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T lymphocytes as potential therapeutic drug carrier for cancer treatment

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Abstract

The aim of our research is the application of human immune cells (T lymphocytes) as target directed drug carrier. Thereby, the inclusion of therapeutical nanoparticles into immune cells is a new strategy for a localized chemotherapy. The autonomous targeting of diseased sites makes immune cells to perfectly controlled drug delivery systems.

The study's aim was to demonstrate the feasibility to mobilise immune cells as therapeutic drug carrier systems which can be combined with existing immunotherapies.

Therefore, Jurkat cells as well as T lymphocytes were used to identify the smoothest procedure for loading nanoparticles into immune cells. Different loading processes, incubation times and nanoparticle concentrations were compared. Nanoparticles coated with cytotoxic antibiotic doxorubicin were used in first release experiments. A time dependent liberation of doxorubicin from carrier cells was discussed as first therapeutic approach.

Keywords: T lymphocytes; Nanoparticles; Doxorubicin

1. Introduction

Nanotechnology is a promising field revealing exciting prospects in the therapeutic application. Characteristics such as biodegradability and minimal toxicity predestine magnetic nanoparticles as intracorporal drug carrier systems (Asmatulu et al., 2005).

The concept of drug targeting and controlled drug delivery can be achieved by surface modifications and choice of appropriate particle materials of these particles (Dinauer et al., 2005). Application possibilities in nanomedicine are manifold. Alexiou et al. (2002) demonstrated that particles with magnetic characteristics can be retained at the target site by applying an external electromagnetic field. Nanoparticles composed of biodegradable polymers are proposed for application in oral chemotherapy (Dong and Feng, 2005; Win and Feng, 2004). Nanosized micelles are of advantage for drug transports with narrow therapeutic indexes (Rapoport, 2004). Because of their small size, nanoparticles are suitable for intravenous administration (Uchegbu and Schätzlein, 2003).

In the present therapy approach therapeutic nanoparticles are loaded into immune cells for a target specific and sheltered transport to the diseased site.

This allows to potentate benefits of nanoparticle administration with advantages of immune system for disease control.

Transport inside of immune cells will protect nanoparticles that would otherwise be removed by the reticuloendothelial system within few hours depending on their size and surface characteristics (Brain et al., 1999; Moghimi and Szebeni, 2003; Mitragotri and Chambers, 2004).

The immune system's ability to recognize and attack transformed cells is exploited by immunotherapies. The autonomous targeting of diseased sites makes immune cells to perfectly controlled drug delivery systems. Drug loading of immune cells can be combined with diverse immunotherapies.

Thus, in adoptive cell transfer (ACT) autologous tumor reactive T cells are proliferated ex vivo and then reinfused into the

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patient's body (Dudley and Rosenberg, 2003; Klebanoff et al., 2005). Other treatments aim to increase the number of tumor targeting T lymphocytes by vaccinating cancer patients with tumor specific antigens (Coulie and Connerotte, 2005).

A combined chemotherapy, adoptive transfer of tumor-reactive T cells and IL-2 treatment evoked a tumor size reduction of 50% in more than 50% of the treated melanoma patients contributing to a clinical trial (Rosenberg and Dudley, 2004).

Dudley et al. (2005) conclude an improved therapy efficacy of ACT compared to other treatments such as high-dose IL-2 administration or chemotherapy.

Another immune therapeutic approach is the use of artificially engineered antibodies, i.e. bispecific antibodies which link tumor cells with immune cells (Birschwein et al., 2006).

A synergistic effect on tumor regression could be provoked in all described treatments by additional release of anti-cancer drugs from T lymphocytes at the target site.

Advantages of drug transportation by tumor reactive T lymphocytes are summarized: (1) localized drug targeting, (2) complementation and amplification of cytotoxic T cell effect by anti-tumor drugs such as angiogenese inhibitors, cytostatica, etc., (3) detection and attack of metastases not yet diagnosed, (4) autonomously working system, (5) high specifity and (6) generation of long-term memory T cells (by loading only a certain proportion of T lymphocytes with drugs).

These are valuable benefits compared to other therapy approaches aiming to release therapeutics exclusively at the tumor site. The here described therapeutic system is an ideal new way to combine chemotherapy and target specific immuntherapy, in order to apply minimal chemotherapeutical drug doses that work maximal target-specific.

The study's aim was to demonstrate the feasibility to mobilise immune cells as therapeutic drug carrier systems which can be combined with existing immunotherapies.

Therefore, the smoothest procedure for loading nanoparticles into immune cells was identified. Different loading processes, incubation times and nanoparticle concentrations were compared.

The cell's particle tolerance was monitored in cell growth studies with loaded cells. Also the mode of particle uptake was analysed for incubation and electroporation based on convocal microscopy studies. Uptake behaviour of JURKAT cells and isolated T lymphocytes was compared to test whether the results from JURKAT cell lines can be transferred to T lymphocytes isolated from human blood. JURKAT cells represent an often used and easy to handle model for human T lymphocytes (Fraser et al., 2004). The efficiency of nanoparticle uptake was quantified by fluorescence activated cell sorting (FACS) analysis. Furthermore, nanoparticles coated with cytotoxic antibiotic doxorubicin were used as a first therapeutic approach. These particles were loaded into T lymphocytes the effect of doxorubicin on the carrier cells was tested over several days. A first release strategy of the drug has been approached.

