Cellular Therapy and Transplantation (CTT). Vol. 10, No. 3-4, 2021
doi: 10.18620/ctt-1866-8836-2021-10-3-4-71-77
Submitted: 18 August 2021, accepted: 12 November 2021

Dextran sulfate coated $CaCO_3$ vaterites as the systems for regional administration of doxorubicin to rats

Natalia N. Sudareva [1,2], Olga M. Suvorova [1], Dmitry N. Suslov [3], Oleg V. Galibin [2], Aleksandr D. Vilesov [1,2]

[1] Institute of Macromolecular Compounds RAS, St. Petersburg, Russia
[2] Pavlov University, St. Petersburg, Russia
[3] Granov Russian Research Center of Radiology and Surgical Technologies, St. Petersburg, Russia

Dr. Natalia N. Sudareva, Institute of Macromolecular Compounds RAS, St. Petersburg, Russia
E-mail: nnsas@mail.ru

Citation: Sudareva NN, Suvorova OM, Suslov DN et al. Dextran sulfate coated $CaCO_3$ vaterites as the systems for regional administration of doxorubicin to rats. Cell Ther Transplant 2021; 10(3-4): 71-77.

Summary

Doxorubicin (DOX) is a water-soluble anthracycline antibiotic possessing high anti-cancer activity. It is possible to achieve decrease in DOX concentration in blood (below the cardiotoxic level) during therapy by forming a depot containing DOX delivery systems that provide prolonged release of the drug. To this purpose, porous calcium carbonate particles (vaterites) coated with polyanion (dextran sulfate) were used. Due to submicron sizes of carriers, they do not freely enter bloodstream. The toxic drug is distributed in the organism only after entering the blood due to release from the delivery systems. Upon intraperitoneal administration of the DOX-containing delivery systems to rats inoculated with Seidel hepatoma, an efficient DOX concentration has been achieved which inhibited tumor growth and reduced the amount of ascitic fluid. Time profiles of DOX release into bloodstream of healthy rats were studied by HPLC after intraperitoneal administration of 4 mg of DOX, using various delivery systems. The drug injected in the form of dextran sulfate coated submicron carbonate cores was released within two weeks, and its concentrations were under the toxicity levels. When the nano-sized DexS+DOX conjugate was used for the drug delivery, DOX was found in rat blood at significantly higher concentrations. Irrespective of drug concentration in plasma, the results of physical examination and autopsy of rats performed on day 21 after intraperitoneal administration of DOX by various delivery systems indicated the absence of any negative reactions in animals.

Keywords

Doxorubicin, drug delivery system, $CaCO_3$, dextran sulfate, polymer-drug conjugate, blood plasma.

Introduction

Doxorubicin is a very potent anticancer antibiotic that, unfortunately, demonstrates considerable cardiotoxicity, accumulation in liver and rapid clearance [1]. Use of various delivery systems for DOX in chemotherapy makes it possible to decrease negative influence of the drug and provides its prolonged release. In our previous work [2], it has been shown that porous carbonate vaterites modified with various polyanions can be successfully applied for encapsulation of DOX and facilitate prolonged *in vitro* release of the substance into blood plasma. Besides, it has been proven that these delivery systems (DS) are bio-resorbable and safe to use [3, 4]. The advantages of these DS in chemotherapy are related to some peculiar features of defense mechanisms of tumor cells against chemotherapeutic compounds. Drug efflux is among these mechanisms which does not affect DS (unlike free DOX). Phagocytosis of drugs by a cell is preceded by opsonization. Mechanism of opsonization (sorption of various plasma components on surface of delivery system) is impaired when a DOX-containing DS is coated with polyelectrolytes. As a result, opsonization is reduced, and the time of passive DS circulation is prolonged in the area of high capillary permeability and disturbed lymphatic drainage (typical of tumor tissues [5]); i.e., the drug is retained in the tumor tissue.

Among other delivery systems for DOX, polymer-drug conjugates (PDC) have been described [6]. In our earlier work [2], the profiles of DOX release into blood plasma have been compared for PDC (DexS+DOX) *versus* DexS coated $CaCO_3$ cores.

