A Promising, Novel Radiosensitizer Nanodrug Complex for Oral Cavity Cancer: Cetuximab and Cisplatin-Conjugated Gold Nanoparticles

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Background: Nanomedicine has provided promising tools for the imaging, diagnosis, and treatment of cancer. Gold nanoparticles (GNPs) may be useful in enhancing the efficacy of radiotherapy, such as radiosensitization, in cancer therapy.

Aims: To develop a nanodrug complex containing cetuximab (C225, CTX) and cisplatin (CDDP) conjugated with GNPs and to investigate its cytotoxic effects on oral cavity cancer cells when combined with radiotherapy.

Study Design: In vitro cell culture study.

Methods: The GNPs were synthesized and successfully conjugated with cetuximab and cisplatin. Cell viability was monitored by the xCELLigence real-time cell analysis (RTCA) single-plate (SP) system in GNP-treated UPCI-SCC-131 cells for 48 hours. Cells with/without GNPs were irradiated with 6 MV X-rays, and colony formation was assayed to investigate the long-term effects of GNPs and the nanodrug complex after irradiation on radiotherapy-resistant oral cavity cancer cells.

Results: The GNPs entered the tumor cells, and GNP–CDDP (P < .0001) and GNP–CDDP–CTX (P < .0001) were shown to cause a decrease in cell viability. GNP and GNP–CTX combined with radiotherapy led to greater reduction on UPCI-SCC-131 colony numbers, than radiation alone (P = .0369) and radiation with free CTX, with sensitizing enhancement ratios of 1 : 2 and 1 : 9, respectively.

Conclusion: The cetuximab and cisplatin-conjugated gold nanodrug complex has a great potential to increase cytotoxicity and overcome resistance to radiotherapy, in the treatment of oral cavity cancer.

INTRODUCTION

Oral squamous cell carcinoma (OSCC), a highly aggressive cancer type, is the most common type of head and neck cancer, with morbidity and mortality worldwide.1,2 Unfortunately, despite advances in the field of cancer treatment, there has been no improvement in the oncological outcomes (locoregional control, overall- and disease-free survival). Currently, the treatment modalities for OSCC are surgery, radio-chemotherapy, and combined treatments. Surgery is frequently performed though significant challenges persist, particularly oral cavity reconstruction and locoregional failure. Moreover, the radio-resistant biology of OSCC is the major limitation for combined radiotherapy±chemotherapy. Therefore, new and innovative treatment strategies are urgently required to overcome these clinical problems and enhance the efficiency of cancer treatment.

Recent advances in nanotechnology have enabled the development of several nanoparticles (1-100 nm) for use in diagnosis, gene/drug delivery, bioimaging, and cancer therapy.3-5 Metal-based nanoparticles with high atomic numbers, which may improve the therapeutic index of radiation therapy, are promising agents for cancer treatment because of their small size, drug-carrying capacity, photothermal effects, and ability to increase sensitivity...
to radiotherapy. Among them, gold nanoparticles (GNPs), well-known metal-based nanoparticles, are suitable candidates as nanocarriers due to their surface area, drug-carrying capacities, and biocompatibility. The literature indicates that GNPs may reduce cancer viability in a dose-dependent manner, and increase the radiosensitivity of cells due to their photoelectric absorption of photons. Therefore, the conjugation of GNPs with other molecules such as antibodies, anti-cancer agents, or vitamins might enhance the effects of radiotherapy and increase tumor cytotoxicity.

Currently, it is well known that the epidermal growth factor receptor (EGFR) is highly expressed in cancer cells in various cancers including breast, colorectal, head, and neck cancers. Cetuximab (CTX) (Erbitux®, Merck) is a chimeric monoclonal antibody against EGFR and has been widely used in the treatment of head and neck cancers in recent years. It enables binding of the extracellular region of EGFR with high affinity, thereby inhibiting EGFR activation, stimulating apoptosis, and inhibiting cell cycle progression, tumor cell invasion, and angiogenesis. The combined treatment with CTX shows synergistic activity with conventional methods such as radiotherapy and chemotherapy.