2. Materials and methods

2.1. Isolation of monocytes and lymphocytes from buffy coats

Twenty-five microliters of buffy coat, treated with EDTA (Sigma-Aldrich Chemie GmbH, Germany) is diluted 1:1 with 25 ml 1× PBS-buffer in 50 ml tubes (Falcon GmbH, Germany) and centrifuged at 400 × *g* and 20 °C for 35 min (gradient centrifugation using Eppendorf centrifuge 5810 R).

Cells in the interphase were transferred into new Falcon tubes containing 10 ml Ficoll-Paque (Amersham Bioscience GmbH, density 1.077 g/ml) and recentrifuged at 300 × *g* and 20 °C for 10 min (twice). Cells in the resulting pellet were quantified by aid of a Neubauer haemocytometer (Optik Labor) (total number of cells/ml = counted number of cells in one large square × 10^4) and adjusted to a working concentration of 1×10^7 cells/ml with 1× PBS.

2.2. Cell culture

A lymphocytic JURKAT cell line (DSMZ GmbH, Germany) was cultivated in RPMI-Medium (Gibco Co., Germany) supplemented with 10% FCS (Invitrogen) 1% penicillin streptomycin at 37 °C in humidified air with 5% CO_2.

2.3. Nanoparticles

TargetMAGs are biocompatible magnetic nanoparticles applied for the delivery of anticancer drugs in locoregional tumor therapy (Lübbe and Bergemann, 1996).

TargetMAG-doxorubicin nanoparticles (chemicell, Germany) consist of a multi-domain magnetite core and a crosslinked starch matrix with terminal cation exchange phosphate groups for reversible binding of the positively charged doxorubicin (Bergemann et al., 1999). The particles have a hydrodynamic diameter of 50 nm, a weight to volume of 25 mg/ml and are coated with 3 mg/ml Doxorubicin.

TargetMAG-AC/GL (chemicell, Germany) are green-fluorescent nanoparticles (extraction/emission: 502 nm/625 nm) with a hydrodynamic diameter of 50 nm. The particles have a supermaramagnetic maghemite core and a starch coating with terminal phosphate groups. The fluorescent dye is irreversibly imbedded into the starch matrix.

2.4. Loading nanoparticles into cells

Incubation: 1×10^7 cells/ml were incubated with 15 μg/ml targetMAG-AC/GL nanoparticles (chemicell) for a specific time period. Incubation was finished by centrifugation at 2.2 × 1000 rpm for 4 min and the cells in the resulting pellet were washed and resuspended with 1× with PBS and stored on ice.

Electroporation: 1×10^7 cells/ml in RPMI medium free of FCS, antibiotics and glucose and 15 μg/ml targetMAG-AC/GL nanoparticles were used; electroporation was performed with 20 pulses of 10 μs length and at 500 V (electric field strength of 2.5 kV/cm) in Electro Square Porator EMC 830 (BTX, San

Diego). The electroporation procedure was finished by centrifugation at 2.2 × 1000 rpm for 4 min and the cells in the resulting pellet were washed and resuspended with 1× with PBS and stored on ice.

2.5. Monitoring particle uptake by confocal microscopy

Intracellular uptake of nanoparticles was verified by confocal fluorescence microscopy: Jurkat cells (1×10^7 cells/ml) were incubated in RPMI-medium containing 15 μg/ml targetMAG. After 30 min incubation at RT, cells were washed twice with PBS and stored on ice in the dark until confocal analysis. Fluorescence images of different cell slices were obtained using a confocal laser scanning microscope (BioRad MRC 1024, Munich, Germany) with a 522/35 bandpass filter for fluorescence detection. Pictures were taken after excitation at 488 nm with a step size of 0.2–0.5 μm between confocal sections (original magnification 63×).

Cells of the control group incubated without nanoparticles showed no fluorescence (data not shown).

2.6. Measuring fluorescent-intensity by flow cytometry

1×10^7 cells/ml were loaded with particles either stained with a green fluorescence dye (extraction/emission: 502 nm/625 nm) or coated with doxorubicin (auto-fluorescence maximum of doxorubicin bound to the particles at 508 nm). The amount of cells containing fluorescent nanoparticles was quantified by fluorescence-assisted cell sorting (FACS Calibur, Becton Dickinson, Heidelberg, Germany; equipped with Argon laser with 488 nm excitation wavelength). By specific gating only loaded cells from T lymphocyte populations were analysed.

2.7. LDH test

The LDH test (cytotoxicity test) is available as experimental kit from Roche Diagnostic GmbH, Germany. The enzyme lactate dehydrogenase (LDH) occurs in the cytoplasm of all cells, and it will diffuse out of the cell into cell-culture-medium in case of cell damage (cytotoxicity marker). The amount of LDH released from cells following insertion of doxorubicin coated nanoparticles was quantified in order to determine cytotoxicity over time.

