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Enhanced solubility and intestinal absorption of cisplatin by coating with nano-hydroxyapatite

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ABSTRACT

We examined oral administration of the poorly water-soluble anti-cancer drug cisplatin (CDDP), using a novel formulation prepared by coating CDDP with nano-hydroxyapatite (CDDP/HAP formulation). Solubility testing showed that dissolution of CDDP from the formulation after 6 h was about 2.5 times that of untreated CDDP raw material. In single-dose oral administration testing in rats, the blood concentration of CDDP as shown by the area under the blood concentration time curve (AUC(0–24)) in the CDDP/HAP formulation group was about 7 times that in a CDDP group at the same dose and AUC(0–72) values for the CDDP/HAP formulation group were concentration-dependent. The AUC(0–72) value for the formulation group was lower at the same dose but almost 1.5 times higher at double the dose compared with rats treated intravenously with CDDP. Growth inhibition of cancer cells was no less when using the CDDP/HAP formulation than with CDDP, and in Lewis lung carcinoma-inoculated mice, the life span of those treated orally with CDDP/HAP formulation was equivalent to that of mice treated intravenously with CDDP. Our results suggest that the formulation using nano-HAP may provide an oral substitute for intravenous infusion of CDDP in cancer patients, so that patient quality of life may be improved.

1. Introduction

Oral administration of drugs is the most desirable route because it is convenient and maintains high quality of life (QOL). However, the drug needs to be soluble and capable of permeating in the intestinal cavity for it to be transported into the bloodstream. It is reported that 40% of commercial drugs [1] and 70% of novel compounds [2] are classified into class II or class IV of the Biopharmaceutical Classification System [3], i.e. have problems of poor solubility and/or permeability. These drugs have limited bioavailability and show inter-individual variability and lack of dose linearity [4,5], and they are not suitable for oral delivery. Drugs with high antitumor activity in particular show a similar tendency.

Cisplatin (CDDP) was first synthesized as an experimental Pt-complex material in 1845. It has a high antitumor effect for many types of cancer [6,7]. CDDP has been the drug of choice for several cancers since its approval for clinical use in the USA in 1978 [8], but must be delivered by intravenous infusion with massive fluid replacement over a few hours because of its nephrotoxicity [9]. Thus there is a problem with QOL in the case of intravenous infusion. There have been two studies reported concerning oral administration of CDDP, one study involving a CDDP-loaded solid dispersion in rats [10], and another involving oral or intravenous CDDP delivery in 32 human patients [11], which examined only the pharmacokinetics of CDDP. But no oral formulation of CDDP is commercially available.

In an earlier study, we reported that the dissolution and intestinal absorption of bezafibrate were improved and the toxicity was reduced by coating the surface of bezafibrate with nano-hydroxyapatite (nano-HAP) [12]. Hydroxyapatite, a calcium phosphate compound, is the main mineral component of bones and teeth, and is used widely in medical and dental applications including toothpaste, orthopedic implants and artificial bone. Because of its excellent biocompatibility and high adsorption capability for protein [13] and for many drugs [14,15], HAP has been widely studied as a potential slow-release carrier for therapeutic agents including proteins, anti-cancer drugs [16] and antibiotics [17]. In the present research using CDDP, we demonstrate for the first time that both drug solubility and intestinal absorption can be enhanced by the use of a nano-HAP coating, suggesting that QOL could be improved by the oral administration of CDDP using our formulation.

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2. Materials and methods

2.1. Materials

All chemicals, materials and equipment were purchased from Japanese suppliers. CDDP was obtained from Medical & Biological Laboratories Co., Ltd, and disintegration test solution 2 and platinum standard solution from Kanto Chemical Co., Inc. Hydrochloric acid, nitric acid, sodium hydroxide, diethyl ether, diethyl phthalate, PBS(−), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), cellulose acetate phthalate, dimethyl sulfoxide (DMSO) and fetal bovine serum were purchased from Wako Pure Chemical Industries, and Dulbecco's modified eagle's medium (DMEM) from Nihon Pharmaceutical Co., Ltd. Somnopentyl® was obtained from Kyoritsu Seiyaku Co., Novo-Heparin® from Mochida Pharmaceutical Co., Ltd, and Medium 199 from Lonza Japan Ltd.

2.2. Preparation of nano-HAP

To prepare the nano-HAP used in this study, micron-level HAP (SKM-1, Sangi Co., Ltd) was mixed at 20% concentration in distilled water. The suspension was ground with a wet grinding mill (DYNO-MILL KDLA, Shinmaru Enterprises Co.), using zirconia beads 0.3 mm in diameter. The primary particle size of the resulting powder obtained after drying in an evaporator was confirmed to be nano-scale by scanning electron microscope (SEM, S-4500, Hitachi Ltd.). The particle size was further assessed, after resuspending as a 3% solution in distilled water, by dynamic light scattering measurement (Microtrac 9340UPA, Nikkiso Co., Ltd.).

