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Uptake kinetics of radiolabelled 1,8-dihydroxyanthraquinone and acridinone derivatives in cultures of breast cancer cells

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The purpose of this study was to radiolabel 9(10H)-acridinone (IA), 10-methylacridinone (IMA), and 1,8-dihydroxyanthraquinone (IDA) with I-123 and to evaluate their uptake characteristics in breast cancer cells. Cell cultures of the human adenocarcinoma breast cell line MCF-7 were incubated both with the I-123-labelled substance and with thallium-201 for incubation periods up to 240 min. Tracer uptake was quantified as percent of the applied activity normalized to one million cells. Uptake of Tl-201 was inversely related to cell density and in good agreement with known data. Similarly, uptake both of IA and IDA was higher with low cell density ranging from 0.41 % to 0.17 % with total cell counts varying between 5.4 and 29.3 million cells. In IMA results yielded somewhat higher values ranging from 0.76 % to 0.13 % with total cell counts varying from 2.8 to 29.0 million cells. Uptake of all substances correlated well with obtained octanol-buffer-partition ratios. Thus, acridones are easy to label with radioactive iodine and are promising for non-invasive evaluation of active efflux mechanisms known in doxorubicine resistant tumors.

Key words: breast neoplasms; tumour cells, cultured; acridinones; anthraquinones; thallium radioisotopes

Introduction

Anthracycline derivatives like doxorubicin (DOX) and iododoxorubicin (IDOX) are efficient anticancer drugs in various tumours, e.g. breast cancer.[1–5] However, this group of drugs is also known to exhibit multi drug resistance by active efflux mechanisms via transmembranous glycoprotein p170.[6–9] Therefore, in the search for potential in vivo tumour tracers applicable prospectly to predict anti-tumour-drug uptake and resistance DOX and IDOX have been radiolabelled and their uptake has been studied both in vitro and in vivo in our laboratory previously showing relatively low tumour uptake in vitro if compared to Tl-201 and a rather quick deiodination in man.[10,11]

In the search for related tumour tracers with a higher initial uptake and perhaps a more stable in vivo label anthraquinone and acridinone derivatives – which are considered structure analogues of DOX – were selected for this study. Both anthraquinone and acridinones also

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exhibit cytotoxic antitumour effects as shown recently in vitro[12–16] and both may be easily labelled with I-123.

Therefore, the aim of our study was to radiolabel anthraquinone and two acridone derivatives and to evaluate their uptake kinetics in anthracycline sensitive cell cultures of breast cancer in relation to the uptake of Tl-201 as an established in vivo tracer with known cellular uptake characteristics.

Materials and methods

Radiolabelling

9(10H)-acridinone (IA), 10-methylacridinone (IMA), and 1,8-dihidroxyanthraquinone (IDA) were purchased from Aldrich (Steinheim, Germany) and radiolabelled with I-123 by electrophilic substitution by the Iodogen method.[17–19] After plating 1 mg of 1,3,4,6-tetrachloro-3α-6α-diphenylglycoluril (Pierce, Oud-Beijerland, The Netherlands) onto the bottom of a test rube, 0.123, 0.166, and 0.098 μmol of IA, IMA, and IDA, respectively, dissolved in a mixture of 20 μl acetone and 80 μl buffer was added. Iodination was then started by adding 5 MBq of iodine-123 iodide (Amersham Buchler, Braunschweig, Germany). The contents of the tube were mixed gently and allowed to react for 60 min at 20 °C. Purification was accomplished using high-performance liquid chromatography with an isocratic elution technique yielding a radiochemical purity of more than 98 % for each substance. Octanol-buffer-partition ratio of the radiolabelled substances and of I-123-labelled doxorubicine were measured in the conventional manner.[20] Chemical structures both of the radiolabelled compounds and of doxorubicin are shown in Figure 1. Note that the exact position of substitution of iodine-123 in bencoic rings is not known to date.

Cell cultures

Tumour cell lines of an anthracycline sensitive human breast adenocarcinoma (MCF-7) were obtained from Deutsches Krebsforschungszentrum (Heidelberg, Germany) and cultured at

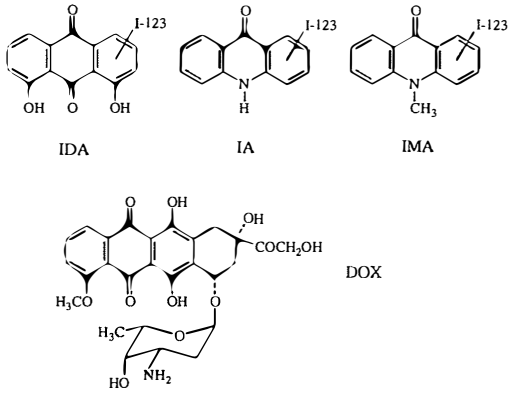

Figure 1. Chemical structure of the radiolabelled substances investigated as compared to doxorubicin (DOX). IA: [I-123]-Iodacridinone, IMA: [I-123]-Iod-10-methylacridinone, IDA: [I-123]-Iod-1,8-dihydroxyanthraquinone.