2.7.1. Preparation steps

Two hundred microliters cell suspension ($(0.2–2) \times 10^4$ cells/ml) were incubated at 37 °C, 5% CO_2 for 24 h in RPMI-medium (Gibco) using a 96-well plate (Nunc GmbH). Thereafter, the medium was replaced by a medium containing doxorubicin-coated nanoparticles. Incubation time of cells with particles was 5 min and was finished by centrifugation at 2.2 × 1000 rpm for 4 min. The cells in resulting pellet were washed twice and resuspended with 1× with PBS. Then, percentage of damaged cells carrying doxorubicin coated nanoparticles was determined after different time steps according to the kit protocol. *Controls*: Untreated cells in RPMI medium (untreated control), untreated cells in RPMI medium with 1% Triton X-100 (dead control). Extinction at 490 or 492 nm, and also at 600 nm as reference, was measured with an ELISA reader. The cell cytotoxicity was determined in relation to the probe's absorption. Cytotoxicity in percent: ((probe absorption − background absorption) − untreated probe absorption)/(Triton probe absorption − untreated probe absorption).

2.8. Monitoring of drug release by fluorescence microscopy

TargetMAG-doxorubicin nanoparticles (chemicell, Germany) were loaded into Jurkat cells by incubation as described before (Section 2.4). Uptake of doxorubicin coated nanoparticles, doxorubicin distribution and doxorubicin release from the cell were visualised by fluorescence microscope (olympus IX 71; fluorescence filter U-MWIBA-2 for green fluorescence: excitation from 460 to 495 nm and emission 510–550 nm).

3. Results

3.1. Loading human immune cells with fluorescent labelled nanoparticles

The aim of this study is to develop a new target oriented cancer therapy. Thereby, T lymphocytes are used as autonomous tumor directed drug delivery systems. Loading drug coated nanoparticles into immune cells is key factor in the new approach.

For uptake studies nanoparticles with a superparamagnetic maghemite core and a starch coating with terminal phosphate groups were used. The fluorescent dye is irreversibly imbedded into the starch matrix. The hydrodynamic diameter averages 50 nm.

The cells were loaded by incubation and electroporation and uptake monitored by confocal laser scanning microscopy (clsm) (Fig. 1).

Fluorescent nanoparticles can be detected in the Jurkat cells and thus successful particle loading has been shown.

3.2. Biocompatibility of nanoparticles

For a safe therapeutic application of nanoparticles inside the human body, it is important to test their biocompatibility. Therefore, cell proliferation of loaded cells was monitored over several days.

The cells were incubated with different nanoparticle concentrations (25 and 50 μg/ml) and cell growth was studied over five days (Fig. 2).

No early death or damage reactions could be detected following loading with negatively charged 50 nm sized nanoparticles compared to untreated control cells. Particle loaded and untreated cells show a normal stationary phase followed by an exponential phase ending in plateau phase.

3.3. Loading nanoparticles into: comparison of incubation and electroporation

A key step in the new therapy approach is the effective loading of drug coated nanoparticles into T lymphocytes. Therefore,

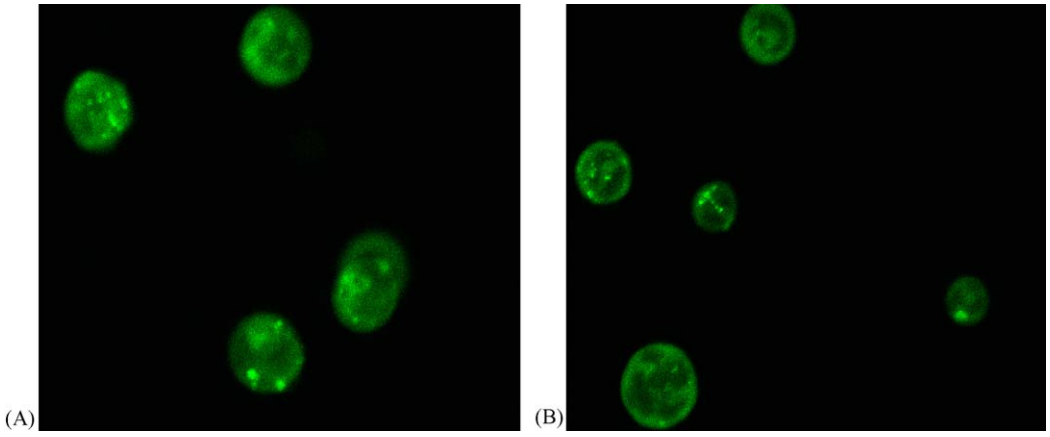

Fig. 1. JURKAT cells loaded by incubation (A) or electroporation (B) with 50 μg/ml green fluorescent target MAG-AC/GL, confocal microscope 488 nm (40× magnification).

the most effective and at the same time most gentle loading procedure has to be identified.

Incubation and electroporation were chosen as suitable alternatives and compared in time parallel approaches.

Thereby, incubation is a very simple approach of mixing particles and cells together and thus inducing uptake by endocytosis. However, the uptake efficiency is the same as for the more sophisticated electroporation process. In both electroporation as well as incubation a cell loading efficiency of almost 100% was achieved as detected by flow cytometric measurements.