Ascitic tumors (particularly, Seidel hepatoma, SH) are widely used as models for *in vivo* studies of regional intraperitoneal chemotherapy. These tumors are included into the list recommended by the Russian Ministry of Health and the State Pharmacological Committee for studies of antitumor mechanisms [7].

Revtovich et al. [8] compared antitumor activity of cisplatin and its prolonged pharmaceutical form based on dextran phosphate hydrogel in the rats inoculated with Seidel hepatoma. They have demonstrated higher efficiency of the modified drug.

When free DOX or DOX-containing delivery systems are used in chemotherapy, it is important to control concentration of the substance in blood. Administration of free DOX results in its distribution through all the organs and tissues. When delivery systems are used, distribution of the drug in organism largely depends on DS structure (i.e., drug release rate) and its administration route (intravenous, intraperitoneal, subcutaneous, etc. [9]). The amount of introduced drug and physiology of an organism are also of importance.

Comparing the literature data on DOX concentration in blood plasma after intravenous administration to different tumor-bearing mice and women, we can see that the amount of introduced DOX exerts relatively low influence on its content in plasma, whereas tumor type is a considerable factor [10-13].

The aims of the present work included (*i*) studies of the opportunity to use the proposed delivery model of antitumor preparation in laboratory animals; (*ii*) time course evaluation of DOX release into blood following intraperitoneal administration, using drug delivery systems based on $CaCO_3$ cores coated with DexS, or DexS-DOX conjugates (HPLC studies of DOX release); (*iii*) comparison between the profiles of DOX release into rat blood (both in healthy rats and animals with Seidel hepatoma), and general condition of animals, thus discerning effects of the released DOX amounts upon malignant cells in tumor-bearing animals and upon intact rats.

Materials and methods

Reagents

Doxorubicin hydrochloride purchased as the "Sindroxocin" preparation, containing 17% of doxorubicin (DOX) and 83% of lactose, was from Actavis (Hafnarfjordur, Iceland). For experiments, doxorubicin salt with protonated amino group (–$NH3^+$) was used. Salts ($CaCl_2 \times 2H_2O$, Na_2CO_3), acetone, and dextran sulfate (M_w = 9-20 kDa) were purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis of carbonate cores

Preparation techniques for porous carbonate vaterites, methods for coating vaterites with DexS polyanion and introducing DOX into these carriers are described in [14]. Briefly, porous vaterites ($CaCO_3$ cores) were prepared by co-precipitation. Equal volumes of 1 M aqueous solutions of $CaCl_2 \times 2H_2O$ and Na_2CO_3 were rapidly mixed at stirring (1000 rpm) with an RW 20 anchor-type mechanical stirrer (Kika-Werk, Switzerland). The mixture was stirred for 30 s. The suspension was then filtered through Schott filter glass (#16), washed thrice with distilled water and with acetone/water mixtures with increasing acetone concentrations (33%, 50%, and 100%). The precipitate was dried in thermostat at 40-50°C until a constant weight was achieved.

Doping of carbonate cores with DexS and DOX loading into delivery systems

The cores were coated with a polyanion (sodium salt of dextran sulfate, DexS). $CaCO_3$ cores (50 mg) were added to 1 mg/mL aqueous solution of DexS (10 mL). The suspension was stirred using a Multi Bio RS-24 rotor (Biosan, Latvia) for 1 h; the solid fraction was filtered off using a Schott glass filter (#16), washed thrice with distilled water and dried at 30°C.

DOX was loaded into doped $CaCO_3$ under continuous stirring of the mixture of $CaCO_3$ suspension and DOX solution (C=2 mg/mL) for 24 hours. The DOX/($CaCO_3$+DexS) ratio was 0.4. After mixing, the suspension was centrifuged at 8000 rpm for 3 min, and the DOX amount in supernatant was determined.

