linked immunosorbent assay (ELISA), using a Multiskan MCC/340P plate spectrophotometer at a reading wavelength of 570 nm and a reference wavelength of 690 nm. Each experiment was performed in triplicate.

Statistical analysis

Data were analyzed using the SPSS version 12.0 statistical package (SPSS® Inc., Chicago, IL). A descriptive study was made of each variable. One-way analysis of variance (ANOVA) was used to verify the equality of several measures, when the number of quantitative variables compared was greater than two, verifying in each case whether the variances were homogeneous. Statistical significance was accepted for $p \leq 0.05$.

Results

Effects of curcumin upon PECA/PJ15 cell viability

The application of curcumin to the PECA/PJ15 tumor cells during 24, 48 and 72 h of incubation without irradiation exerted an inhibitory effect upon the cell viability. The curcumin concentration associated with the lowest cell viability was 5.25 μM, with statistically significant differences for 24 h ($p = 0.002$), 48 h ($p < 0.001$) and 72 h of incubation ($p < 0.001$) (Figure 1).

Effects of combined curcumin and irradiation upon the PECA/PJ15 cell line

Curcumin and 1 Gy of irradiation. The greatest cell viability reducing effect with 1 Gy of irradiation occurred after 24 h of incubation with a curcumin concentration of 3.75 μM (Figure 2).

Curcumin and 2.5 Gy of irradiation. The greatest cell viability reducing effect with 2.5 Gy of irradiation occurred after both 24 and 48 h of incubation with a curcumin concentration of 4.5 μM (Figure 3).

Curcumin and 5 Gy of irradiation. The greatest effect upon cell viability with 5 Gy of irradiation occurred after both 48 and 72 h of incubation with a curcumin concentration of 3.75 μM (Figure 4).

Discussion

The agents most commonly used in chemotherapy regimens for head and neck cancer are cisplatin or carboplatin, often administered in combination with taxanes and/or 5-fuorouracyl—the duration of

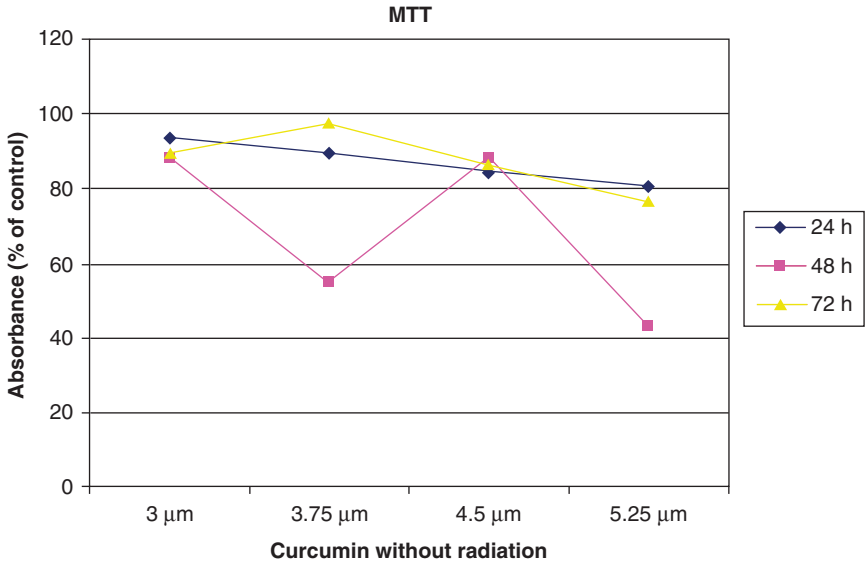

Figure 1. Cell viability after 24, 48 and 72 h of incubation with the application of curcumin in the absence of irradiation (24 h, $p = 0.002$; 48 h, $p < 0.001$; 72 h, $p < 0.001$).

therapy being limited due to toxic effects [15]. Consequently, new therapeutic strategies need to be identified and evaluated in pre-clinical models before entering clinical trials.