Due to the clinical importance of CTX, researchers have shown great interest in drug-loaded nanoparticles that are actively targeted to specific overexpressed surface proteins, such as EGFR, to enhance their anti-cancer potential. The purpose of this experimental study is to develop a new, innovative nanodrug complex using GNPs as a nanocarrier, cisplatin (CDDP) as a cytotoxic agent, and CTX for active targeting; and to evaluate the cytotoxic and radiosensitization effects of this nanodrug complex in a radiotherapy-resistant oral cavity cancer cell line in vitro.

MATERIAL AND METHODS

GNPs Synthesis, Characterization, and Conjugation with CTX and CDDP
In the literature, sodium citrate (Na₃C₆H₇O₇) is frequently used as a reducing agent for the synthesis of GNPs. Initially, 72 µL of hydrochloroauric acid (HAuCl₄) (Sigma-Aldrich, Taufkirchen, Germany) solution was added to 95 mL purified water and heated to 280°C on a magnetic stirrer. Following this procedure, sodium citrate solution (Sigma-Aldrich, Taukirchen, Germany) was added to 5 mL of distilled water in a bath type sonicator for 30 minutes. Next, the sodium citrate solution was heated to 60°C. When the hydrochloroauric acid solution reached 97°C, the solution containing sodium citrate was added at 1 mL/s. The mixture was held on a heating plate (280°C) for 10 minutes. The heating was stopped when the solution turned the color of red wine. After cooling, 8-arm-PEG-NH₂ was added into the mixture and mixed using a probe-type ultrasonic mixer for 5 minutes under temperature control (not exceeding 25°C). The nanodrug complex was obtained by binding of 25 µg cetuximab (CTX) (Merck, Darmstadt, Germany) and conjugation of 5 µg cisplatin (Pt(NH₃)₂Cl₂) (CDDP) (Sigma-Aldrich, Taufkirchen, Germany) to GNPs surfaces functionalized with PEG-NH₂ (Figure 1). The method detailed below was performed in the adsorption of C225 and CDDP on PEG-NH₂/Au nanoparticle surfaces. First, 10 mL of DI water solution containing C225 at initial concentrations of 50 µg/mL was prepared. To this solution, Au/PEG-NH₂ nanoparticles functionalized with 50 µg PEG-NH₂ were added, and mixed with a magnetic stirrer for 4 hours at constant temperature without changing the pH of the solution. C225, which was not adsorbed to Au/PEG-NH₂ surfaces, was centrifuged at 5000 rpm for 15 minutes and removed from the upper phase of the centrifuge tube. All procedures were carried out in a completely dark environment to prevent drug degradation. Similarly, for the binding of cisplatin to the Au/PEG-NH₂/C225 carrier system conjugated with C225, 10 mL of DI water solution containing CDDP at a concentration of 50 µg/mL was prepared and added to the solution containing Au/PEG-NH₂/C225 carrier for 4 hours. It was mixed with a magnetic stirrer over a long period. Unbound CDDP was centrifuged and removed from the Au/PEG-NH₂/C225/CDDP carrier system. The characterization of the GNPs was performed by scanning electron microscopy (SEM) and UV-visible spectroscopy.

Cell Culture
The radiotherapy-resistant oral cavity cancer cell line (UPCI-SCC-131) and fibroblast cell line (NIH-3T3) were purchased from DSMZ (Braunschweig, Germany) and ATCC (American Type...
The effect of GNPs on cell viability was monitored by xCELLigence Single Plate (SP) System (RTCA SP Analyzer, Roche Applied Science, Mannheim, Germany, and ACEA Biosciences, San Diego, CA). UPCI-SCC-131 and NIH-3T3 cells were seeded at 1 × 10^6 cells/flask for 24 hours, then exposed to 25 µg/mL of GNPs for 48 hours. Cells were trypsinized and washed with PBS, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for at least 2 hours at room temperature, and then post-fixed in 1% OsO₄ at 4°C for 60 minutes. After fixation, the cells were cut into small blocks (1 mm³) and dehydrated in a graded series of ethanol (50%, 70%, 80%, 90%, 96%, and 100%), and blocks were placed in 1,2-propylene oxide (Merck, Haarlem, Netherlands)-Epon resin overnight. Semi-thin sections of 700 nm in thickness were cut using an Ultracut E ultramicrotome (Leica Microsystems, Wetzlar, Germany) and were stained by toluidine blue. Ultra-thin sections were obtained from selected blocks, mounted on copper grids, and examined using a transmission electron microscope (Zeiss-Sigma 500, Germany).

