Polybutylcyanoacrylate Nanoparticles and Drugs of the Platinum Family: Last Status

Dashti Rahmat Abadi Fatemeh · Hasan Ebrahimi Shahmabadi · Anita Abedi ·
Seyed Ebrahim Alavi · Fatemeh Movahedi · Maedeh Koohi Moftakhari Esfahani ·
Tahereh Zadeh Mehrizi · Azim Akbarzadeh

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Abstract Cisplatin and carboplatin drugs from platinum-containing family are anti-cancer drugs. Using these drugs causes side effects. Targeted and selective prescription decreases side effects of the drugs. This can be achieved using nanotechnology. In this study, cisplatin and carboplatin drugs were loaded on polybutylcyanoacrylate nanoparticles using emulsion polymerization method. To determine amount of loaded drug onto nanoparticle, spectrophotometry method was used. Evaluation of cytotoxicity of such nanoparticles was performed on MCF-7 cell line using MTT assay. Loading percentage of cisplatin and carboplatin drugs on nanoparticles were estimated 4 and 6 %, respectively. Cytotoxicity survival rate for cisplatin and nanoparticle containing cisplatin at the lowest concentration ($p < 0.01$) (20 μM) were estimated 64 ± 1 and 67 ± 0.5 %, respectively. These values at the highest concentration ($p < 0.01$) (160 μM) were measured 28 ± 0.7 and 31 ± 0.4 %. Additionally for carboplatin and nanoparticles containing carboplatin at the concentration ($p < 0.01$) (20 μM)

amounts were estimated to be 80 ± 0.6 and 84 ± 0.6 %, while at the concentration ($p < 0.01$) (160 μM) were identified to be 44 ± 0.5 and 51 ± 0.2 %, respectively. Probably, due to low level of loading, cytotoxicity of both drugs at nano particle status was decreased in comparison with their standard form.

Keywords Polybutylcyanoacrylate · Drug delivery · Emulsion polymerization · Cisplatin · Carboplatin

Introduction

Platinum-containing drugs are used for treatment of various types of cancer such as head and neck, ovarian and lung [1]. The oldest and most commonly used platinum-containing drug is cis diammine dichloro platinum (II). This drug is activated and positively charged via hydrolysis. This process leads to aqueous and much active compounds. Such compounds are able to form DNA-additional compounds complexes later to cause cell death [1]. Common problems associated with clinical use of cisplatin are cumulative toxicities such as kidney toxicity, auditory toxicity and peripheral neuropathy [2]. Rapid deactivation of the drug binding to plasma and tissue proteins and intrinsic or acquired resistance of a batch of tumor cells, limits the effectiveness of cisplatin therapy [1]. Drug loading on stable hemal carriers changes pharmacokinetics of the drug. This action results in increase of drug accumulation in tumor due to an increase in entrapment and permeability of blood vessels. For more selective delivery of cisplatin to tumors, this drug is used as conjugation of drug-dissolved polymer, colloid nanocarriers like pegylated liposomes, poly aspartic acid-poly ethylene glycol micelles, poly caprolactone-poly

D. R. A. Fatemeh · A. Abedi
Department of Inorganic Chemistry, North Tehran Branch,
Islamic Azad University, Tehran, Iran

H. Ebrahimi Shahmabadi · S. E. Alavi · F. Movahedi ·
M. Koohi Moftakhari Esfahani · A. Akbarzadeh (✉)
Department of Pilot Nanobiotechnology, Pasteur Institute of
Iran, Tehran, Iran
e-mail: azimakbarzadeh1326@gmail.com

T. Zadeh Mehrizi (✉)
Department of Immunology, Faculty of Medicine, Shahid
Beheshti University of Medical Science, Tehran, Iran
e-mail: mehrizi.t@gmail.com

ethylene glycol micelles, poly caprolactone-poly 2-(*N,N*-dimethylamino)ethyl methacrylate micelles [3–9]. No carrier able to deliver cisplatin to tumors effectively is developed yet. It comes from low level of hydrophobicity and lipophilicity of this drug [10]. Polybutylcyanoacrylate (PBCA) nanoparticles (NPs) are ideal carriers for delivery of this drug due to biodegradability and ability to change bio distribution of drugs. Additionally, they are easy to prepare and needless of any sort of purification [11–14]. Firstly, synthesis of such NPs was reported by Couvreur et al. [12]. Afterwards, several studies were reported around molecular weight distribution, toxicity, pharmacokinetics, bio distribution, stability and conjugation of different drugs [11, 15–21].

In this work, we studied the use of this NP as a carrier for two drugs of platinum family, cisplatin and carboplatin.

