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Influence of the formulation on the tolerance profile of nanoparticle-bound doxorubicin in healthy rats: Focus on cardio- and testicular toxicity

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Abstract

A toxicological study of doxorubicin bound to poly(butyl cyanoacrylate) or human serum albumin nanoparticles coated with polysorbate 80 was performed in healthy rats. The doxorubicin formulations were injected at a therapeutic dose regimen (3 × 1.5 mg/kg with a 72 h interval), and the animals were followed up for 15 or 30 days. The overall result of this study suggests that the surfactant-coated nanoparticle formulations of doxorubicin have favorable toxicological profiles. Specifically, these formulations display a considerably reduced cardio- and testicular toxicity, as compared to a free drug.

Keywords: Cardiotoxicity; Doxorubicin; Human serum albumin; Nanoparticles; Poly(butyl cyanoacrylate); Polysorbate 80; Rat; Testicular toxicity

1. Introduction

The anthracycline antibiotic doxorubicin is one of the most effective anticancer drugs, which is extensively used for the treatment of many neoplastic diseases. However, its clinical usefulness is often limited by severe adverse side effects, the most important of which are cardiomyopathy and congestive heart failure (Singal et al., 2000; Minotti et al., 2004). Over the past decades, substantial efforts have been directed towards better understanding of the mechanisms of activity and toxicity of doxorubicin and other anthracyclines and to the improvement of their therapeutic index.

Several strategies have been used, the search for a "better anthracycline" being first in the list. Indeed, nearly 2000 analogs were synthesized and evaluated; yet only few of them have reached the stage of clinical development and approval. Second generation analogs like mitoxantrone, epirubicin or idarubicin exhibit a decreased cardiotoxicity; however, these agents were not able to totally alleviate the risk of inducing cardiomyopathy (Anderlini et al., 1995; Ryberg et al., 1998; Gonsette, 2004).

Another approach attempts to counteract the destructive action of free radicals (reactive oxygen species) generated by the anthracycline molecule upon deglycosylation and intercalation of the aglycones into biologic membranes (Zucchi and Danesi, 2003; Minotti et al., 2004). Free radical generation also may occur via non-enzymatic pathways and is mediated by doxorubicin complexes with intracellular iron, which can reduce molecular oxygen, producing a burst of reactive oxygen species (Myers, 1998). Hence, this approach involves application of adjuvant cardioprotective therapy using agents that could defend cardiomyocytes against anthracycline-derived reactive oxygen species, such as antioxidants (i.e. thiol containing agents, piperidine nitroxides or vitamins A, E, and C) or iron chelators (i.e. dexrazoxane) (Hasinoff et al., 2003). However, the cardioprotective efficacy of these agents is often quite limited.

Further, it has been shown that the severity of doxorubicin-induced cardiotoxicity is directly correlated to the peak drug

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concentration in the heart and plasma rather than to plasma AUC (Danesi et al., 2002). Indeed, safety and efficacy of doxorubicin could be improved by replacing bolus administration with slow infusions, which correlated with the pharmacokinetic findings demonstrating that both plasma and left ventricular peak concentrations of doxorubicin and its active metabolite doxorubicinol were significantly lower after slow infusion than after bolus dosing (Cusack et al., 1993). Accordingly, a number of strategies are focused on the optimization of the drug biodistribution. One way to solve this problem is the development of the drug delivery systems that enhance distribution of anthracyclines within tumors, whereas exposure of healthy tissues to potentially toxic levels of the drug is decreased. Thus, liposomal formulations considerably improved the pharmacokinetics and therapeutic index of doxorubicin (Safra et al., 2000; Batist et al., 2001).

While liposomes are the most advanced example of this strategy, the nanoparticle-based delivery systems represent a promising alternative which enables broadening of the spectrum of drug activity. Thus, binding of doxorubicin to poly(alkyl cyanoacrylate) nanoparticles allowed overcoming of the multidrug resistance (Cuvier et al., 1992; Colin de Verdiere et al., 1997). Furthermore, recent studies suggest that the nanoparticle-based formulation of doxorubicin has a potential for the systemic chemotherapy of brain tumors, whereas the efficacy of free doxorubicin against this type of tumors is restricted due to the drug inability to cross the blood–brain barrier. Indeed, polysorbate 80-coated poly(butyl cyanoacrylate) (PBCA) nanoparticles enabled the brain delivery of doxorubicin after intravenous administration (Gulyaev et al., 1999) and yielded a high anti-tumoral effect against intracranial glioblastoma in rats (Steiniger et al., 2004).

