Introduction

Taxanes are mitotic inhibitors stabilizing microtubules by promoting their assembly and preventing depolymerization (11). Recently, it has been experimentally demonstrated that paclitaxel and docetaxel may also act as radiosensitizing agents, promisingly enhancing the cytotoxic effect of radiation in the treatment of cervical cancer (9).

Docetaxel is a highly lipophilic agent that can be dissolved in oil solutions such as lipiodol (ethylesters of iodinated fatty acids of poppy seed oil). The lipiodol is widely used to selectively deliver cytotoxic agents or radioiodine to some tumors (3,5). Due to the molecular structure of docetaxel (it has two free benzene rings), it may be possible to substitute its molecule with radionuclide iodine 131 dissolved in lipiodol (Fig. 1). The primary reason of docetaxel labeling with radionuclide iodine 131 was an attempt to observe the selective biodistribution using the whole body scintigraphy with gamma camera (8). This radionuclide has its own cytotoxic potential owing to the emitted β- and γ-radiation (1), and therefore it may be presumed that the combined cytotoxic effect of the radiolabeled docetaxel might be accelerated when compared to that of docetaxel or radioiodine alone. To verify this hypothesis, we carried out a series of cytotoxicity tests, comparing the influence of docetaxel, radiolabeled docetaxel and radionuclide 131I-lipiodol on behavior and viability of human cervical cells in vitro.

Materials and Methods

Human cervical cell line HeLa Hep2 (EATCC, No. 86030501, Porton Down, United Kingdom) was cultivated as stationary monolayer in plastic tissue-culture dishes (Nunclon, Roskilde, Denmark). Cells were grown in Dulbecco’s modified Eagle’s medium – DMEM (Sevapharma, Prague, Czech Republic), supplemented with 10% bovine serum (Bioveta, Ivanovice, Czech Republic), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were passaged every third day using 0.25% trypsin.

Docetaxel (Taxotere® inj.) was obtained from Rhône-Poulenc Rorer, Canada. Radiolabelling with iodine 131 radionuclide was performed by Nuclear Research Institute (Řež u Prahy, Czech Republic). Radiochemical purity of labeled docetaxel measured by thin layer chromatography was determined as exceeding 95%.

Summary: The cytotoxic potentials of the lipiodol emulsion with dissolved 131I-docetaxel, the 131I-lipiodol emulsion and non-labeled docetaxel were tested on the HeLa Hep2 cell line during 24 hours. The pilot study confirmed that the radio-labeled docetaxel was significantly more toxic than the radionuclide or docetaxel alone.
**Fig. 2A:** The effect of 200 μg/ml docetaxel, 131I-lipiodol and 131I-docetaxel solutions at 10x dilution on viability of HeLa Hep2 cells during 24h as measured by WST-1 assay. Presented values are means ± SD of sixteen independent spots. *denotes significant difference from control culture at P<0.05, # denotes significant difference from cultures treated with docetaxel at P<0.05 using one way-Anova test, with post test Dunnet’s.

**Fig. 2B:** The photomicrograph of HeLa Hep2 cells treated with 131I-docetaxel at 10x dilution after 24 hours (final conc. 200 μg/ml and activity 2 MBq). The cells are detached, with the remaining ones showing various perturbations. White arrow indicates blebbing membrane. Phase contrast 400x.

**Fig. 2C:** The effect of 20 μg/ml docetaxel, 131I-lipiodol and 131I-docetaxel solutions at 100x dilution (final conc. 20 μg/ml and activity 0.2 MBq) on viability of HeLa Hep2 cells during 24h as measured by WST-1 assay. Presented values are means ± SD of sixteen independent spots. *denotes significant difference from control culture at P<0.05, # denotes significant difference from cultures treated with docetaxel at P<0.05, + denotes significant difference from cultures treated with 20 μg/ml docetaxel at P<0.05 using one way-Anova test, with post test Dunnet’s.