DOX load (L) was calculated using the following equation: $L = (m_i - m_s)/m_P$, where m_i is the initial amount of DOX (mg), m_s is the amount of non-encapsulated DOX in supernatant solution (mg), m_P is the amount of particles (mg).

DOX concentrations were determined using the calibration curves obtained from optical density measurements in the corresponding solvents at λ=480 nm. The measurements were carried out using a SF-2000 spectrophotometer (LOMO, St. Petersburg, Russia).

Polymer-drug conjugates and their loading

Polymer-drug conjugates (DexS+DOX) were prepared according to the technique described in [2]. The DOX solution (2 mg/mL) and solution of DexS polyanion (1 mg/mL) were mixed in volumes that provided an equimolar ratio of functional groups. Then the mixture was subjected to ultrasound treatment for 2 min and centrifuged at 8000 rpm for 10 min. The supernatant was removed, the residue was freeze-dried. The DOX concentration in supernatant was determined spectrophotometrically. The PDC load was determined by subtracting the amount of DOX in supernatant from total amount of introduced DOX followed by dividing this quantity by the weight of the residue. All the measurements were carried out thrice.

In vivo experiments with rats

Two groups of rats were used in the experiments with intraperitoneal administration of various DOX delivery systems. The first group (experimental) included the rats with inoculated Seidel hepatoma; DOX encapsulated into calcium carbonate cores doped with DexS polyanion was administrated intraperitoneally (i.p.). The second group consisted of healthy rats; they were treated i.p. with DOX incorporated

into different delivery systems: (*i*) submicron-sized calcium carbonate cores coated with DexS polyanion ($CaCO_3$+DexS); (*ii*) nanosized polymer-drug conjugates (DexS+DOX). Two reference groups were also included, i.e., the first reference group consisted of non-treated animals with inoculated Seidel hepatoma (no DOX included in the $CaCO_3$+DexS delivery system was introduced). The second reference group consisted of healthy rats that were treated with free DOX (without delivery systems).

Thirty-six male and female outbred rats with body weight ranging from 256 to 312 g were used in the experiments ("Rappolovo" nursery for laboratory animals). All manipulations with animals were performed under general anesthesia: Sol. Zoletil 50 (0.05 mL *per* 0.1 kg of body mass), Sol. Rometarum 20 mg/mL (0.0125 mL *per* 0.1 kg of body mass, intramuscularly). The animals were caged (2-5 individuals in a cage), had free access to water and food (4R F18 prolonged keeping formula for rodents, Macedonia, Italy). The animals were fed the standard diet for laboratory rats used in the vivarium of A. M. Granov Russian Research Center of Radiology and Surgical Technologies, St. Petersburg, Russia.

The animals of experimental and reference groups were examined daily; consumption of water and food was registered, body temperature and weight were measured. Behavior of animals and life expectancy were estimated. Immediately after death, ascitic fluid was collected, and its volume was determined. All the manipulations with animals were performed in accordance with State Standard 33216-2014 "Regulations for work with laboratory rodents and rabbits".

Transplantation of ascitic Seidel hepatoma (SH)

Resuspended cells of ascitic Seidel hepatoma (freshly defrosted and washed from dimethyl sulfoxide) were injected into abdominal cavity of rats from the 1st group. Ascitic fluid containing Seidel hepatoma cells (1 mL) was introduced intraperitoneally using a needle (21 G). The dosage was calculated using the Freireich quotient [15].

Introduction of DOX into rats

Doxorubicin (both free and encapsulated in delivery systems) was administered to anaesthesized animals using Zoletil 50, 0.05 mL per 0.1 kg of body mass i.p.). The drug was injected in 1.5 mL of 5% glucose solution, containing DOX in $CaCO_3$+DexS cores, or DOX in polymer drug conjugates (DexS+DOX), or free DOX (2 or 4 mg of DOX *per* 1 animal of the 1st and 2nd groups, respectively). The drug preparations were injected by means of 21-gauge needles. For the animals of the 1st experimental group, the tumor cells and DOX-containing delivery systems were applied simultaneously. Examination of animals from the 1st group was described elsewhere ("*In vivo* experiments with rats").