**Transmission Electron Microscope**

A total of 2 × 10⁶ cells were plated in 25 cm² flasks for 24 hours, then exposed to 25 µg/mL of GNPs for 48 hours. Cells were trypsinized and washed with PBS, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for at least 2 hours at room temperature, and then post-fixed in 1% OsO₄ at 4°C for 60 minutes. After fixation, the cells were cut into small blocks (1 mm³) and dehydrated in a graded series of ethanol (50%, 70%, 80%, 90%, 96%, and 100%), and blocks were placed in 1,2-propylene oxide (Merck, Haarlem, Netherlands)-Epon resin overnight. Semi-thin sections of 700 nm in thickness were cut using an Ultracut E ultramicrotome (Leica Microsystems, Wetzlar, Germany) and were stained by toluidine blue. Ultra-thin sections were obtained from selected blocks, mounted on copper grids, and examined using a transmission electron microscope (Zeiss-Sigma 500, Germany).

**Cell Viability Assay**

The effect of GNPs on cell viability was monitored by xCELLigence Single Plate (SP) System (RTCA SP Analyzer, Roche Applied Science, Mannheim, Germany, and ACEA Biosciences, San Diego, CA). UPCI-SCC-131 and NIH-3T3 cells were seeded at 1 × 10⁶ cells/well into gold-coated 96-well E-plates and exposed to GNPs for 48 hours. The cells were automatically monitored every 15 minutes. The cell index value, which measures the relative change in electrical impedance to represent cell viability, was calculated for each sample by the RTCA Software Version 2.0. The percentage of viability was measured by the ratio of the cell index value of control cells to the nanodrug complex-applied cells. Each sample was assayed in triplicate, and 3 independent experiments were performed.

**In Vitro Irradiation**

For irradiation experiments, 5 × 10² cells containing GNPs were irradiated by 0.5, 2, 6, and 8 Gy of 6 MV radiotherapy (Siemens, Primus LINAK, Germany), then seeded in a 24-well plate in MEM media supplemented with 10% FBS. For each sample, 2 sets of 24-well plates were prepared, 1 was irradiated, while the other non-irradiated plate served as a negative control.

**Clonogenic Survival Assay**

 Colony assay was performed to evaluate the therapeutic effects of radiotherapy on cell survival with and without GNPs. After irradiation, cells were kept in a humidified incubator for 10-21 days. Subsequently, they were fixed with 4% paraformaldehyde (Sigma-Aldrich, Taufkirchen, Germany) and stained using 1% crystal violet (Sigma-Aldrich, Taufkirchen, Germany). Finally, the resulting colonies were counted. A colony was defined as a group of more than 50 cancer cells. An image of each colony was captured by UVP Gel Logic System (UVP Inc., Upland, CA).

The plating efficiency (PE) was calculated based on the survival of the control group (0 Gy) and determined by:

\[
PE(\%) = \frac{\text{Number of colonies counted}}{\text{Number of cells seeded}} \times 100
\]

The surviving fraction (SF) was calculated as:

\[
SF = \frac{\text{Surviving colonies}}{\text{Cells plated} \times \text{PE}}
\]

The contribution of GNPs and nanodrug complex for the increment of radiotherapy efficiency was measured by dose enhancement factor (DEF), calculated as the ratio of doses needed to give the same surviving fraction as that of the radiation-only control cells at the doses of 0.5, 2, 6, and 8 Gy. The sensitization enhancement ratio (SER) was calculated as the radiation dose required for radiation alone, divided by the dose needed for different concentrations of GNP + radiation at a survival fraction.

