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Journal of Controlled Release 102 (2005) 235–245

journal of controlled release

www.elsevier.com/locate/jconrel

Interactions of carboplatin with fibrin(ogen), implications for local slow release chemotherapy

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Received 1 August 2004; accepted 31 August 2004
Available online 11 November 2004

Abstract

The effect of carboplatin (CPt) on fibrin(ogen) clot formation and the possible use of this combination for local slow release chemotherapy were examined. CPt significantly reduced thrombin-induced fibrin clotting time (CT) and increased clot turbidity in a concentration-dependent manner. When CPt was mixed with physiological levels of fibrinogen (>1 mg/ml), electron-dense nanoparticles (3 nm) were formed, as demonstrated by both optical particle counter and transmission electron microscopy (TEM). Upon thrombin-induced coagulation, the CPt nanoparticles were trapped within the fibrin mesh. At higher fibrinogen levels (>5 mg/ml), the 3-nm CPt nanoparticles aggregated, so that ~2% and ~0.5% of the CPt on the fibrinogen appeared as larger particles of 10 and 50 nm, respectively. Dialysis experiments showed that 60–70% of the CPt was released from the fibrin clot within one hour as a non-particulate soluble form, while ~30% of particulate CPt were retained. Up to 5 mg/ml this portion of firmly attached CPt was dependent of the initial drug level. CPt released from the fibrin by either diffusion or by fibrinolysis exhibited cytotoxic activity towards retinoblastoma (RB) cell lines (Y-79 and Weri RB1) equivalent to free drug. Our study indicates that CPt enhances fibrin clot formation and suggests the use of fibrin with high dose CPt for slow release chemotherapy against localized tumors such as retinoblastoma.

Keywords: Carboplatin; Nanoparticles; Fibrin(ogen); Release kinetics; Clot turbidity; Chemotherapy

1. Background

The interaction of drugs with plasma proteins is of great interest in evaluating the drug activity in vivo, its pharmacokinetics and possible side effects of systemic intravenous administration. The possible interactions of CPt with plasma fibrin(ogen) at any

Abbreviations: CPt, carboplatin; Fibrin(ogen), fibrinogen and/or fibrin; CisPt, cis-platinum; RB, retinoblastoma.

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doi:10.1016/j.jconrel.2004.08.029

concentrations have not been previously reported. This issue may have implications on the cytotoxic effects of the drug by systemic administration. The interactions of the CPt with fibrin(ogen) would also establish the possible use of higher concentrations of fibrin sealant for slow delivery of CPt to treat localized tumors.

Besides the interest in the physiological effects of CPt on coagulation, fibrinogen and thrombin, as blood plasma products, can be used as fibrin sealants for different clinical applications, including drug delivery. For example, antibiotics have been combined with fibrin sealants for slow drug release in various types of in vitro and wound healing situations [1–9] and in ocular surgeries [10].

The family of Pt based drugs applied in clinical practice is composed mainly from Cis-platinum (cis-Pt) and carboplatin (CPt). Both drugs are highly cytotoxic for normal tissues, which limit their dose used in treating cancer patients with solid tumors [11–13]. Therefore, if the drug can get incorporated in the fibrin clot without interfering with the coagulation process it could be used for local slow release and drug delivery of high-dose chemotherapy to the tumor area.

The most common Pt-based drug, cisplatin (cisPt), is chemically reactive. It hydrolyzes rapidly and covalently binds to many proteins through the free SH or SS moieties [13–15]. Moreover, it was demonstrated that cisPt interacted strongly with SS disulfide bridges in fibrinogen and opened more than 4 (out of 29) such linkages. This resulted in conformational changes and reduced fibrin clot turbidity [16]. Such high reactivity may underlie some of the clinical side effects of cisPt treatments, which include dose- and time-dependent reduction of platelet reactivity, increased plasma viscosity associated with deep vein thrombosis [17,18] possibly leading to further complications such as renal failure [19].

Carboplatin (CPt) is considered to be clinically milder as it induces less severe side effects [12,13]. Besides its efficacy for the systemic treatment for wide range of malignant diseases, there were attempts to use CPt for the treatment of localized tumors such as retinoblastoma (RB), both by direct local administration and by systemic treatment [20–22]. The efficacy of systemic chemotherapy for the treatment of RB was relatively poor because targeting and penetration of the drugs into the eyeball was limited. It was proposed to inject locally high drug doses into the conjunctiva or eye vitreous in order to reach high local doses with minimal systemic toxicity [23–25]. Nevertheless, such treatments had limited success due to the rapid clearance of the drug. Therefore, it was suggested that a carrier such as fibrin sealant might enable such treatments with slow drug release [26].

In the current study, the interactions of high dose CPt with fibrin(ogen) with or without the thrombin were examined, both in terms of coagulation and as a tool for slow drug release. In combination of CPt with high fibrin concentration, the rate drug release and the mechanism of interaction of CPt with fibrin(ogen) were studied. The cytotoxicity of the slowly released CPt was assayed in vitro with RB-WERI retinoblastoma cell line as model for cells of localized ophthalmologic tumors.

2. Methods and materials

2.1. Reagents and CPt

CPt (Paraplatin) in concentration of 10 mg/ml was purchased from Bristol–Myers Squibb (Cermoneta, Italy). Human fibrinogen and thrombin were obtained from the New York Blood Center and Vitex (New York, NY). Fibrinogen samples were also supplied from the Scottish Nation Blood Transfusion Services (Edinburgh, UK) Baxter Immuno (Vienna) and Omrix (Tel-Hashomer, Israel).

2.2. Clotting-time measurement

The interaction of CPt with fibrinogen was tested in small disposable cuvettes by adding CPt to either low or high fibrinogen concentrations (3.8 or 40 mg/ml), without or with CPt in final reaction volume of 200 μl in PBS in pH 7.2. To activate the fibrinogen, a small aliquot of thrombin was added to reach a final concentration of 0.5 U/ml with 4 mM Ca^{2+} and the clotting time (CT) was determined manually by the “tilt tube” method (4 repetitions per point) similar to a method that was previously described [27].