37 °C and pH of 6.8–7.4 in plates with 75 cm^2 ground area in a modified L-15 Leibowitz medium (Biochrom KG, Berlin, Germany) containing in addition 10 Vol.-% fetal calf serum (Boehringer, Mannheim, Germany), 20 mmol/l L-glutamine (Biochrom KG, Berlin, Germany), and 0.1 mg/l gentamycine (Biochrom KG, Berlin, Germany), respectively. Cells were considered to be appropriate for experiments in both exponential and stationary growth state when containing 6–8 million and 20–30 million cells per culture plate with 25 cm^2 ground area, respectively.

Experimental protocol

10–20 kBq of the I-123-labelled IA, IMA, IDA or 10–20 kBq thallium-201 dissolved lin 30 ml culture medium were added to each culture plate, respectively. After incubation using different time intervals ranging from 1 to 240 min, with 5 test tubes each per incubation interval. Tracer uptake was stopped by removing the culture medium, washing and cooling with 10 ml of 4 °C saline solution. After correcting for physical decay, uptake of the radiolabelled substances under investigation was measured in a well counter at 159 ± 32 keV and 80 ± 16 keV for I-123 and Tl-201, respectively. The number of cells was counted in every 10th culture plate

randomly chosen from the culture plates. Uptake was then expressed as percent of the amount of activity in 3 ml incubation medium normalized to one million cells. Results are given as mean ± one standard deviation.

Results

Thallium-201

Uptake kinetics of Tl-201 are shown in Figure 2. The uptake was inversely related to the number of cells in the culture plates. In exponential growth state two measurements with 7.5 and 10.3 million cells were performed yielding maximum uptake values of 1.35 ± 0.15 % at 30 min and 0.97 ± 0.06 % at 60 min, respectively. In stationary growth state maximum uptake amounted to 0.46 ± 0.05 % at 240 min.

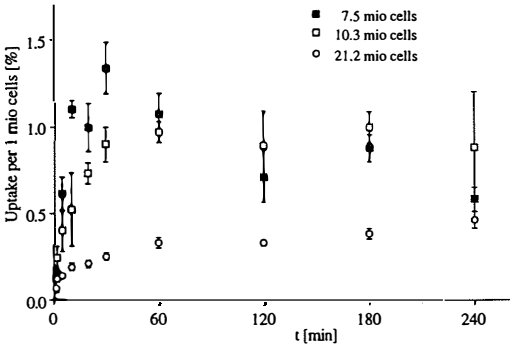

Figure 2. Uptake of Tl-201 in cell cultures of human breast cancer at different incubation intervals given in percent of the activity applied per million cells. Symbols denote mean ± SD according to different total cell counts in exponential (squares) and stationary (circles) growth state. Note, that uptake decreases with increasing total cell count.

I-123 labelled 9(10H)-acridinone

In Figure 3 uptake data of IA is depicted. Increased uptake of IA is obtained at low cell counts in the culture plates as compared to decreased uptake seen in exponential growth state. At 7.6 and 8.8 million cells maximum uptake was 0.41 ± 0.02 % and 0.29 ± 0.02 % after 60 min of incubation, respectively, while maximum uptake was 0.17 ± 0.01 % when 29.3 million cells were exposed to IA.

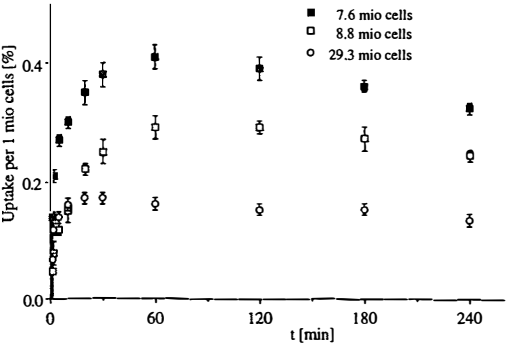

Figure 3. Uptake of 9(*10H*)-acridinone in cell cultures of human breast cancer at different incubation intervals given in percent of the activity applied per million cells. Symbols denote mean ± SD according to different total cell counts in exponential (squares) and stationary (circles) growth state. Note, that uptake decreases with increasing total cell count.

I-123 labelled 10-methylacridinone

Time-activity curves related to IMA are shown in Figure 4. In short, maximum uptake in exponential growth state was reached at about 60 min incubation interval amounting to 0.76 ± 0.07 % and 0.40 ± 0.01 % for 2.8 and 4.6

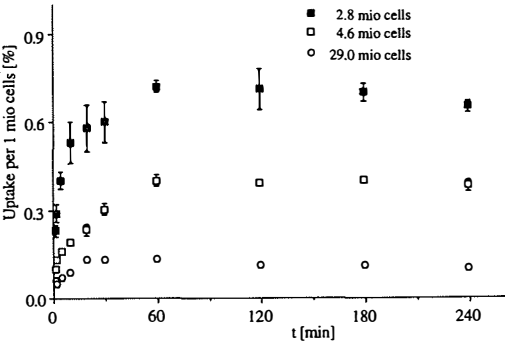

Figure 4. Uptake of 10-methylacridinone in cell cultures of human breast cancer at different incubation intervals given in percent of the activity applied per million cells. Symbols denote mean ± SD according to different total cell counts in exponential (squares) and stationary (circles) growth state. Note, that uptake decreases with increasing total cell count.

million cells, respectively. In contrast, maximum uptake at 60 min incubation interval was 0.13 ± 0.01 % when cell count yielded 29.0 million cells in the culture plates.