**Immunocytochemical Staining for EGFR Expression**

EGFR antibody (NCL-L-EGFR, Novocastra Laboratories Ltd., Newcastle, UK) was obtained from Leica Biosystems. For immunocytochemistry, UPCI-SCC-131 cells were seeded into a 25 cm² flask at a density of 1 × 10⁴ cells/well into gold-coated 96-well E-plates and exposed to GNPs (10, 20, 25, 50, and 100 µg/mL) and nanodrug complex for 48 hours. The cells were automatically monitored every 15 minutes. The cell index value, which measures the relative change in electrical impedance to represent cell viability, was calculated for each sample by the RTCA Software Version 2.0. The percentage of viability was measured by the ratio of the cell index value of control cells to the nanodrug complex-applied cells. Each sample was assayed in triplicate, and 3 independent experiments were performed.

**RESULTS**

**The Characterization of GNPs**

The shape and size of GNPs were determined using SEM (Figure 2). The results suggested that GNPs were spherical, and the core diameters were approximately 15 nm (Figure 2A). The shape of the ultraviolet-visible (UV-Vis) spectra is shown in Figure 2B for single GNP, GNP with cisplatin (GNP–CDDP), GNP with cisplatin and doxorubicin (GNP–CDDP–DOX), GNP with cisplatin and etoposide (GNP–CDDP–ETO), and GNP with cisplatin, doxorubicin, and etoposide (GNP–CDDP–DOX–ETO).
cetuximab (GNP-CTX), and GNP with cisplatin and cetuximab (GNP-CDDP-CTX). Figure 2B clearly shows that unconjugated GNP of 15 nm size gave a maximum peak at approximately 525 nm wavelength. On the other hand, the maximum peak of the nano-carrier systems produced with the agents conjugated to the particle surface was at a wavelength of 200 nm, and had a higher absorbance value.

Intracellular Localization of GNPs in UPCI-SCC-131 Cells
Nanodrug complexes have a key role in cytotoxicity due to their internalization by the cells. Prior to irradiation assay, transmission electron microscopy (Zeiss Sigma 500 electron microscope; Oberkochen, Germany) was used to determine the intracellular distribution of 15 nm GNPs, 48 hours after exposure. Figure 3 shows the oral cavity cancer cells that were treated with 25 µg/mL GNPs. After internalization, autophagosomes were detected in all images.

Immunocytochemical Staining
EGFR protein expression levels in UPCI-SCC-131 cells were determined by the immunocytochemical staining method. While purple stains are indicative of nucleus stained with hematoxylin & eosin, brown stains are indicative of EGFR expression. Immunocytochemistry data showed that EGFR expressions were scored as ++++, which is indicated to be strongly positive, as a result of staining (Figure 4).
Real-Time Cell Viability in GNP-Treated UPCI-SCC-131 and NIH-3T3 Cells

The xCELLigence assay was used to assess the effects of GNPs on the cell viability of the oral cavity cell line (Figure 5). The results showed that the dose-dependent effects of 15 nm GNPs were not cytotoxic to UPCI-SCC-131, and the EC$_{50}$ values were 20 µg/mL. According to nanodrug complex results, GNP–CDDP (25 µg/mL-2.5 µg/mL) decreased the cell viability compared to free CDDP at the dose of 2.5 µg/mL ($P < .0001$) and control ($P < .0001$). Similarly, we found that the GNP–CDDP–CTX nanodrug complex was more cytotoxic than CDDP–CTX on cell viability ($P = .0001$). Both CTX ($P = .2313$) and GNP–CTX ($P = .0007$) were non-toxic compared to control cells (Figure 6). We also evaluated the cell viability of the fibroblast cell line, NIH-3T3, containing the nanodrug complex. As can be seen in Supplementary Figure 1, no significant cell cytotoxicity was observed for 48 hours after GNP and GNP–CDDP treatment. However, CDDP, GNP–CDDP, and GNP–CDDP–CTX groups all indicated a decrease in cell viability.