Along with visual inspection, peripheral blood (1.0 mL) was taken from tail vein of rats of the 1st group 24 h, at 4, 7, 14, 17 and 21 days after drug injection. Before blood sampling, the rat was examined, weighed, its body temperature was measured, then it was anesthetized and fixed in a holder for immobilizing rodents. Plasma was obtained from the blood specimens 10 min after blood sampling by centrifugation for 15 min at 1500 rpm. The supernatantses were frozen and stored in closed vessels at -40°C for further analysis.

Determination of DOX content in plasma of rats

Content of doxorubicin (DOX) in rat blood plasma was determined by high-performance liquid chromatography (HPLC) using Prominence-I LC 2030C 3D Plus instrument (Shimadzu) equipped with an RF-20A fluorimetric detector and a 5 μm Luna C18 column (Phenomenex). The excitation wavelength was 475 nm, emission wavelength was 555 nm. Analysis was performed in the gradient elution regime (with acetonitrile) in 0.01 N Na-formiate buffer (pH 3.68). Duration of experiment: was 20 min, at the detection limit of 1 ng/mL. All the measurements were carried out thrice.

Results and discussion

First group of rats

The influence of DOX delivery systems based on porous calcium carbonate cores coated with DexS ($CaCO_3$+DexS) on development of Seidel hepatoma was studied in laboratory rats.

The first experimental group included 4 male and 4 female rats with body mass varying from 256 to 306 g. The first reference group consisted of 5 male and 3 female animals (266 to 312 g). The animals were followed up as described under Materials and Methods.

Six animals from the first experimental group died within a period from 10 to 14 days after the procedure (median, 14 days). All animals of the reference group died within a period from 8 to 13 days after the beginning of the experiment; the lifetime median was 8 days. Physical examination revealed ascites in animals of both groups starting from the 4th day after starting the experiment. The volume of ascites determined during autopsy in the reference group varied from 31 to 122 mL (median, 66 mL), compared with volume of 28 to 34 mL (median: 31 mL) in the 1st experimental group. The difference in ascites volumes between the two groups was statistically significant ($p < 0.05$, according to the Mann-Whitney criterion). Two animals from the first experimental group survived for more than two weeks and were withdrawn from the experiment on day 207.

Changes in appearance and behavior of animals of the reference group were seen at 8 days and were observed for 2-3 days until death of animals. Their fur became lackluster and scraggly. The animals were depressed, lacking curiosity and reaction to other animals, low motor activity, with absence of vertical postures. The amount of consumed food decreased, whereas the amount of consumed water remained the same. If an animal died within 8 days after injecting hepatoma cells, the above changes were not observed.

In the animals treated with doxorubicin (the 1st experimental group), exterior and behavior started to change later (in 10 days). The changes in fur appearance were similar to those in reference group. The animals were depressed, moved slowly (crawled); reddish eyelids and developed yellowish crusts (simple blepharitis) were observed. Of note, no eye pathology was seen in the animals of the reference group. Two animals of experimental group that died within 10 days after treatment did not show these changes.

In two surviving animals from the 1st experimental group (that survived for 207 days after implantation of hepatoma cells and injection of DOX in $CaCO_3$+DexS delivery systems), ascites was revealed in 4 days (physical examination). From 10 to 14 days of the experiment, changed appearance (dull and scraggly fur), behavior and motion activity (depression, absence of vertical postures) were observed. Slight reddening of eyelids, decrease in food consumption and weight loss also occurred. Then the above sickness symptoms disappeared, the animals started to feed normally and gained weight. No signs of Seidel hepatoma and ascites were revealed during autopsy.

The observations described above allowed us to make the following conclusions: DOX applied intraperitoneally in the DS based on calcium carbonate cores doped with DexS affects development of the tumor, manifesting as decreased volume of ascitic fluid. Low percentage of survival (only two animals) may be explained by low dose of antitumor preparation (2 mg per animal). In this series of experiments, concentrations of doxorubicin in blood were not determined.