**Fig. 2D:** The photomicrograph of HeLa Hep2 cells treated with 131I-docetaxel at 100x dilution (final conc. 20 μg/ml and activity 0.2 MBq) after 24 hours. No viable cells are visible. Phase contrast 400x.
Nonactive lipiodol oil solution (ethiodized poppy seed oil) was obtained from LIPIODOL® Ultra-Fluid (BYK, Germany), and 131I-lipiodol was from LIPIOCISTM (CIS Bio International, France). One ml of 131I-lipiodol or lipiodol with dissolved 131I-docetaxel (10 mg) was added dropwise to the 4 ml solution containing Poloxamer 188 (Synperonic PE/F 68, ICI Surfactants, Cleveland, England), Polysorbate 20 (Tween 20, ICI Surfactants, Cleveland, England), and water and ultrasonicated with 40 W input in pulses lasting 2 seconds with 0.5 second pauses for 2 minutes. The 20% emulsions were used for the experiments. The radioactivity of both emulsions was determined at 20 MBq/ml. The emulsion of 131I-lipiodol and lipiodol with dissolved 131I-docetaxel were diluted with DMEM 10x (500 μl of 20 % emulsion to 5 ml of medium) and 100x (50 μl of 20 % emulsion to 5 ml of medium). Docetaxel was prepared as 200 μg/ml stock solution in non-serum DMEM. The concentrations of 200 μg/ml and 20 μg/ml, respectively, which corresponded to the concentrations in 10x and 100x dilutions of lipiodol emulsion with dissolved 131I-docetaxel were used.

HeLa Hep2 cells were seeded onto thirty Petri dishes at concentration of 1 x 10^6 cells/ml and left in an incubator for 24h at 37 °C and 5% CO₂. After 24 hours, the cells were rinsed with a fresh medium and exposed for 24h to 5 ml of medium containing tested emulsions (five Petri dishes each) at appropriate concentration. At regular intervals, the medium was aspirated and the cell morphology was examined under the Olympus CK2 phase contrast microscope.

To test the viability, WST-1 colorimetric assay, which is based on the cleavage of the tetrazolium salt to colored formazan by mitochondrial dehydrogenases in viable cells was employed. HeLa Hep-2 cells at concentration 6,000 cells/well in 200 μl of DMEM containing 10% bovine serum were seeded in two 96–well microtiter plates, with the first column of wells without cells (blank). The cells were incubated 24h at 37 °C and in 5% CO₂. After incubation, the medium was replaced with a medium containing tested emulsions and cultivated for 24h at 37 °C and 5% CO₂. After this period, 100 μl of WST-1 was added. The cells were further incubated for 2h. The absorbance was recorded at 450 nm with 650 nm of reference wavelength by a spectrophotometer. In all cases, the absorbance of the tested substance in medium alone was recorded to determine whether it interfered with the assay. Each tested solution was tested in sixteen independent spots. Statistical analysis was carried out with a statistical program GraphPad Prism, using one-way Anova test with Dunnet’s post test for multiple comparisons. Results were compared with control samples, and means were considered significant if P<0.05.

**Results**

All tested emulsions proved to be toxic for HeLa Hep-2 cells in the interval of 24 hours. In comparison with 131I-lipiodol or non-labeled docetaxel, 131I-docetaxel proved to be significantly more efficient (Fig. 2A, C), producing the first observable changes as soon as 2h after the beginning of the treatment. There was a difference in the observed morphological appearance of HeLa Hep-2 cells exposed to differing concentrations of 131I-docetaxel. While the 10x dilution inflicted rapid changes including cell shrinkage and nuclear fragmentation (Fig. 2B), the 100x dilution had a less dramatic effect involving membrane blebbing and slower cell degradation (Fig. 2D).

**Discussion**

Our results demonstrate that it is possible to label docetaxel with 131I radionuclide and such a preparation may be emulsified in lipiodol medium standardly used for the delivery of cytostatic drugs to selected tumors (Fig. 1). Furthermore, such a radiolabeled 131I-docetaxel in the therapeutic concentrations acts on the exposed cervical cells with a higher speed and efficacy than 131I-lipiodol or docetaxel alone. In addition, our observations suggest that 131I labeled docetaxel might induce two different types of cell death – apoptosis or necrosis depending on the employed concentration. Whether this effect is due to the amplified radiosensitization of the exposed cells caused by docetaxel, with the radiation only completing the entire process, or some other mechanisms are involved remains to be elucidated (2,6,9). The use of a radioactively labeled cytostatic drug may be beneficial due to several reasons. Firstly, by combining two different mechanisms of action; i.e. microtubule stabilization and direct DNA interference it could greatly enhance tumor cell killing properties of such therapy while preventing the rapid development of resistance which so often burdens otherwise promising potential of taxol-based therapy (4,11). Secondly, the synergistic functioning of this preparation may allow reduction in overall therapeutic doses and adjustment of treatment regimen in individual patients, thereby reducing size effects of the therapy (4).

Thus it seems that radiolabeled 131I-docetaxel as well as other 131I-taxanes (7,10) holds a promising therapeutic potential whose mechanism and efficacy might be worth of further investigation both in vitro as well as in vivo.

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