Clonogenic Survival Assay

Clonogenic survival assay was performed to measure the cytotoxic effects of GNPs, based on the ability of a single cell to form a colony, and to compare the radiation sensitizing efficacies of GNPs on UPCI-SCC-131 cells.20 Figure 7A and 7B demonstrate the number of colonies formed in 25 µg/mL of GNP, GNP–CTX, CTX, GNP–CDDP, CDDP, GNP–CTX–CDDP, CDDP–CTX, and control cells (without any agent). The effects of GNPs at the minimum doses (0.5 and 2 Gy) decreased the surviving fractions ~1.2-fold compared to control cells. With an increased radiation dose of GNP–CTX, a decrease in cell survival was detected when compared with control and CTX alone (Figure 7C). The cells treated with the nanodrug complex, including GNP–CDDP, GNP–CDDP–CTX, and the drugs (CDDP and CDDP–CTX) had no surviving colonies.

The DEF is the ratio of the dose with radiation alone to the dose with radiation+GNPs (Table 1). Notably, DEF values >1 indicate that GNPs are functioning as radiosensitizers. On the other hand, DEF values <1 show GNPs are radioprotectors. According to DEF values, GNPs were radiosensitizers at all doses starting from 0.5 Gy. While the GNP–CTX complex was a radioprotector at a dose of 0.5 Gy, it had a radiosensitizing effect at doses of 2, 6, and 8 Gy. However, no colony formation was detected in cells
containing GNP–CDDP and GNP–CDDP–CTX after RT, therefore, DEF values for these nanodrug complexes were calculated as zero. The SER was calculated as 1.2 and 1.9, for GNP and GNP–CTX, respectively.

**DISCUSSION**

Nanotechnology plays a significant role in medical approaches such as targeted therapies using nanocarriers, drug delivery systems, early detection, and diagnosis. The major problem associated with cancer treatment is the undesirable side effects and multidrug resistance, but toxicity may be significantly reduced by nanoparticle-mediated drug delivery and chemotherapeutic agents with high specificity in the target tissue.

GNPs can enhance the response to radiation therapy by exhibiting a radiosensitizing effect, and can also be used as nanodrug carriers due to their easy conjugation with other biomolecules such as chemotherapeutic agents or antibodies. In this study, we were able to develop a new and innovative nanodrug complex by successfully conjugating CTX, CDDP, and GNPs. Moreover, our literature survey demonstrated that this was the first experimental study involving an examination of the cytotoxic and radiosensitizer effects of the GNP–CTX–CDDP nanodrug complex evaluated on OSCC (UPCI-SCC-131 cells) in vitro at different megavoltage energies.

The human EGFR is associated with proliferation, angiogenesis, apoptosis, and metastasis, and is overexpressed in over 90% of head and neck cancers when compared with healthy cells. Because of its role, EGFR-targeted therapies may be utilized for patients with cancer. Cetuximab is a monoclonal antibody that binds specifically to the extracellular domain of human EGFR, and is approved for use as monotherapy after platinum-based therapies or in combination with radiotherapy. In the current context, we also determined that
TABLE 1. Dose Enhancement Factor (DEF) Values* of Gold Nanoparticles (GNPs) After Radiation

| Radiation Doses (Gy) | Control | GNP | GNP–CTX | GNP–CDDP | GNP–CDDP–CTX |
|----------------------|---------|-----|---------|----------|--------------|
| 0.5                  | 1       | 1.20| 0.87    | 0        | 0            |
| 2                    | 1       | 1.18| 1.84    | 0        | 0            |
| 6                    | 1       | 3.13| 4.25    | 0        | 0            |