Materials and Methods

Materials

Butylcyanoacrylate (BCA) monomer was purchased from Evobond® Tong Shen Enterprise Co., Ltd. (Taiwan) while

Synthesis and Characterization of PBCA NPs Containing Cisplatin and Carboplatin

The two drugs were loaded during synthesis of NPs by emulsion polymerization [22]. To summarize in details, this procedure includes adding 1 % BCA monomer to polymerization environment containing 0.01 N chloridric acid, cisplatin/carboplatin (1 mg/ml), polyethylene glycol (0.25 %) and 2 % of 70000 dextran. Polymerization environment was agitated (500 rpm) for 4 h. Then pH was adjusted to 5.5 by NaOH (0.1 N). Agitation was continued for one more hour to form final shape of NPs. Size and Zeta potential of NPs was determined by dynamic light scattering method using Zeta sizer (ZEN 3600, Malvern Instruments Ltd., Worcestershire, UK).

Resultant suspension was centrifuged for 2 h at 4 °C and 21, 500 rpm (GRX- 220 high speed refrigerated centrifuge, TOMY, Saitama, Japan) to separate supernatant. To eliminate not attached drug, rinsing NP with distilled water was repeated for one more time. To determine amount of drug-loaded NPs, the amount of platinum concentration in supernatant was measured using spectrophotometry. It was defined according to [Formula 1](#):

$$\text{Percentage of loading} = \frac{(\text{Initial cisplatin} - \text{cisplatin in supernatant})}{\text{Initial cisplatin}} \times 100 \quad (\text{Formula 1})$$

dextran, chloridric acid and sodium hydroxide were purchased from Merck. MTT, penicillin/streptomycin and cisplatin/carboplatin was purchased from Sigma, Isopropanol from Dr. Mojallali Chemical Complex Co. (Iran), DMEM and FBS from Gibco (US) and poly ethylene glycol (600 Da) from Kimiagaran emrooz Co. (Iran). MCF-7 cells were supplied from Pasteur institute of Iran. In this study, all chemicals and reactants were of analytical grade and the water was used in distilled form.

Methods

Zeta sizer was used to determine the size and scanning electron microscope (SEM) was used to verify the size in nano scale. Loading efficiency was assayed by means of spectrophotometer and cytotoxicity by MTT assay as described below.

In order to lyophilize and store NP, dissolved mannitol (200 mg/ml) (as an agglomeration inhibitor and cold protector) was added to remained sediment equiponderant to used monomer.

NPs containing no drug were prepared in a same way except that no drug was added. To study the shape of the NPs, the scanning electron microscope (XL30, Philips, Netherlands) was used.

MTT Assay for Evaluation of Cytotoxicity Effect of Free Drug and NPs Containing Drug

MTT assay was applied [23] to determine cytotoxicity effect of cisplatin and carboplatin as well as comparing the effect with drug loaded nanocarriers.

MCF-7 cells were cultivated in DMEM with dilution rate of 1×10^4 for each well in a 96-well plate. Culture medium containing 10 % fetal bovine serum and 1 % penicillin/streptomycin was incubated at 37 °C with 10 %

CO₂. After 24 h of cell cultivation and considering cell adhesion, the supernatant was removed. Afterwards identical amounts of cell and concentrations of 0, 20, 40, 80 and 160 $\mu\text{mol/l}$ of cisplatin (in form of free drug and drug loaded on nanocarriers) were treated. After 48 h incubation, 100 μl MTT solution (0.5 mg/ml PBS, pH 7.4) was added to each well and incubation took place for 3 h at 37 °C. Later, MTT solution was removed and 200 μl isopropanol (100 %) was poured in wells in order to dissolve formed formazan crystals. Thereupon absorbance was measured in wavelength of 570 nm using Spectrophotometer (BioTek Instruments, VT, USA). All the experiments were triplex and repeated for three times. Cytotoxicity was calculated as the portion of treated cells absorption to control cells absorption. MTT was also performed for carboplatin and carboplatin loaded nanocarrier in a similar approach.

Results

Ten min after adding BCA monomer, the color of the medium started to be milky which comes from polymerization of the monomer. This color change occurred for both nanocarriers. Size, size distribution and Zeta potential of nanocarriers containing cisplatin were estimated to be 287 nm, 0.21 and -8 mV, respectively while these values were 319 nm, 0.24 and -7.2 mV for nanocarriers containing carboplatin. Scanning electron microscope images, verified formation of nanocarriers for both drugs (Figs. 1, 2).

The resultant images represent separate, spherical NPs evident between mannitol tabular parts. They have a smooth surface and are approximately homogenous. From appearance point of view, there was not any obvious difference between two NPs.

Considering results of drug loading, cisplatin loaded onto the NPs is less than carboplatin. This amount was 4 and 6 % for cisplatin and carboplatin, respectively.

Results of cytotoxicity assay showed that synthesizing NPs not only did not improve cytotoxicity, but also reduced it compared with free drug. In maximum and minimum concentration of cisplatin (160 and 20 μM), cytotoxicity decreased 10 and 9 % in comparison with standard drug. Such a decrease was equal to 20 and 13 % for carboplatin in minimum and maximum concentration.