Electroporation parameter of 20 pulses with a pulse length of 10 μs at 500 V (electric field strength of 2.5 kV/cm) were tested before as the most efficient for particle uptake and cell survival (data not shown).

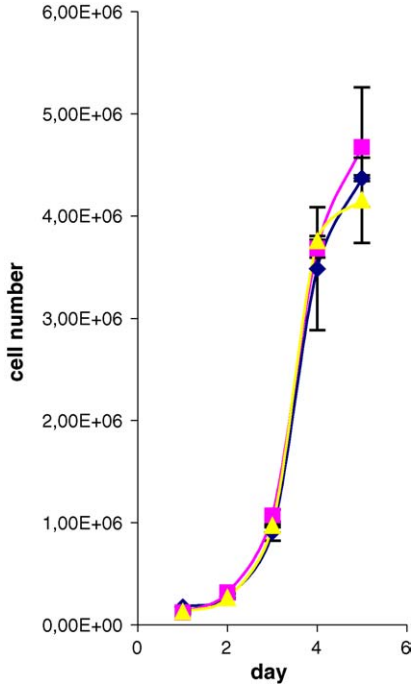

Fig. 2. Monitoring the cell survival (cell proliferation) of negatively charged 50 nm sized nanoparticles over five days and two different concentrations: 25 and 50 μg/ml. (●) Untreated control cells, (■) cells loaded with 25 μg/ml and (▲) cells loaded with 50 μg/ml.

However, because incubation is an easy to handle and very gentle method to load nanoparticles into the T lymphocytes, it was chosen as ideal loading procedure.

3.4. Mechanism of particle uptake into the cells

Nanoparticle uptake and localisation were investigated by clsm. Particles marked with fluorescent dye could be visualized at different levels inside the cell (Fig. 3).

Thus, nanoparticles assembled in endosome-like structures are detected, but fluorescence of smaller spots inside the cytoplasm and at the outer cell membrane can also be observed.

This distribution pattern is equally evoked by both loading processes, i.e. incubation and electroporation performed in time parallel approaches. Successful particle uptake in the cell can thus be confirmed.

3.5. Incubation as time dependent process

The minimal incubation time was determined in order to saturate cells with nanoparticles. Fluorescence labelled particles were supplied in surplus, i.e. at a concentration of 15 μg/ml and loading efficacy into the cell was compared following different incubation times. For both T lymphocytes and their model cell line, Jurkat cells, a high loading efficiency of more than 90% was already detected after 5 min (Table 1). Loading efficacy could not be significantly increased by longer incubation, i.e. up to 1 h.

Table 1
Efficacy of nanoparticle uptake into JURKAT cells after different incubation times and determined by FACS analysis

Incubation time	Loading efficiency (mean value)	Standard deviation (S.D.)
10 s	97.62	2.393
15 s	98.27	1.764
30 s	98.85	1.678
1 min	98.93	1.143
2 min	98.70	1.387
5 min	98.50	1.604
1 h	98.90	1.230

Data represents mean of two independent experiments ± S.D., $n = 6$.

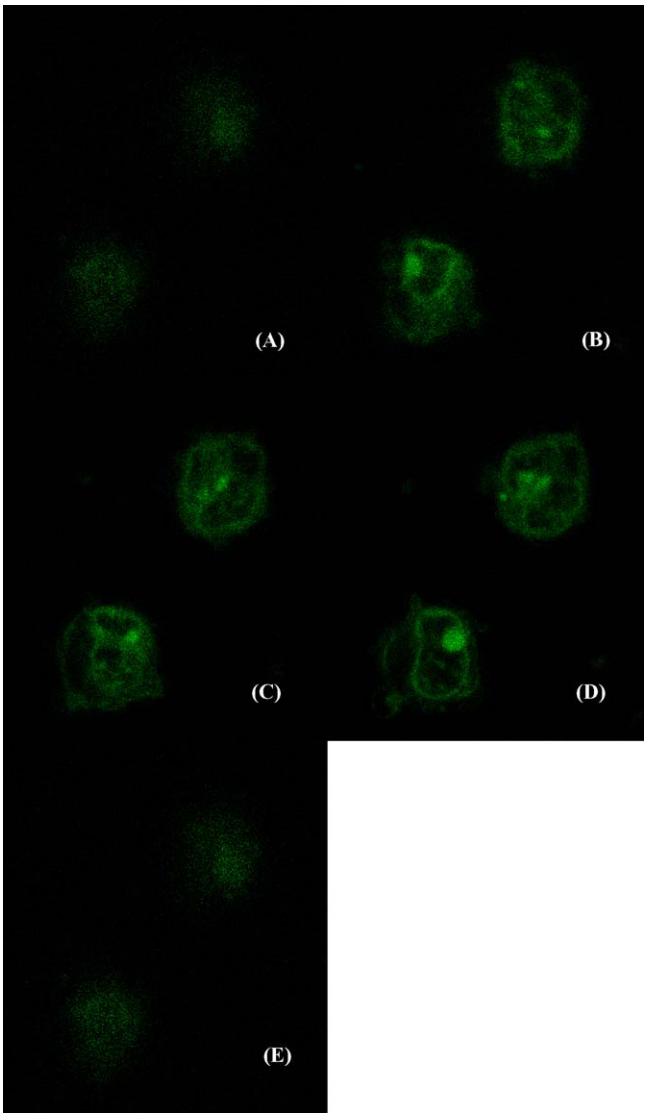

Fig. 3. Confocal images of different optical sections (A–E) from Jurkat cells after incubation with fluorescent nanoparticles (original magnification 63× with 3× zoom).