**DEF >1: GNPs act as radiosensitizers, DEF<1: GNPs act as radiosensitizers. CDDP: Cisplatin; CTX: Cetuximab.**

FIG. 7. A-C. Radiosensitization effects of GNPs in UPCI-SCC-131 cells (A) Images of colonies containing functionalized GNPs exposed to irradiation at different megavoltages was determined using clonogenic survival assay (B) Numbers of colonies was decreased with increasing doses of radiotherapy (C) The effects of GNPs on UPCI-SCC-131 cell survival was calculated by surviving fractions (SF), which showed the actual efficacy of the radiation effect on the cells. Data are expressed as mean ± SD (n = 3). GNP, Gold nanoparticle; CDDP, Cisplatin; CTX, Cetuximab.
EGFR protein expressions in UPCI-SCC-131 cells showed strong positivity (Figure 4), therefore, we specifically aimed to design a nanodrug complex using GNP, CTX, and CDDP, a cytotoxic chemotherapeutic agent particularly used as a radiosensitizer in head and neck cancers. In a recent article, Eskiizmir et al. determined the effects of CTX with IC_{50} doses of 400 μg/mL on cell viability in UPCI-SCC-131 cancer cells.\(^{21}\) In this context, CTX was used as a specific targeting agent rather than a cytotoxic agent; therefore, the much lower dose of 25 μg/mL was preferred for this study.

The transmission electron microscope, which is used to determine the localization of GNP within the cell, clearly revealed the efficient entry of GNPs into the cell. Furthermore, it was shown that GNPs were located in the autophagosome throughout the cell. According to xCELLigence results, different concentrations of PEGylated GNPs, of 15 nm diameter, were non-toxic on cell viability compared to control cells at 48-hour incubation; therefore 25 μg/mL was the chosen concentration of GNPs as a nanocarrier. The toxicity of nanoparticles depends on different factors such as their size, surface, and shape. It was shown that small sizes of gold clusters (1.2 and 1.4 nm) had higher cytotoxicity on necrosis and apoptosis than 15 nm GNPs.\(^{22,23}\) In concordance with our results, Leve et al. failed to show the cytotoxic effect of GNPs on colorectal cancer cells (Caco-2, HT-29, and HCT-116) for 24 and 48 hours.\(^{24}\) On the basis of the present cell viability results, GNP–CDDP and GNP–CDDP–CTX showed the greatest reduction in obtaining statistically significant differences in comparison with control group for this study. Davidi et al. developed a nanosystem which acts as a radiosensitizer, drug carrier, and tumor-imaging agent for head and neck cancers. They synthesized cisplatin- and glucose-coated nanoparticles (20 nm), and similar to our results, they showed that their nanosystem penetrated tumor cells and led to a decrease in cell viability.\(^{25}\) We have also investigated the effects of the nanodrug complex on cell viability in a normal cell line, NIH-3T3, and found no statistically significant difference in cell viability on treatment with GNP and GNP–CDDP. However, GNP–CDDP–CTX may have shown a synergistic effect and enhanced the cytotoxic activity of the nanodrug complex, and this may lead to a reduction in the cell viability of NIH-3T3. These are noteworthy contributions to the literature. Vecchia et al.\(^{26}\) evaluated the cytotoxicity of citrate-capped GNPs in NIH fibroblast (NIH-3T3) and found that GNPs tended to decrease cell viability in a dose-dependent manner. In another in vitro study, Chueh et al.\(^{27}\) showed that GNPs reduced cytotoxicity as a survival mechanism by autophagy induction on NIH-3T3 cells. On the other hand, it was reported that doxorubicin-conjugated GNPs decreased the cell viability of NIH-3T3 cells compared to control cells.\(^{28}\) Hence, the parameters such as target cell type, size, or concentration of the nanoparticles need to be carefully chosen when assessing the toxicity of nanoparticles.

