

DOCETAXEL LABELED WITH IODINE 131 RADIONUCLIDE - A POSSIBLE NEW ANTICANCER DRUG? (THE PILOT STUDY)

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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.

Key words: *In vitro*; Taxanes, Lipiodol, Docetaxel, I31-iodine, Cytotoxicity

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 radionuclide 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 μ g/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%.

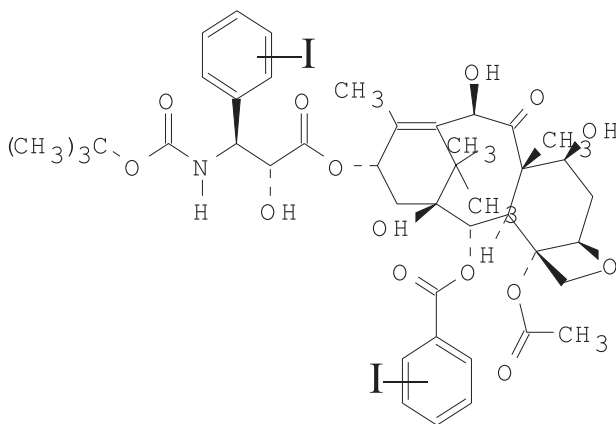


Fig 1: The proposed chemical structure of docetaxel labeled with ¹³¹I.

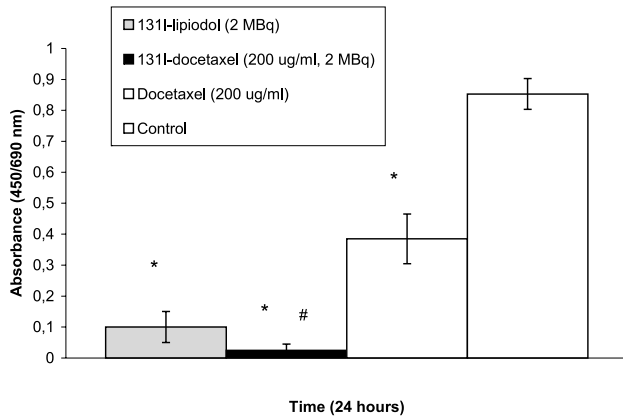


Fig. 2A: The effect of 200 µg/ml docetaxel, ¹³¹I-lipiodol and ¹³¹I-docetaxel solutions at 10x dilution on viability of HeLa Hep2 cells during 24h as measured by WST-1 assay. Presented values are means ± SD of sixteen independent spots. *denotes significant difference from control culture at P<0.05, # denotes significant difference from cultures treated with docetaxel at P<0.05 using one way-Anova test, with post test Dunnet's

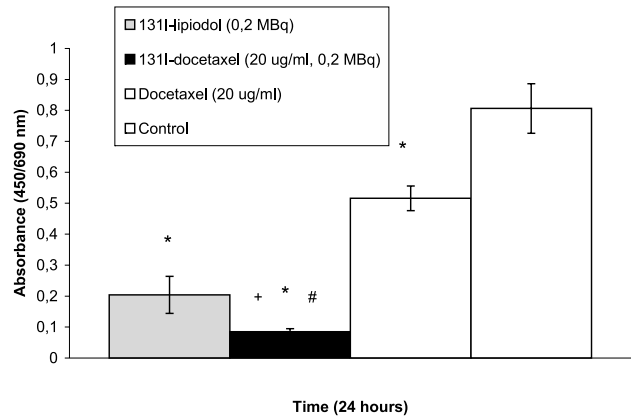


Fig. 2C: The effect of 20 µg/ml docetaxel, ¹³¹I-lipiodol and ¹³¹I-docetaxel solutions at 100x dilution (final conc. 20 µg/ml and activity 0.2 MBq) on viability of HeLa Hep2 cells during 24h as measured by WST-1 assay. Presented values are means ± SD of sixteen independent spots. *denotes significant 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

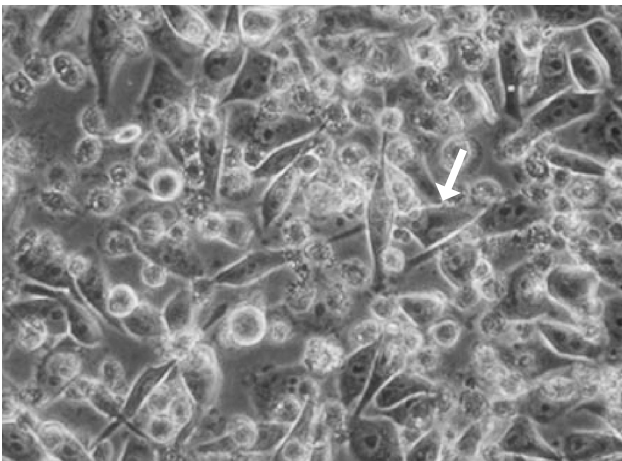


Fig. 2B: The photomicrograph of HeLa Hep2 cells treated with ¹³¹I-docetaxel at 10x dilution after 24 hours (final conc. 200 µg/ml and activity 2 MBq). The cells are detached, with the remaining ones showing various perturbations. White arrow indicates blebbing membrane. Phase contrast 400x.

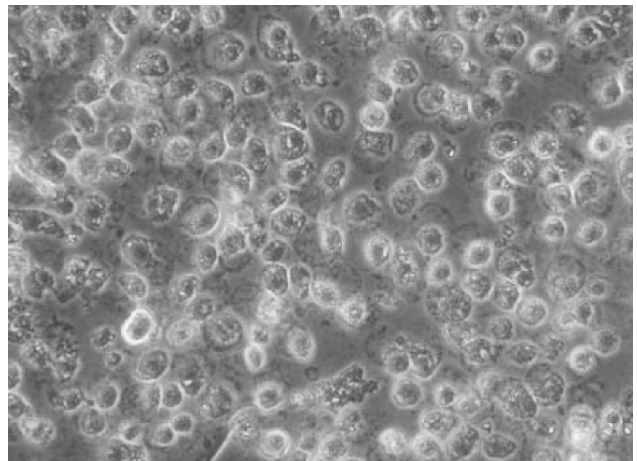


Fig. 2D: The photomicrograph of HeLa Hep2 cells treated with ¹³¹I-docetaxel at 100x dilution (final conc. 20 µg/ml and activity 0.2 MBq) after 24 hours. No viable cells are visible. Phase contrast 400x.

Nonactive lipiodol oil solution (ethiodized poppy seed oil) was obtained from LIPIODOL® Ultra-Fluid (BYK, Germany), and ¹³¹I-lipiodol was from LIPIOCIS™ (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 (Syneronic PE/F 68, ICI Surfactants, Cleveland, England), Polysorbat 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 1×10^6 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 Hep2 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 scanning multiwell 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 Hep2 cells in the interval of 24 hours. In comparison with 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 standarty 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.

Acknowledgments

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