3.6. Particle loading as concentration dependent process

Beside the incubation time, the particle concentration is also a critical parameter to achieve maximal loading of nanoparticles into cells. Loading efficacy was monitored in T lymphocyte populations by flow cytometric measurements.

The lowest tested particle concentration of 5 μg/ml JURKAT cells already resulted in a loading of almost 100% of the cell population. Incubation with higher particle concentration did not increase the particle uptake of single cells.

3.7. Loading cells with doxorubicin causes cell damage

Doxorubicin is a cytotoxic anthracycline antibiotic often used in systemic chemotherapy in order to fight cancer.

Target specific doxorubicin deposition is aimed in this approach based on T lymphocytes as delivery system for drug coated nanoparticles.

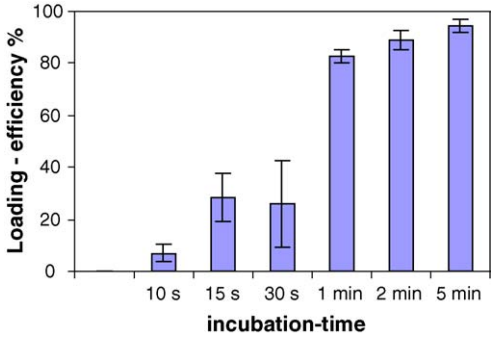

Fig. 4. Uptake of doxorubicin-coated nanoparticles into T lymphocytes after different incubation times were determined by FACS analysis. Data represents mean ± S.D., $n = 6$.

Doxorubicin nanoparticles consist of a multi-domain magnetite core and a crosslinked starch matrix with terminal cation-exchange phosphate groups for reversible binding of the positively charged doxorubicin (Bergemann et al., 1999). The particles have a hydrodynamic diameter of 50 nm.

Incubation of Jurkat cells with doxorubicin coated nanoparticles resulted in a significant increase ($p < 0.05$) of loading efficiency over 10 s to 5 min, i.e. up to 96% following 5 min incubation time (Fig. 4).

Thereafter, a time retarded release of the drug from the carrier cell allows drug transportation to diseased sites. Diffusion of doxorubicin inside the carrier cell will cause cell damage and thus induce drug release in a time dependent manner. Cell cytotoxicity was determined over time starting from loading Jurkat cells with doxorubicin coated nanoparticles. One hour after loading doxorubicin coated nanoparticles into the cells about 5% of the cells died. The number increased to 20% after 5 h and finally to 60% after about 15 h (Fig. 5).

Thus, a time dependent release of the doxorubicin from the particle inside the carrier cell has been shown to result in cell death and drug release (Figs. 5 and 6).

First results based on human T lymphocytes expressing CD 8-independent, p53A2.1-specific TCR and experiments with bispecific antibodies showed that cytotoxic effector T lymphocytes still target and bind cancer cells after being loading with drug coated nanoparticles. In other words, drug loading into

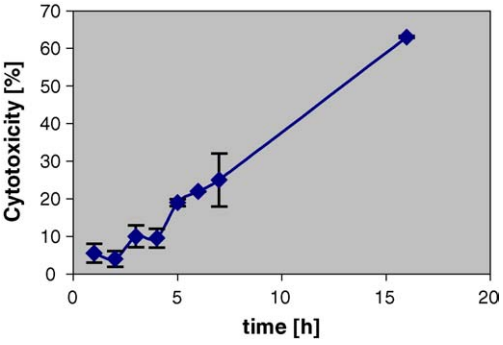

Fig. 5. Drug release from JURKAT cells and induced cell damage over time. Cell damage was determined by LDH-cytotoxicity-test. Data represents mean ± S.D., $n = 6$.

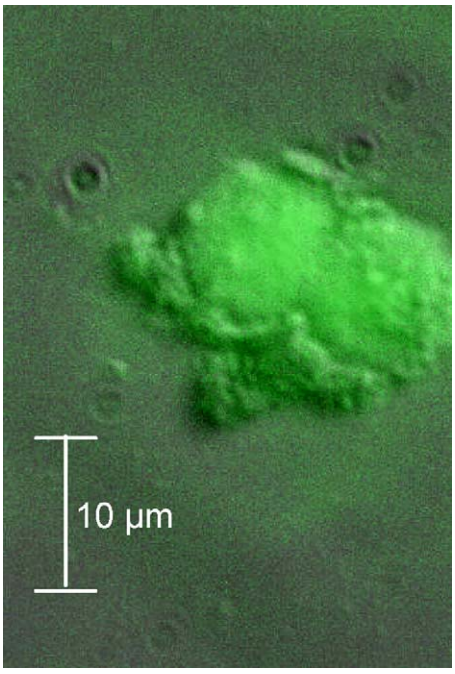

Fig. 6. Drug release from Jurkat cells 2.5 h after loading doxorubicin coated nanoparticles. Fluorescence microscope 460–490 nm (original magnification 40× with zoom).

cells does not compromise the physiological function (data not shown).