Since the animals tolerated treatment with DOX in ($CaCO_3$+-DexS) delivery systems relatively well, and the applied dose (2 mg of doxorubicin *per* animal) inhibited development of Seidel hepatoma, one may assume that the amount of injected preparation in DS can be increased. Therefore, the next experiment with laboratory animals was designed.

Second group of rats

Time dynamics of DOX release in blood of laboratory animals after i.p. administration of two types of delivery systems were studied. The two kinds of delivery systems were used: porous calcium carbonate cores doped with DexS ($CaCO_3$+-DexS) (DS1), and DexS-DOX conjugates (DS2).

The 2nd experimental group of rats included 17 healthy animals (body weight 270-310 g). Six rats (2 females, 4 males) were treated with DOX in DS1 (4 mg *per* animal); the remaining eleven animals (6 females, 5 males) were treated with DOX in DS2 (4 mg of DOX *per* animal). The 2nd reference group for treatment with free DOX consisted of 3 rats (2 females, 1 male) weighing from 260 to 280 g.

Upon i.p. administration of $CaCO_3$+DexS delivery systems containing DOX, the animals were active, no inflammation symptoms were observed in the injection area. During the experiment, no changes in the state of fur, eyes, neither abnormal behavior nor reactions were registered. The body temperature was typical of healthy rats. Starting from the 2nd day, consumption of food and water was common for the animals kept in the vivarium (from 3 to 8 mL daily *per* 100 g of body mass, and from 4.4 to 4.7 g of food daily *per* 100 g of body mass). One of 11 rats that were given DOX in DS2 (DexS-DOX conjugates) died at 12 days after drug administration. Autopsy of the dead rat revealed neither macroscopic changes in internal organs, nor defects of DOX administration.

Concentration of DOX in rat blood plasma after administration of various delivery systems

In what follows, we describe the results of experiments involving healthy rats; free DOX and two types of DOX-containing delivery systems were injected intraperitoneally. Like as in the first experimental series, DOX was encapsulated in calcium carbonate cores doped with DexS and included in conjugates with this polyanion. During i.p. administration, the delivery systems enter intercellular fluid which is nearly similar to blood plasma. In our earlier works [14,16], it has been demonstrated that vaterites were gradually destroyed in plasma, resulting into increased release rate of encapsulated compounds from porous carrier. Moreover, treatment of vaterites with DexS made it possible to reach prolonged release of DOX into blood plasma. Scanning electron microscopy demonstrated that structural changes in hybrid delivery systems occurring in plasma correlated with DOX release profiles [14]. After introduction of various delivery systems, DOX concentration in rat blood plasma was determined and compared with the concentration reached after administration of free DOX. Time profiles of DOX concentrations are seen in Fig. 1.

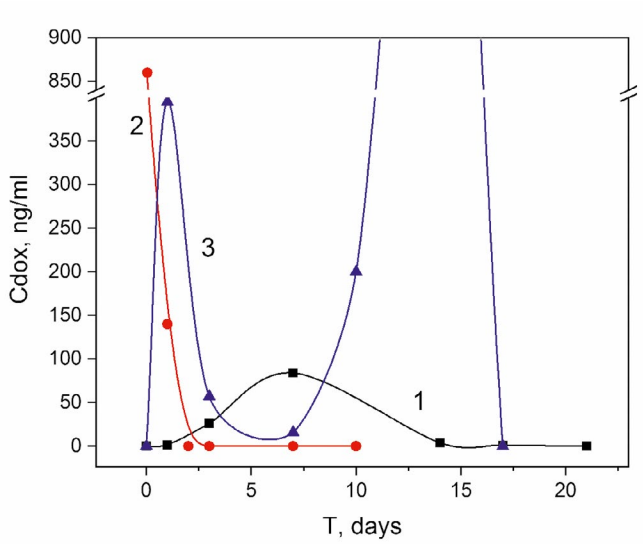

Figure 1. DOX concentrations in plasma after intraperitoneal administration to the rats: $CaCO_3$+DexS+DOX (1); free DOX (2); DexS+DOX (3)

Before analysis of the obtained results, let us compare the literature data on DOX concentrations in blood of various tumor-bearing animals. It should be noted that the results obtained in experiments with rats cannot be directly used in pharmacology. The authors of [17] emphasize that it is necessary to estimate interspecies differences in distribution and elimination of drugs.