Furthermore, studies illustrated a higher level of toxicity for cisplatin (both free form and loaded on NP) in comparison with carboplatin (Figs. 3, 4). Both drugs demonstrated higher toxicity in free form. In other words, loading drugs on NPs leads to an enhancement in cell survival (Figs. 5, 6).

Fig. 1 Scanning electron microscope micrograph from PBCA nanocarrier loaded by cisplatin (magnification: 15000 times)

Fig. 2 Scanning electron microscope micrograph from PBCA nanocarrier loaded by carboplatin (magnification: 7500 times)

Discussion

Platinum-containing anticancer drugs are given intravenously and during 1 day, 65–98 % of platinum is bound to proteins in plasma [24, 25]. Binding cisplatin to proteins results in a decrease in renal excretion of platinum and accumulation of it in tissues. It is also assumed that binding cisplatin to proteins leads to auditory and kidney toxicity [26]. Due to this fact, targeted delivery of cisplatin causes the reduction in side effects and enhancement in efficiency [27]. Polymer-based NPs are colloidal polymer

Carbopt-NP: Carboplatinum-loaded NP
Cispt-NP: Cisplatinum-loaded NP

Fig. 3 Comparing cytotoxicity effect of free cisplatin and carboplatin and cisplatinum and carboplatinum loaded onto PBCA nanocarriers on MCF-7 cells after 48 h incubation. Results are presented as mean $\pm$ 5 % error from at least three independent experiments

Fig. 4 Comparing cytotoxicity effect of free cisplatin and carboplatin in equal concentrations on MCF-7 cells after 48 h incubation. Results are presented as mean $\pm$ 5 % error from at least three independent

Fig. 5 Cytotoxicity effect of free cisplatin and cisplatin loaded onto PBCA nanocarriers on MCF-7 cells after 48 h incubation. Results are presented as mean $\pm$ 5 % error from at least three independent experiments

Fig. 6 Cytotoxicity effect of free carboplatinum and carboplatinum loaded onto PBCA nanocarriers on MCF-7 cells after 48 h incubation. Results are presented as mean $\pm$ 5 % error from at least three independent experiments

particles in size of less than micron. The therapeutic product can be embedded, encapsulated or conjugated to their surface. They have been applied in several in vitro studies as appropriate carriers for targeted delivery of biomolecules, drugs, gens and vaccines which leads to enhancing efficiency and reducing side effects of the drug. Poly-alkylcyanoacrylate polymers are member of this group. These polymers are the only ones capable of overcoming the phenomenon of drug resistance [28, 29]. It is also possible to change their surface by surfactants or covalent bond with polyethylene glycol chains to produce hidden NPs [18, 30]. Different drugs such as dalargin and loperamide are loaded on NPs by simple absorption. Additionally, methotrexate or doxorubicin are attached to NPs by adding during the process of polymerization [31, 32].

Moreover, such NPs have two interesting features make them appropriate candidates for drug delivery systems. Firstly, they are more stable in in vitro conditions compared with several biodegradable polymers however they decompose rapidly in in vivo conditions. Such a feature makes it possible to store them in laboratory and control their release in in vivo conditions. Secondly, they can be synthesized by different methods. Surface properties can be modified in a way to control interaction of NPs with living structures which improves in vivo control [33].

In this study, we synthesized PBCA NPs containing cisplatinum and carboplatinum. As far as we know, there is just one study on loading cisplatinum onto such carriers and nothing was found for carboplatinum [34]. So we have reported this for the first time.

On the other hand, loading efficiency was around 5 % which is in accordance with Egea MAs reported value however in this work the diameter of cisplatinum NP was 20 nm larger [34].

In this work, produced NPs had a high quality considering size and size distribution. It was recognized that in same concentrations, cisplatin had a higher level of toxicity compared with carboplatin in both free and loaded on nanocarrier form which was reported for free form previously [35]. Both drugs had less cytotoxicity in NP form probably due to low level of loading which was more evident for NPs containing carboplatin.

Loading level of carboplatin was identified to be higher than cisplatin presumably because of solubility which is less for cisplatin. In spite of low level of loading, such NPs can remain hidden due to preparation in presence of polyethylene glycol. This results in existence of NPs in the blood for a longer time and subsequently higher efficiency. On the other hand, since NPs are used for tumor therapy and due to special properties of tumor (EPR effect), drug accumulation in loaded form is 10–100 times more than free form [36]. Mentioned points can compensate low level of loading and cause increase in *in vivo* drug efficiency.

Totally, results of this work illustrated that emulsion polymerization cannot be a good choice for loading these drugs onto PBCA NPs and it would be essential to examine other methods such as miniemulsion polymerization.

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