4. Discussion

4.1. First step in developing a new therapy

This study aims to develop a target directed chemotherapy based on T lymphocytes which deliver the drug autonomously to the diseased sites.

T cells play an essential role in immunosurveillance and destruction of cancer cells (Dunn et al., 2004). However, clinical success of immunotherapies is so far limited. Thus, vaccination with peptides or dentritic cells derived from tumor cells resulted in detectable immune response, but it was too weak and transient to eradicate tumors (Wang and Wang, 2005). In the here presented approach antigen-specific T lymphocytes are used as drug delivery systems in order to enhance the natural tumor killing efficacy. Negatively charged nanoparticles with a hydrodynamic diameter of 50 nm and coated with doxorubicin have been demonstrated to be suitable for incorporation into T lymphocytes and lymphocytic JURKAT cells.

The advantage of using T lymphocytes as drug delivery entities is obvious. Inside the carrier cells the particles are protected against clearance by the endoreticulo system (Brain et al., 1999). This is in contrast to freely circulating polymeric particles that are cleared within a few minutes to hours depending on their size and surface characteristics (Mitragotri and Chambers, 2004). Furthermore, tumor specific T lymphocytes or alternatively immune cells combined with bispecific antibodies specifically target cancer tissue and thus support controlled drug release. High therapy effect by cell promoted drug targeting and low side effects is the consequence and benefits over other chemotherapies (Asmatulu et al., 2005). This therapeutic system will work autonomously and target even metastasis not yet diagnosed. By loading only part of the T cells the generation of memory T cells is ensured that protect the body against new tumor formation.

In this study, doxorubicin coated nanoparticles have been loaded into immune cells, but also other drugs such as angiogenesis inhibitors, mitose inhibitor, etc. will be tested aiming to synergistic tumor destruction together with cytotoxic T cell action.

The nanoparticles used in this study were most effective for uptake into cells. This was determined with fluorescence labelled nanoparticles which can be easily detected by clsm and in flow cytometric studies. Also good biocompatibility has been shown in cell experiments.

The corresponding tolerance experiments were only performed over five days because after cell division and particle distribution in growing number of cells detection is difficult.

4.2. Comparing electroporation and incubation as loading methods

Effective loading of nanoparticles into T lymphocytes is a key step of the here proposed drug targeting approach. The gentle introduction of medicament coated particles without compromising the physiological function is important to maintain the targeting ability of the carrier cells.

Two methods have been compared: (i) electroporation, the formation of transient artificial permeability pathways in the cell membrane induced by electric current and consequently introducing of extracellular molecules into the cytoplasm (Teissie et al., 1999) and (ii) endocytosis, a natural process characterized by membrane invagination and detachment of newly formed vesicles inside the cytoplasm in order to deliver large molecules into cells (Conner and Schmid, 2003).

Incorporation of diverse macromolecules into cells has been shown by electroporation. Baron et al. (2002) for example succeeded in inserting antibodies into cells. However, the main disadvantage is the high killing rate of cells during the procedure which has been described up to about 50–90% (Baron et al., 2002).

The uptake of nanoparticles into T lymphocytes was studies by confocal microscopy. Single images of different cell levels provided details on intracellular distribution. Following both loading procedures, i.e. electroporation and incubation, particles were detected free in cytoplasm and in small accumulations which are supposed to be endosome-like structures.

Endocytotic processes have already been described for the internalisation of a large variety of substances and nanoparticles in eukaryotic cells (Berry et al., 2004; Rejman et al., 2004).

Since incubation is a very smooth process, this method will be used in the future for therapeutic loading of T cells with drug coated particles.

4.3. Comparing different incubation times

The minimal incubation time for effective loading of T lymphocytes or Jurkat cells, respectively, with particles has been determined. Nanoparticles were supplied in surplus, i.e. at a concentration of 15 μg/ml.

After 5 min cells were already saturated with particles. No significant increase in uptake could be determined after a longer incubation period.

Win and Feng (2004) studied the uptake of polymeric nanoparticles by human colon adenocarcinoma cell line Caco-2 for an oral chemotherapy approach. They determined a significant increase in particle uptake at an incubation period beyond 2 h. Nevertheless, this study showed a high incubation efficiency into freshly isolated leukocytes and in lymphocytic JURKAT cell lines more earlier by using starch-coated 50 nm sized negatively charged nanoparticles.

4.4. Comparing different particle concentrations

In this study, no significant differences could be shown in uptake behaviour comparing different particle concentrations 5–20 μg/ml at 5 min incubation time. A small but not significant increase on the way from 5 μg/ml towards 15 μg/ml, observed in JURKAT cells and buffy coat cells could be shown, which means a rise in uptake efficiency from 97% to 99%. Nevertheless, a particle concentration of 5 μg/ml is already suffient for a successful loading of T lymphocytes with nanoparticles. It has to be proven, whether 5 μg/ml drug coated nanoparticles is adequate for therapeutical approaches and with that, which local concentration will be needed at tumor site (still in process).