Another main goal of the present study was to examine the systematic effects of combined therapy by stimulating X-ray containing GNPs. The UPCI-SCC-131 cell line is known to have ATM (ataxia-telangiectasia mutated) deficiency, causing resistance to radiotherapy.\(^{31}\) Colony formation assay showed that there was a reduction in colony numbers after the treatment with GNPs and nanodrug complex combined with radiotherapy in UPCI-SCC-131 cells. In particular, GNP–CTX with radiotherapy improved the efficacy of radiotherapy, compared to the effects of GNP or free CTX alone on cell viability. This showed that GNP–CTX in combination with radiotherapy produced a great synergism on UPCI-SCC-131 cell survival even at minimal radiotherapy doses such as 2 Gy. Popovtzer et al. evaluated the effectiveness and toxicity of targeted GNP with CTX followed by conventional 6 MV radiotherapy in head and neck squamous cancer cells (HNSCC).\(^{32}\) Their results showed that a GNP/CTX injection was associated with a significant improvement in tumor radiosensitivity, relative to the other modalities. In our study, cells containing CDDP (GNP/CDDP–CTX, GNP–CDDP, CDDP–CTX, and free CDDP) failed to form colonies after radiotherapy, possibly related to the use of high-dose cisplatin (2.5 μg/mL).

We also aimed to measure the DEF in the presence of the GNPs at different radiotherapy doses. The DEF (2 Gy) and DEF (6 Gy) of GNP were found as 1.18 and 3.13, respectively. This revealed that the combination of GNPs and radiotherapy showed enhanced cytotoxic effects in vitro. In conjunction with this, DEF values for GNP–CTX were calculated as 1.84 and 4.25 at 2 and 6 Gy, respectively. The DEF of GNPs at all radiotherapy doses was above 1, which shows that 15 nm GNPs and functionalized GNP–CTX may be good radiosensitizers, even at concentrations as low as 25 μg/mL, when used with megavoltage X-ray irradiation. Previous studies have reported that using the GNP-based radiotherapy enhanced the therapeutic index for the treatment of cancer with high DEF and SER values.\(^{33,34}\) The sensitization enhancement ratio (SER), which is used to evaluate how effectively radiosensitizers decrease cell survival, was calculated as 1.2 and 1.9 for GNP and GNP–CTX, respectively, at 2 Gy. These high SER values indicate that GNPs were able to reach a maximum radiosensitization.

This study showed that the PEGylated GNP nanodrug complexes are potentially effective in generating high cytotoxicity at low doses by significantly potentiating radiotherapy activity in RT-resistant oral cavity cancer cells, and successfully conjugating with CTX to achieve an active targeted nanodrug complex. It is also clearly possible to develop the nanodrug complex with active/pas-sive targeted radiosensitizing properties.

The 2 major advantages of our nanoparticle system are their excellent biocompatibility and low biological toxicity compared with other traditional agents such as cisplatin. When coupled with in vitro X-ray irradiation, the nanodrug system showed more effectiveness at low doses, thus potentially decreasing the extent of radiotherapy for patients.

For the first time in the literature, in this study, we successfully synthesized the GNP-conjugated cisplatin and cetuximab complexes as nanodrugs, and evaluated the efficiency of combination therapy with radiotherapy in the presence of the nanodrug complex for the radiotherapy-resistant oral cavity cancer cell line.

\(^{285}\)
Based on in vitro radiotherapy, PEG-coated GNP may be used for drug delivery and improve the efficiency of possible applications in radiotherapy. These findings will provide a valuable approach to apply the highly effective nano-radiosensitizer for the treatment of oral cavity cancers. Further investigation is needed into the differential radiosensitizing efficacies of GNP at other concentrations, or with larger or smaller sizes; however, this work will require in vivo studies.

Ethics Committee Approval: As stated by The Scientific and Technological Research Council of Turkey (TUBITAK), ethics committee approval is not required for in vitro cell culture studies.

Data Sharing Statement: Author elects not to share data.

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Supplementary FIG. 1. Cell viabilities of NIH-3T3 cells studied by using the x-CELLigence RTCA system, and the cells were treated in medium containing 25 μg/mL GNPs, 2.5 μg/mL CDDP, and 25 μg/mL CTX. Data are expressed as mean ± SD (n = 3). *P ≤ .05