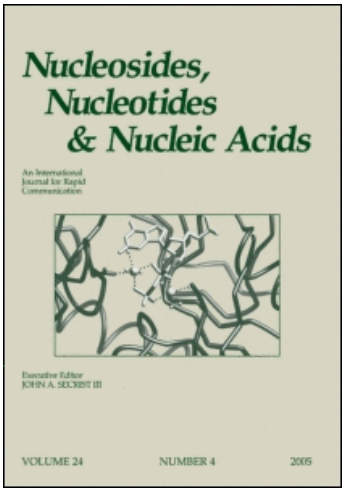

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Schott, H. , Heβ, W. , Leitner, B. and Schwendener, R. A.(1988) 'Antibody Cell-Targeting of Liposomes Carrying Ara C Derivatives', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 721 — 723
To link to this Article: DOI: 10.1080/07328318808056317
URL: http://dx.doi.org/10.1080/07328318808056317

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 721-723 (1988)

ANTIBODY CELL-TARGETING OF LIPOSOMES CARRYING ARA C DERIVATIVES

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Abstract: The prodrug N[4]-oleoyl ara C was incorporated into the membranes of functionalized unilamellar liposomes which were coupled to monoclonal antibodies. Cell-targeting of the prodrug-liposome-antibody complexes was investigated in vitro.

The cytostatic effect of 1-β-D-arabinofuranosylcytosine (ara C) can be improved by its chemical derivatization to lipophilic prodrugs[1,2] and by subsequent incorporation into the bilayers of unilamellar liposomes[3]. To optimize the cytostatic effects monoclonal antibodies which are able to selectively recognize tumor cells were linked to liposomes loaded with the prodrug N[4]-oleoyl ara C for example. The linkage was performed on a preparative scale via two different routes.

According to the first route, the liposomes containing N[4]-oleoyl ara C and functionalized by carboxylate groups were covalently coupled to [^{125}J] labelled antibodies using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimid x HCl (EDC) as condensation agent[4]. The condensation reactions were performed during 5.5 h at room temperature. The reaction mixtures were purified by chromatography on an Ultrogel AcA 22 column[5]. From the radioactivity measured for the isolated prodrug-liposome-antibody complexes the number of antibodies coupled per liposome was calculated.

The second route employs the biotin-avidin-system. Biotinylated[^{3}H] labelled liposomes containing the prodrug were complexed with biotinylated antibodies via avidin molecules[8]. Avidin is able of binding up to four biotinylated compounds.

Cell-binding assays showed, that depending on the optimum concentration and temperature, for example, the liposome-antibody complex (N^4-oleoyl ara C : liposome : antibody (Tü 7) = 5200 : 1 : 1) synthesized via EDC-condensation was bound up to 5-30 times better by the human target cells BJAB and U 937 than by the non-target mouse cells NS 1 (see TABLE 1).

The specific targeting of the liposome-antibody complex, composed of N^4-oleoyl ara C : liposome : biotin : avidin : biotin-antibody (8000 : 1 : 2500 : 165 : 0.6) was proved with the MHC class I mouse antibody B8-24-3. The results summarized in TABLE 2 showed that 6 times more antibody as well as 6 times more liposome-antibody complex were

TABLE 1: Conditions and results of the incubation of the target cell-lines U 937 or BJAB and the non-target mouse cell-line NS 1 with the complex: N^4-oleoyl ara C -liposome-antibody (Tü 7). 10^6 cells were incubated at 4°C resp. 37°C with the corresponding reagents dissolved or suspended in 1ml 150 mM NaCl, 1 mM NaH_2PO_4 (pH 7.3) each.

Cell-line	Amounts of the complex incubated (cpm)	bound (cpm)	(%)	Calculated number of molecules per cell liposome	N^4-oleoyl-ara Cx10^6	Ratio of complex bound by each cell line at 4°C	37°C
BJAB	852400	1145	0.13	3400	17.0		1.0
U 937	852400	2139	0.26	6600	33.0		2.0
NS 1	852400	1522	0.18	4500	22.5		1.3
BJAB	85240	347	0.41	1000	5.0		1.0
U 937	85240	1206	1.41	3500	17.5		3.5
NS 1	85240	346	0.41	1000	5.0		1.0
BJAB	8520	364	4.27	1100	5.5	1.0	1.0
U 937	8520	1091	12.80	3200	16.0	21.1	3.0
NS 1	8520	51	0.60	200	1.0	4.4	0.1

TABLE 2: Conditions and results of the incubation of the murine target cell-line EL 4 and the non-target human cell-line U 937 with the reagents A: mixture of biotinylated antibody ([^{3}H]biotin-B8-24-3 : B8-24-3 = 1 : 5); B: complex of N^+-oleoyl ara C-liposome-biotin-avidin-biotin-antibody (B8-24-3); C: complex of N^+-oleoyl ara C-liposome-biotin-avidin-[^{3}H]biotin. 10^7 cells were incubated at 4°C for 30 min with the corresponding reagents dissolved or suspended in 1 ml 150 mM NaCl, 1 mM NaH_2PO_4 (pH 7.3) each.

Cell-line	Reagent	Amounts of the reagent incubated (cpm)	bound (cpm)	(%)	Calculated number of molecules per cell antibody	liposome	N^+-oleoyl-ara Cx10^6
EL 4	A	49600	300	0.6	142000	---	---
U 937	A	49600	50	0.1	24000	---	---
EL 4	B	44000	1740	4.0	116000	180000	1400
U 937	B	44000	300	0.7	20000	30000	240
EL 4	C	23700	140	0.6	---	4400	35

bound by the target mouse cells (EL 4) than by the non-target human cells (U 937) which served as a negative control. The activity of the coupled antibody was confirmed by the result that the EL 4 cells bound 40 times more liposomes if these were linked with the B8-24-3 antibody compared to liposomes which were not linked with an antibody but derivatized with [^{3}H] biotin.

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