Free DOX is rapidly assimilated by organisms of DBA_2 mice. After intravenous introduction of 7 mg/kg of the drug, its concentration in plasma decreases by a factor of 100 within an hour (down to 0.2 nM/mL=116 ng/mL [10]). A 10-fold decrease in plasma DOX concentration (down to 1 μg/mL) was observed within 4 h, when almost similar amount of free DOX (5 mg/kg) was administered intravenously (i.v.) to tumor HeLa-bearing mice, [11]. Upon i.v. injection of DOX (10 mg/kg) to tumor MCF-7 bearing mice, the drug disappeared during 4 days. At 12 hrs, 70 ng/mL of DOX was found in plasma. Signs of cardiotoxicity (release of characteristic enzymes) were observed in 2 weeks after DOX administration [12]. The results of studies involving breast cancer patients treated with standard amounts of DOX showed that

the concentrations of DOX in plasma ranged between 12 and 620 ng/mL. The risk of cardiomyopathy in such patients was under 4% [13]. Hence, DOX concentration in plasma after treatment may vary from 10 to 600 ng/mL. The total amount of drug in blood is directly proportional to animal size and, therefore, to blood volume.

One may assume that determination of DOX concentrations in rat blood plasma within the mentioned range, together with physical examination of animals, allows comparing effects of various DOX delivery systems after i.p. administration. Moreover, we should consider the fact that DOX is not only present in blood, but distributed in all organs of an animal.

Comparison of time profiles of DOX release from DexS-coated $CaCO_3$ cores and from DexS+DOX conjugates into human blood plasma is discussed in [2]. In the case of conjugates, the DOX release profile (unlike that from doped carbonate cores) demonstrated burst release of the drug for the first 24 h. Later on, gradual increase in the released amount of drug (up to 70%) was observed for 2 weeks. As shown in Fig. 1, (curve 3) injection of the fast-releasing carriers is associated with higher DOX levels in the bloodstream.

The authors of [11] compared time profiles of DOX concentration in blood plasma of HeLa-bearing mice after i.v. administration of free DOX *versus* DOX incorporated into nanoparticles of various compositions. Similar results were obtained in the present work. High amounts of DOX were released from DS2 nanocarriers into plasma during the first hour after administration, unlike the case of free DOX injection (Fig. 1, curve 2). Increased plasma DOX concentration early after injection may be caused by release of DOX molecules that were weakly bound to the carriers. Similar situation is possible in the case of polymer-drug conjugates formation; despite equimolar ratio of components, complete attachment of DOX to DexS did not occur, due to considerable difference in sizes of molecules.

Calcium carbonate cores doped with DexS provided prolonged release of DOX with gradually increased concentration (Fig.1, curve 1). Note that DOX concentrations in plasma lie in the nanogram range typical of humans and animals treated with DOX.

Besides, the size range of the DOX delivery systems should be taken into account; they determine ability of the drug to penetrate into organs and tissues. Of course, nanosized conjugates have advantages over submicron-sized doped carbonate cores. Probably, this factor is responsible for increased concentration of released DOX by the end of second week after injection of the drug within polymer-drug conjugates.