Curcumin is a dietary antioxidant derived from turmeric (*Curcuma longa*) and has been known since ancient times to possess therapeutic properties [5]. Curcumin prevents head and neck cancer. In this sense, the experimental studies of Tanaka et al. [18] showed that male F344 rats fed with dietary curcumin (0.5 g/kg) during the tumor initiation and post-initiation stages exhibited a 91% reduction in the frequency of 4-nitroquinoline-1-oxide-induced tongue carcinoma, together with a decrease in the incidence of oral pre-neoplasms.

Another study conducted by Azuine et al. [19] showed that curcumin alone or in combination with catechin inhibited methyl-(acetoxymethyl)-nitrosamine and reduced the number of oral squamous cell carcinoma lesions.

Radiotherapy plays an important role in the management of cancers, contributing to secure local control of tumors following surgery in patients with early stage cancer. However, radiotherapy alone fails to suppress tumors, which recur and become radioresistant. The factors conditioning such radioresistance in patients with recurring malignancies are not clear.

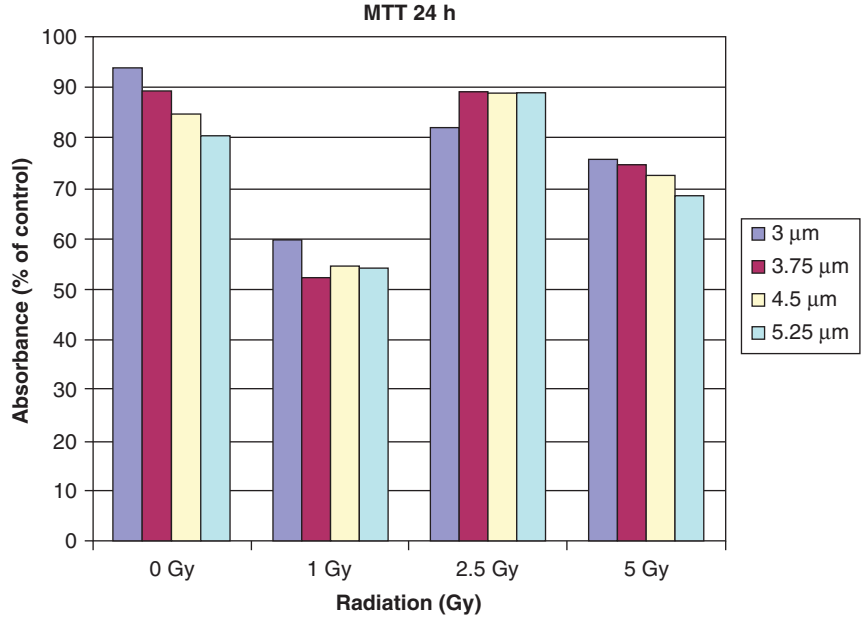

Figure 2. Effects of combination curcumin and irradiation after 24 h of incubation (1 Gy, $p = 0.010$; 2.5 Gy, $p = 0.042$; 5 Gy, $p = 0.042$).

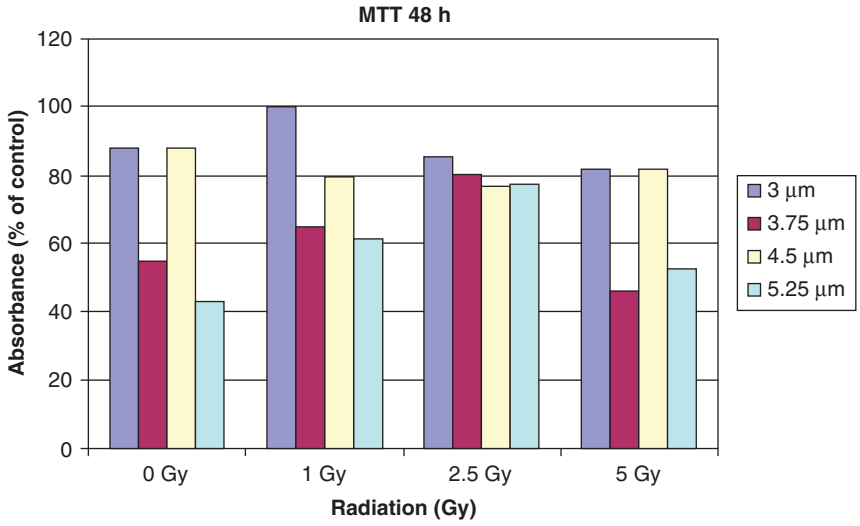

Figure 3. Effects of combination curcumin and irradiation after 48 h of incubation (1 Gy, $p < 0.001$; 2.5 Gy, $p = 0.282$; 5 Gy, $p < 0.001$).