The toxicological study of the above formulation revealed that binding of doxorubicin to the nanoparticles did not increase its acute toxicity (Gelperina et al., 2002). Moreover, the pharmacokinetic studies demonstrated that after intravenous injection of doxorubicin bound to polysorbate 80-coated PBCA nanoparticles the cardiac concentrations were significantly lower, as compared to the drug in solution (Gulyaev et al., 1999).

Another promising carrier system is based on human serum albumin (HSA) nanoparticles. Albumin is the most ubiquitous protein in the human body and is a natural carrier for many substances. HSA-nanoparticles represent a versatile colloidal drug carrier system with high drug loading capacity in combination with biodegradability and biocompatibility. The results of the recent studies of paclitaxel bound to albumin nanoparticles demonstrated a favorable safety profile of this formulation (Gradishar et al., 2005).

The objective of the present study was to gain further insight into the toxicological properties of the PBCA- and HSA-based nanoparticulate formulations of doxorubicin in a rat model, which appears to be most adequate among animal models for characterization of anthracycline-related toxicity (Mazue et al., 1995). The study was focused on the evaluation of cardiotoxicity and testicular toxicity, which is another important yet unabated adverse effect of doxorubicin.

2. Materials and methods

2.1. Chemicals

n-Butyl-2-cyanoacrylate (Sicomet® 6000) was obtained from Sichel–Werke (Hannover, Germany). Human serum albumin (HSA, fraction V, purity 96–99%, 65,000 Da), glutaraldehyde 8% solution, and dextran 70,000 were obtained from Sigma (Steinheim, Germany). Polysorbate 80 (Tween® 80) was supplied by ICI Chemicals (Essen, Germany); doxorubicin was from Sicor (Rho, Italy). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Preparation and characterization of nanoparticles

2.2.1. Preparation of doxorubicin-loaded PBCA nanoparticles

Doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles (Dox-PBCA) were manufactured by anionic polymerization (Couvreur et al., 1979; Kreuter, 1983). One percentage of *n*-butyl-2-cyanoacrylate was added to a 1% dextran solution in 0.01N HCl under constant stirring. After 40 min, doxorubicin was added to the mixture to obtain a final doxorubicin concentration of 0.25%. Stirring was continued for 2.5 h, and then the polymerization was completed by neutralization with 0.1N NaOH. The mixture was filtered through a G1 sintered glass filter (Schott, Mainz, Germany) and lyophilized; 3% (w/v) mannitol was used as a cryoprotector.

2.2.2. Characterization of doxorubicin-loaded PBCA nanoparticles

The particle size and polydispersity of the size distribution were measured by photon correlation spectroscopy (PCS) using a Malvern zetasizer 3000HSA (Malvern Instruments Ltd., Malvern, UK). The samples were diluted with purified water and measured at a temperature of 25 °C at a scattering angle of 90°. An average particle diameter of 240 ± 40 nm was found. The average surface charge was -13.0 ± 2.1 mV.

The drug content was measured spectrophotometrically after dissolution of the freeze-dried formulation in DMSO. The percentage of the nanoparticle-bound drug was calculated as the difference between the drug content and amount of the free drug after its separation by ultrafiltration of the redispersed formulation, as follows: 400 μl of the suspension were centrifuged for 15 min at $16{,}100 \times g$ in centrifugal membrane filter devices (Microcon 30 kDa, Millipore, USA). The concentration of the free drug in the filtrate was assessed by spectrophotometry (480 nm). It was shown that $56 \pm 1.2\%$ of the drug in suspension was associated with the nanoparticles. The loading capacity was 137.5 ± 3.5 μg Dox per 1 mg PBCA.

Empty PBCA-NP were synthesized using the same technique and had an average diameter of 250 ± 30 nm. The average surface charge was -17.0 ± 2.5 mV.