Conclusions

Intraperitoneal administration of 2 mg of DOX in DS based on calcium carbonate cores doped with DexS in rats inoculated with Seidel hepatoma resulted in increase of life expectancy by 1.75 times and in decrease in ascites volume in laboratory animals. It is expected that increase in the dosage up to 4 mg per animal will lead to more efficient inhibition of tumor growth. This dose was used in the studies of dynamics of DOX release into blood plasma after i.p. administration of the drug to intact rats using delivery systems of various structures. In 2 days after introduction of free DOX to rats, the drug disappeared from blood plasma. Application of the delivery systems made it possible to prolong presence of the drug in blood. When similar amounts of DOX (4 mg per animal) were introduced by means of various delivery systems, DOX was present in blood plasma at different amounts, depending on the structure of a delivery system. Porous calcium carbonate cores doped with DexS allowed for release of DOX within 2 weeks at the rates under cardiotoxic concentrations. The release profile of DOX in blood plasma after injection of polymer-drug conjugate DexS-DOX had a complex pattern, due to the carrier structure. These DS release significantly higher amounts of DOX into plasma by 14th day after beginning the experiment. Despite the difference in DOX release profiles, neither calcium carbonate, nor conjugate DS caused negative reactions in rats, as confirmed by observations of behavior and physical state of the animals, and autopsy results. Therefore, both studied drug delivery systems could be used for prolonged regional administration of antitumor DOX preparation.

Financial support

The study was performed within the framework of budget-supported research project №AAAA-A20-120022090044-2, Institute of Macromolecular Compounds, RAS.

Conflict of interests

None declared.

References

1. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev. 2004;56:185-229. doi: 10.1124/pr.56.2.6

2. Sudareva N, Suvorova O, Saprykina N, Vlasova E, Vilesov A. DOX delivery systems based on doped $CaCO_3$ cores and polyanion-drug conjugates. J Microencapsul. 2021;38:164-176. doi: 10.1080/02652048.2021.1872724

3. Popryadukhin P, Sudareva N, Suvorova O, Yukina G, Sukhorukova E, Saprykina N, et al. Morphology of target drug delivery systems ($CaCO_3$ vaterites covered with dextran sulfate) in rat muscular tissue. Cell Ther Transplant. 2020;9:78-84. doi: 10.18620/ctt-1866-8836-2020-9-4-78-84

4. Sudareva N, Suvorova O, Saprykina N, Smirnova N, Bel'tiukov P, Petunov S, et al. Two-level delivery systems based on $CaCO_3$ cores for oral administration of therapeutic peptides. J Microencapsul. 2018; 35:619-634. doi: 10.1080/02652048.2018.1559247

5. Youn Y, Bae H. Perspectives on the past, present, and future of cancer nanomedicine. Adv Drug Delivery Rev. 2018;130:3-11. doi: 10.1016/j.addr.2018.05.008

6. Alven S, Nqoro X, Buyana B, Aderibigbe B. Polymer-drug conjugate, a potential therapeutic to combat breast and lung cancer. Pharmaceutics, 2020;12:406. doi: 10.3390/pharmaceutics12050406

7. Guidelines for the experimental (preclinical) study of new pharmacological substances. (Ed. Khabriev R). Medicine Publishers. 2005, 832 p (In Russian).

8. Revtovich M, Istomin U, Krasko O, Treshchalina E, Bichkovsky P, Yurkschtovich T, et al. Antitumor activity of the hydrogel form of cisplatin on Seidel ascitic hepatoma. Russ Biother J. 2016;15:96-101. (In Russian). doi: 10.17650/1726-9784-2016-15-4-96-101

9. Adiseshaiah P, Hall J, McNeil S. Nanomaterial standards for efficacy and toxicity assessment. WIREs Nanomed Nanobiotechnol. 2009;2:99,112. doi: 10.1002/wnan.66

10. Baurain R, Deprez-De Campeneere D, Trouet A. Determination of Daunorubicin, Doxorubicin and their fluorescent metabolites by high-pressure liquid chromatography: plasma levels in DBA2mice. Cancer Chemother Pharmacol. 1979;2:11-14.