4.5. Doxorubicin release

Doxorubicin is an anthracycline antibiotic and one of the most cytostatic drugs used in cancer chemotherapy. Its mode of action is the inhibition of DNA and RNA bio-synthesis (Schwartz and Matuszewski, 2002).

One approach of drug targeting based on doxorubicin is described by Rapoport et al. (2003). Thereby, micelles enriched in doxorubicin circulate in the blood stream and drug release is triggered at the target site by localized application of ultrasound.

In this study, nanoparticles coated with doxorubicin are loaded into T lymphocytes which circulate in the blood stream. The drug will defuse in the carrier cell and destroy about 60% of these cells after 15 h. The blood is pumped through the body about once within one minute. So there is enough time for target finding.

Divers strategies are discussed in literature in order to improve the antigen recognition in T cell based immunotherapy.

Thus, Kuball et al. (2005) describe that retroviral expression of CD8-independent, p53-specific T cell receptor into human T cells imparted the CD8+ lymphocytes with broad tumor-specific activity and turned CD4+ T cells into potent tumor-reactive, p53A2.1-specific T helper cells.

Cell destruction is of advantage in case of transgenic T cells since the application of genetically modified cells is controversially discussed for introduction into the human body. Using body own immune cells it is beneficial to only load a part of them with drugs in order to assure the memory cell generation.

Also, bispecific antibodies (BsAb) offer an elegant way to improve antibody based immunotherapy for cancer by crosslinking tumor cells and selected cytotoxic effector cell populations. Also in this case tumor destruction can be improved by loading therapeutic agents into the effector cells.

In vitro experiments have shown that 9 mg doxorubicin/kg body weight is the maximal tolerated dose (MTD) for systemic chemotherapy in mouse model and that this concentration evoked a successful tumor regression (Barraud et al., 2005).

It can be hypothesised that the medication dose in a drug targeting system will be much lower since the drug is supposed to be deposited mostly at the tumor side. This will increase therapy effect and lower the side effects compared to current chemotherapy.

Doxorubicin coated to polysorbate-coated nanoparticles was already used to treat human glioblastoma by passing the blood–brain barrier (Steiniger et al., 2004).

In conclusion, we succeeded in loading JURKAT cells as well as freshly isolated lymphocytes with fluorescent starch-containing particles having therapeutical potential, and already found efficient incubation parameters to be at an incubation time of 1 min (doxorubicin coated nanoparticles) and a particle concentration of 5 μg/ml. No fundamental differences in uptake behaviour could be found between lymphocytic JURKAT cells and freshly isolated leukocytes, which means that JURKAT cells are representative when analysing immune cells. This approach provides an uptake of nanoparticles into T lymphocytes from over 90%. Therefore, this article represents a very good starting point for a therapy using T lymphocytes as nanoparticle drug delivery system for cancer treatment, pointed out by doxorubicin coated nanoparticles. In a next step tumor-reactive, drug-loaded T lymphocytes will be tested in a mouse model to be efficient to narrow tumor tissue.

Currently, in vitro studies in target effector cell models are investigated and mouse experiments will follow to prove the feasibility of this therapy approach. Doxorubicin but also other drugs with other mode of actions such as angiogenesis inhibitors will be tested.

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References

Alexiou, C., Schmidt, A., Klein, R., Hulin, P., Bergemann, C., Arnold, W., 2002. Magnetic drug targeting: biodistribution and dependency on magnetic field strength. J. Magn. Magn. Mater. 252, 363–366.

Asmatulu, R., Zalich, M.A., Claus, R.O., Riffle, J.S., 2005. Synthesis, characterisation and targeting of biodegradable magnetic nanocomposite particles by external magnetic fields. J. Magn. Magn. Mater. 292, 108–119.

Barraud, L., Merle, P., Soma, E., Lefroncois, L., Guerret, S., Chevallier, M., Dubernet, C., Couvreur, P., Trepo, C., Vitvitski, L., 2005. Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo. J. Hep. 42, 736–743.

Baron, S., Poast, J., Rizzo, D., Mc Farland, E., Kieff, E., 2002. Electroporation of antibodies, DNA, and other macromolecules into cells: a highly efficient method. J. Immunol. Methods 242, 115–126.

Bergemann, C., Müller-Schulte, D., Oster, J., Brassard, L., Lübbe, A.S., 1999. Magnetic ion-exchange nano- and microparticles for medical, biomedical and molecular biological applications. J. Magn. Magn. Mater. 194, 45–52.

Berry, C.C., Charles, S., Wells, S., Dalby, M.J., Curtis ASG, 2004. The influence of transferrin stabilised magnetic nanoparticles on human dermal fibroblasts in culture. Int. J. Pharm. 269, 211–225.

Birschwein, K., Schlereth, B., Guller, C., Wolf, A., Lutterbuese, R., et al., 2006. MT110: A novel bispecific single-chain antibody construct with high efficacy in eradicating establised tumors. Mol. Immunol. 43 (8), 1129–1143.