Several studies have indicated that curcumin may serve as a radio-enhancing agent [13,14,20–22]. In this sense, it was found to enhance the effect of gamma irradiation on hamster ovarian cells and on the PC-3 human prostate cancer cell line. Using cell growth and a colony forming (clonogenic) assay, we previously found that curcumin enhances the effect of ionizing irradiation on squamous cell carcinoma cells *in vitro* [20]. Cheng et al. [22] demonstrated that curcumin, even at high doses (up to 8 g/day), is non-toxic for patients with pre-malignant lesions and as such could hypothetically be given for prolonged periods of time (6–7 weeks of irradiation treatments) with minimal side-effects.

Khafif et al. [21] investigated whether curcumin can sensitize squamous cell carcinoma cells to the ionizing effects of irradiation. Incubation with curcumin only (3.75 µM) for 48 h did not reduce the number of cells or their ability to form colonies in the absence of irradiation. In plates that were exposed to 1–5 Gy of irradiation, however, the cell counts dropped significantly when pre-treated with curcumin—the maximal effect being recorded for the 2.5 Gy irradiation dose. The clonogenic assay revealed a significant decrease in the ability to form colonies following pre-treatment with curcumin at all irradiation doses. Thus, curcumin may serve as an adjuvant to radiotherapy. Findings of several studies suggest that curcumin is radioprotective. The oral administration of curcumin at doses of 5, 10 or 20 mg/kg body weight significantly reduced the frequencies of micronucleated polychromatic erythrocytes in mice subjected to whole-body exposure to 1.15 or 0.05 Gy/s of gamma radiation as determined at 24, 30 or 48 h post-irradiation [23]. Exactly how curcumin affords radioprotection is not fully clear. On

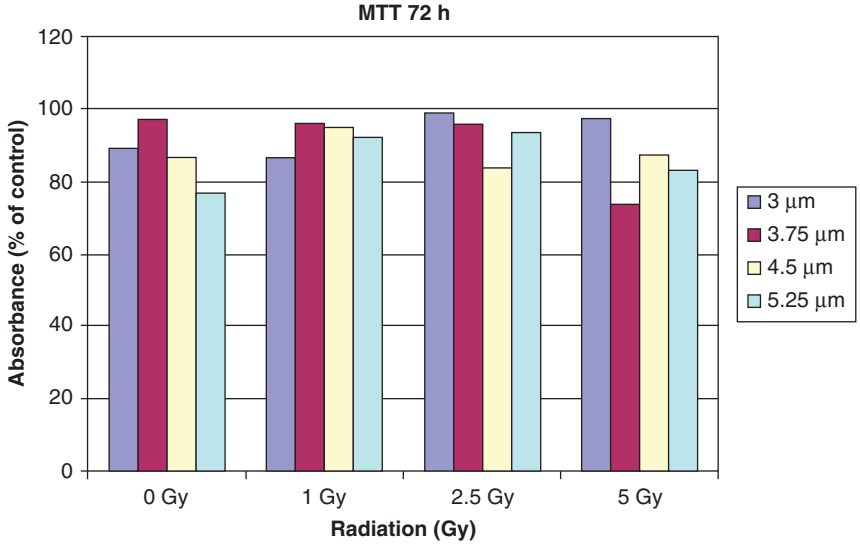

Figure 4. Effects of combination curcumin and irradiation after 72 h of incubation (1 Gy, $p = 0.088$; 2.5 Gy, $p = 0.146$; 5 Gy, $p = 0.004$).

the other hand, the administration of curcumin in cancer patients is claimed to kill the tumor cells effectively by enhancing the effect of irradiation and, at the same time, protecting normal cells against the harmful effects of irradiation.

The available information on curcumin suggests that the radioprotective effect may be fundamentally due to its ability to reduce oxidative stress and inhibit the transcription of genes related to oxidative stress and inflammatory responses, whereas the radiosensitizing activity could be due to the up-regulation of genes responsible for cell death [13].