11. Zhang C, Wu Y, Dong Y, Xu H, Zhao I. Quantification of DOX bioavailability in biological samples of mice by sensitive and precise HPLC assay. Pharm Biol. 2016; 54:55-61. doi: 10.3109/13880209.2015.1014918

12. Mondal L, Mukherjee B, Das K, Bhattacharya S, Dutta D, Chakraborty S, et al. CD-340 functionalized doxorubicin-loaded nanoparticle induces apoptosis and reduces tumor volume along with drug-related cardiotoxicity in mice. Int J Nanomed. 2019;14:8073-8094. doi: 10.2147/IJN.S220740

13. Harahap Y, Ardiningsih P, Winarti A, Purwanto D. Analysis of the Doxorubicin and Doxorubicinol in the plasma of breast cancer patients for monitoring the toxicity of Doxorubicin. Drug Design Devel Ther. 2020;14:3469-3475. doi: 10.2147/DDDT.S251144

14. Sudareva N, Suvorova O, Saprykina N, Tomson V, Suslov D, Galibin O, Vilesov A. Morphology of hybrid doxorubicin delivery systems (dextran sulfate-coated CaCO3 vaterites) in human blood plasma. Cell Ther Transplant. 2021; 10:79-85. doi: 10.18620/ctt-1866-8836-2021-10-1-79-85

15. Freireich E, Gehan E, Rall D, Schmidt L, Skipper H. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother Res. 1966;50:219-224.

16. Sudareva N, Popryadukhin P, Saprykina N, Suvorova O, Yukina G, Galibin O, Vilesov A. CaCO3 vaterite as components of target drug delivery systems. Cell Ther Transplant. 2020;9:13-19. doi: 10.18620/ctt-1866-8836-2020-9-2-13-19

17. Li Y, Zhang C, Zhou S, He M, Zhang H, et al. Species difference in paclitaxel disposition correlated with poor pharmacological efficacy translation from mice to humans. Clin Pharm Adv Appl. 2018;10:165-174. doi: 10.2147/CPAA.S185449

$CaCO_3$ ватериты, покрытые декстрансульфатом, как системы для регионарного введения доксорубицина крысам

Наталья Н. Сударева [1,2], Ольга М. Суворова [1], Дмитрий Н. Суслов [3], Олег В. Галибин [2], Александр Д. Вилесов [1,2]

[1] Институт высокомолекулярных соединений РАН, Санкт-Петербург, Россия

[2] Первый Санкт-Петербургский государственный медицинский университет им. акад. И. П. Павлова, Санкт-Петербург, Россия

[3] Российский научный центр радиологии и хирургических технологий им. акад. А.М. Гранова, Санкт-Петербург, Россия

Резюме

Доксорубицин (ДОХ) – водорастворимый антрациклиновый антибиотик, обладающий высокой противораковой эффективностью. Можно добиться уменьшения концентрации ДОХ в крови ниже кардиотоксического уровня в процессе терапии, формируя депо, содержащее системы доставки ДОХ с пролонгированным высвобождением лекарства. Для этих целей использовали кальций карбонатные пористые ватериты, допированные полианионом декстран сульфатом. Субмикронные размеры носителей не позволяют свободно включаться им в кровеносное русло. Распространяется токсическое лекарство по организму только после попадания в кровь в результате высвобождения из систем доставки. Внутрибрюшинное введение крысам с перевитой гепатомой Зайделя ДОХ-содержащих систем доставки позволило оценить эффективную концентрацию ДОХ, тормозящую рост опухоли и уменьшающую объем асцитной жидкости. Динамику поступления ДОХ в кровь здоровых крыс после внутрибрюшинного введения 4 мг ДОХ в системах доставки различной природы определяли методом ВЭЖХ. Введенное при помощи допированных декстрансульфатом субмикронных карбонатных ядер лекарство высвобождается в кровь крыс в течение двух недель в концентрациях, меньших токсичных значений. При использовании в качестве системы доставки наноразмерного конъюгата декстрансульфат+ДОХ в крови крыс обнаруживается лекарство в значительно больших концентрациях. Независимо от концентрации ДОХ в плазме результаты физикального осмотра, а также аутопсии крыс в течение 21 дня после внутрибрюшинного введения ДОХ в разных системах доставки, свидетельствуют об отсутствии негативных реакций у животных.

Ключевые слова

Доксорубицин, система доставки лекарства, $CaCO_3$, декстрансульфат, конъюгат полимер-лекарство, плазма крови.