Brain, J.D.M., Ramon, M., Decamp, Malcom, M., Warner, Angeline, E., 1999. Pulmonary intravascular macrophages: their contribution to the mononuclear phagocyte system in 13 species. Am. J. Physiol. Lung Cell. Mol. Physiol. 276, L146–L154.

Conner, S.D., Schmid, S.L., 2003. Regulated portals of entry into the cell. Nature 422, 37–44.

Coulie, P.G., Connerotte, T., 2005. Human tumor-specific T lymphocytes: does function matter more than number? Curr. Opin. Immun. 17, 320–325.

Dinauer, N., Balthasar, S., Weber, C., Kreuter, J., Langer, K., von Briesen, H., 2005. Selective targeting of antibody conjungated nanoparticles to leukemic cells and primary T-leukocytes. Biomaterials 26, 5898–5906.

Dong, Y., Feng, S.-S., 2005. Poly(D,L-lactide-co-glycolide)/montmorillonite nanoparticles for oral delivery of anti cancer drugs. Biomaterials 26, 6068–6076.

Dudley, M.E., Rosenberg, S.A., 2003. Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat. Rev. Cancer 3, 666–675.

Dudley, M.E., Wunderlich, J.R., Yang, J.C., Sherry, R.M., Topalian, S.L., Restifo, N.P., Royal, R.E., Kammula, U., White, D.E., Mavroukakis, S.A., Rogers, L.J., Gracia, G.J., Jones, S.A., Mangiameli, D.P., Pelletier, M.M., Gea-Banaclocje, Robinson, M.R., Berman, D.M., Filie, A.C., Abati, A., Rosenberg, S.A., 2005. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J. Clin. Oncol. 23, 2346–2357.

Dunn, G.P., Old, L.J., Schreiber, R.D., 2004. The three Es of cancer immunoediting. Annu. Rev. Immunol. 22, 329–360.

Fraser, S.P., Diss, J.K.J., Lloyd, L.J., 2004. T-lymphocyte invasiveness: control by voltage-gated Na^+ channel activity. FEBS Lett. 569, 191–194.

Klebanoff, C.A., Khong, H.T., Antony, P.A., Palmer, D.C., Restifo, N.P., 2005. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol. 26, 111–117.

Kuball, J., Schmitz, F.W., Voss, R.-H., Antunes Ferreira, E., Engle, R., Guillaume, P., Strand, S., Romero, P., Huber, C., Sherman, L.A., Theobald, M., 2005. Cooperation of human tumor-reactive CD4+ and CD8+ T cells after redirection of their specificity by a high-affinity p53A2.1-specific TCR. Immunity 22, 117–129.

Lübbe, A.S., Bergemann, C., 1996. Clinical experiences with magnetic drug targeting: a phase i study with 4′-epidoxorubicin in 14 patients with advanced solid tumors. Cancer Res. 56, 4686–4693.

Mitragotri, S., Chambers, E., 2004. International application published under the patent cooperation treaty, International Publication Number WO/032970 A2.

Moghimi, S.M., Szebeni, J., 2003. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein binding properties. Prog. Lipid Res. 42, 463–478.

Rapoport, N., Pitt, W.G., Nelson, J.L., 2003. Drug delivery in polymeric micelles: from in vitro to in vivo. J. Control. Release 91, 85–95.

Rapoport, N., 2004. Combined cancer therapy by micellar-encapsulated drug and ultrasound. Int. J. Pharm. 277, 155–162.

Rejman, J., Oberle, V., Zuhorn, I., Hoekstra, D., 2004. Size-dependent intrnalization of particles via the pathway of clathrin- and caveolae-mediated endocytosis. Biochem. J. 377, 159–169.

Rosenberg, S.A., Dudley, M.E., 2004. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. Colloquium Papers 101, 14639–14645.

Schwartz, M.S., Matuszewski, B.K., 2002. Determination of a peptide—doxorubicin, prostate-specific antigen activated prodrug, and its active metabolites in human plasma using high-performance liquid chromatography with fluorescence detection: stabilization of the peptide prodrug with EDTA. J. Chromatogr. B 780, 171–182.

Steiniger, S.C., Kreuter, J., Khalansky, A.S., Skidan, I.N., Bobruskin, A.I., Smirnova, Z.S., Severin, S.E., Uhl, R., Kock, M., Geiger, K.D., Gelperina, S.E., 2004. Chemotherapy of glioblastoma in rats using doxorubicin-loaded nano-particles. Int. J. Cancer 109, 759–767.

Teissie, J., Eynard, N., Gabriel, B., Rols, M.P., 1999. Electropermeabilization of cell emembranes. Adv. Drug Deliv. Rev. 35, 3–19.

Uchegbu, I.F., Schätzlein, A.G., 2003. Generics manufacturers should exploit drug delivery technologies for improved therapeutics. Bus. Briefing Pharmatech., 203–208.

Wang, H.Y., Wang, R.-F., 2005. Antigen-specific CD4+ regulatory T cells in cancer: implications for immunotherapy. Microbes Infect. 7, 1056–1062.

Win, K.Y., Feng, S.S., 2004. Effects of particle size and surface coating on cellular uptake of poly-meric nanoparticles for oral delivery of anticancer drugs. Biomaterials 26, 2713–2722.