2.3. Preparation of formulations

Preparation of the CDDP/HAP formulation was done by mechanical fusion (Mechanofusion AMS-Mini, Hosokawa Micron Group) at 2000 rpm for 15 min. A weight ratio of 1:2 (CDDP:HAP) was used as it was found that at this ratio the surface of CDDP was completely coated with nano-HAP when observed by electron microscope (Fig. 1). The chamber was cooled with a cooling water circulation apparatus to 0 °C and the inside of the chamber purged with argon gas (G3 grade) at 3 L/min during mechanical fusion. A physical mixture of CDDP and nano-HAP used as a control in this study was prepared by mixing CDDP and nano-HAP in a plastic bag at a weight ratio of 1:2. For enteric use in animal studies, to avoid the dissolution of HAP by stomach acids, the CDDP/HAP formulation was subjected to enteric coating in a coating pan, using a coating solution prepared by mixing acetone, cellulose acetate phthalate and diethyl phthalate at a weight ratio of 255:9:36.

2.4. Observation and measurement of formulations

The surface shapes of CDDP, the physical mixture of CDDP and nano-HAP, and CDDP/HAP formulation were observed by SEM (S-4500). To more closely observe the nature of the CDDP/HAP formulation, preparation of a cross section using a cross-section polisher (SM-09010, JASCO Corporation), and its observation by SEM (JSM-741F, JASCO Corporation) were outsourced to the Hosokawa Micron Corporation. The acceleration voltage was 15.0 kV. The crystalline forms of CDDP, the physical mixture, CDDP/HAP formulation and nano-HAP were also measured by powder X-ray diffraction (Empyrean, Spectris Co., Ltd.). The measurement was performed at 45 kV and 40 mA, a scanning angle of 10–40°, and a scanning speed of 4°/min.

2.5. Solubility test

Solubility testing was carried out using distilled water and disintegration test solution 2 (phosphate buffer solution; pH 6.8) as test media. Each test was repeated 12 times. We did not use disintegration test solution 1 (phosphate buffer solution; pH 1.2) because the CDDP/HAP formulation in this study was an enteric coated preparation. Fifty milliliters of each test medium was put in a glass tube and stirred with a 15 mm teflon stir bar at a constant rotation speed of 120 rpm. Respectively, 1.0 g of CDDP, 3.0 g of the physical mixture of CDDP and nano-HAP (containing 1.0 g of CDDP), and 3.0 g of CDDP/HAP formulation (containing 1.0 g of CDDP) were added to each test medium. One milliliter samples were collected into a microtube at 1, 3, 10, 30, 60, 180, and 360 min after the start of the test, which was performed in an incubator at 37 ± 0.5 °C. Each sample was centrifuged for 10 min at 12,000 rpm, and the supernatant was frozen and freeze-dried. The dry sample was then dissolved in 1.0 mL of aqua regia (10%) and the resulting solution diluted with distilled water. The platinum concentration of the sample was determined by inductively coupled plasma atomic emission spectroscopy (ICP) (SPS-1700R, SII NanoTechnology Inc.). Samples were quantified by comparing with authentic standards and the amount of dissolved CDDP was calculated from the determined platinum concentration.

2.6. Pharmacokinetic study after single-dose oral administration

Kwl:SD male rats (7 weeks; Saitama Experimental Animals Supply Co. Ltd.) were used in a pharmacokinetics study involving single-dose oral administration. Testing was performed using, respectively, CDDP, the physical mixture of CDDP and nano-HAP, and CDDP/HAP formulation. The dose of CDDP in each case was 30 mg/kg, so that for the physical mixture and CDDP/HAP formulation 90 mg/kg (corresponding to CDDP 30 mg/kg) was administered. Rats in each case were fasted for 16 h before oral administration. CDDP was completely dissolved in 5 mL water and administered by oral gavage using a sonde. The other preparations were suspended in 5 mL water and administered in the same way. A blood sample of about 500 μL was collected from each rat's tail vein at 0.5, 1.0, 3.0, 6.0 and 24.0 h after administration. The sample was diluted with the same amount of 0.1 mol/L sodium hydroxide and stood at room temperature for 1 h. The platinum concentration of the sample was determined by ICP as described above and the CDDP concentration in the blood was calculated from it. Each group consisted of five rats. All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Sangi Co., Ltd.

2.7. Comparison of intravenous infusion and oral administration

Kwl:SD male rats (7 weeks) were used in a study to compare intravenous infusion and oral administration. The dose of CDDP used for intravenous infusion was 1.25 mg/kg. The required amount, for each animal, was completely dissolved in 15 mL of sterilized physiological saline. To avoid the agglutination of blood, Novo-Heparin® was added to the physiological saline in the ratio of 1 unit/mL. Rats were anesthetized with Somnopentyl® and a Surflo® indwelling needle (Terumo Corporation) was inserted into the femoral vein. The CDDP solution was administered for 5 h with a peristaltic pump (PST-100, AGC Techno Glass Co., Ltd.), and the indwelling needle was then removed. Oral administration of CDDP/HAP formulation was then performed to compare with the intravenous infusion of CDDP. The doses of CDDP/HAP formulation were 3.75, 7.5, 15 and 30 mg/kg (corresponding to CDDP 1.25, 2.5, 5 and 10 mg/kg respectively). After both types of administration, rats were moved to a plastic cage and fed ad libitum. A blood sample of about 500 μL was collected from