I-123 labelled 1,8-dihydroxyanthraquinone

Uptake measurements using IDA yielded maximum uptake between 30 and 120 min after starting the incubation. Uptake amounted to 0.31 ± 0.03 % in exponential growth state counting 5.4 million cells. When cell counts increased to 13.2 and 17.8 million cells uptake amounted to 0.16 ± 0.01 % and 0.20 ± 0.01 %, respectively. Time activity curves showing I-123 labelled 1,8-dihydroxyanthraquinone are shown in Figure 5.

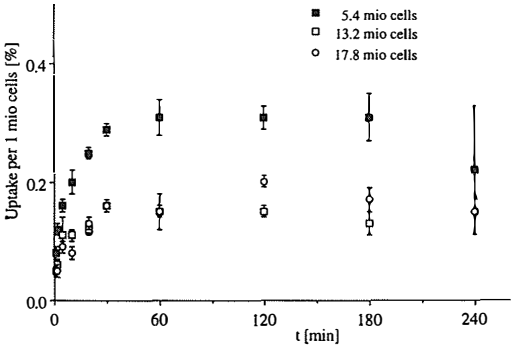

Figure 5. Uptake of 1,8-dihydroxyanthraquinone in cell cultures of human breast cancer at different incubation intervals given in percent of the activity applied per million cells. Symbols denote mean ± SD according to different total cell counts in exponential (filled symbols) and stationary (open symbols) growth state. Note, that uptake decreases with increasing total cell count.

Discussion

The uptake of thallium-201 was measured in cell cultures of anthracyline sensitive human adenocarcinoma of the breast amounting to be about 1–1.5 % of the applied activity per million cells in the culture plate. The amount of the uptake measured was within the known range,[21–23] thus confirming the reliability of the experimental set up chosen.

All substances under investigation showed the same sort of tracer kinetics, i.e. reaching an equilibrium state with a roughly monoexponential time course. Two underlying mechanisms may be responsible for this phenomenon: passive diffusion or ative uptake. Mass flux following passive diffusion is enhanced both by an enlarged diffusion area and by an increased concentration gradient. On the other hand, passive diffusion will decline with increasing diffusion distance.[24] In our experiments both concentration gradient and diffusion distance were constant in culture plates with low and hight total cell counts. However, cell surface exposed to the tracer was variable depending on cell density: in case of low total cell counts the cells in the culture plates showed no direct contact to each other, thus, the cell surface increased, which leads to an increased diffusion area. In fact, uptake values of thallium-201 decreased with increasing total cell counts in the culture plates yielding about 1.3 % at 7.5 million cells, and 0.4 % at 21.2 million cells. The same tendency could be shown for all substances under investigation, i.e. IA, IMA, IDA, as well. Therefore, influx and efflux of these substrates in sensitive breast cancer cells seems to be mainly due to passive diffusion.

However, the eventually reached steady state uptake of the tested tracers varied from each other. In detail, maximum uptake values of IDA, IA, and IMA were about 0.3 %, 0.4 %, and 0.7 %, respectively. It might be supposed that the eventual uptake of these substances simply varies due to their differing lipophilicity.[25] An enhanced diffusion of more lipophilic anthracycline derivatives through biomembranes could be demonstrated[26] as well as an increased effect of more lipophilic sodium channel blockers in isolated myelinated nerves.[27] As a measure of lipophilicity the octanol-buffer-partition ratio was determined and found to be 4.7, 5.2, and 5.4 for IDA, IA and IMA, respectively, which correlates very well with the according uptake data. Moreover, uptake of doxorubicine was measured previously as being 1.5 %,[28–31] and its octanol-buffer-patition ratio was 9.6. Thus, the amount of eventual uptake

of the respective substances seems to be mainly dependent on its lipophilicity.

The uptake kinetics of these anti-tumor substrates are thus of the same kind as the native non-labeled compounds[21] and, therefore, of value to act as tracers for possible drug resistance. Further studies are planned to test this drug group in drug resistant cell lines with and without interventions effecting the efflux capacity of the transmembraneous glycoprotein p170.

Conclusions

IA, IMA, and IDA can be labelled easily with iodine-123 resulting in good yields and radiochemical purity. As to the influx-efflux mechanism passive diffusion seems to be the main underlying process. As fas as the amount of their eventual uptake is concerned, lipophilicity seems to be the determining factor. These substrates are therefore of interest for further studies with drug-resistant cell lines to evaluate their applicability for predicting drug resistance.

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