In our study, curcumin alone exerted an effect upon PECA/PJ15 tongue tumor cell viability—the concentration associated with the greatest reduction in viability being 5.25 μM. The greatest effects upon cell viability corresponded to curcumin at a dose of 3.57 μM and an irradiation dose of 5 Gy, in the studied cell line. Based on the findings of our study, curcumin combined with irradiation was found to be more effective. Thus, curcumin may serve as an adjuvant to radiotherapy. However, since experimental findings cannot be easily extrapolated to clinical practice, further studies are needed, with confirmation of these observations in an *in vivo* model.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Pignon JP, Bourhis J, Domenge C, Designe L. Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data MACH-NC Collaborative Group. Meta-Analysis of chemotherapy on head and neck cancer. Lancet 2000;355:949–55.

[2] Scully C, Bagan J. Oral squamous cell carcinoma: overview of current understanding of aetiopathogenesis and clinical implications. Oral Dis 2009;15:388–99.

[3] Garavello W, Ciardo A, Spreafico R, Gaini RM. Risk factors for distant metastases in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2006;132: 762–6.

[4] Reuter CW, Morgan MA, Eckardt A. Targeting EGF-receptor signalling in squamous cell carcinomas of the head and neck. Br J Cancer 2007;96:408–16.

[5] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70: 461–77.

[6] Aggarwal BB, Takada Y, Oommen OV. From chemoprevention to chemotherapy: common targets and common goals. Expert Opin Investig Drugs 2004;13:1327–38.

[7] Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox Signal 2008;10:511–45.

[8] Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003;66:1022–37.

[9] Srinivasan M, Rajendra Prasad N, Menon VP. Protective effect of curcumin on gama-radiation induced DNA damage and lipid peroxidation in cultured lymphocytes. Mutat Res 2006;611:96–103.

[10] Aggarwal S, Takada Y, Singh S, Myers JN, Aggarwal BB. Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kappaB signaling. Int J Cancer 2004; 111:679–92.

[11] Rogers SJ, Harrington KJ, Rhys-Evans P, O-Charoenrat P, Eccles SA. Biological signifficance of c-erbB family oncogenes in head and neck cancer. Cancer Metastasis Rev 2005;24: 47–69.

[12] Baselga J, Averbuch SD. ZD1839 ('Iressa') as an anticancer agent. Drugs 2000;60:33–40.

[13] Jagetia GC. Radioprotection and radiosensitization by curcumin. Adv Exp Med Biol 2007;595:301–20.

[14] Khafif A, Lev-Ari S, Vexler A, Barnea I, Starr A, Karaush V, et al. Curcumin: a potential radio-enhancer in head and neck cancer. Laryngoscope 2009;119:2019–26.

[15] Moreno-Jiménez M, Valero J, López-Picazo JM, Arbea L, Aristu J, Cambeiro M, et al. Concomitant cisplatin, paclitaxel, and hyperfractionated radiotherapy in locally advanced head and neck cancer: comparison of two different schedules. Am J Clin Oncol 2010;33:137–43.

[16] Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 1987;47:936–42.

[17] Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity. Cancer Res 1987;47:943–6.

[18] Tanaka T, Makita H, Ohnishi M, Hirose Y, Wang A, Mori H, et al. Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis by dietary curcumin and hesperid comparison with the protective effect of beta-carotene. Cancer Res 1994;54:4653–9.

[19] Azuine MA, Bhide SV. Adjuvant chemoprevention of experimental cancer: catechin and dietary turmeric in forestomach and oral cancer models. J Ethnopharmacol 1994;44: 211–17.

[20] Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. Oncogene 2004;23: 1599–607.

[21] Khafif A, Hurst R, Kyker K, Fliss DM, Gil Z, Medina JE. Curcumin: a new radio-sensitizer of squamous cell carcinomacells. Otolaryngol Head Neck Surg 2005;132:317–21.

[22] Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, et al. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res 2001;21:2895–900.

[23] Abraham SK, Sarma L, Kesavan PC. Protective effects of chlorogenic acid, curcumin and beta-carotene against gamma-radiation-induced *in vivo* chromosomal damage. Mutat Res 1993;303:109–12.