Bioautomation, 2007, 8, Suppl. 1, 138 – 145 ISSN 1312 – 451X

Adsorption of Oxaliplatin by Hydroxyapatite

Betsiou M.[1], Sikalidis C.[1*], Papageorgiou A.[2]

[1]*Department of Chemical Engineering*
Aristotle University of Thessaloniki
POBox 1683, 54006, Thessaloniki, Greece
Phone: 2310996185, Fax: 2310996230
E-mail: sikalidi@eng.auth.gr

[2]*Dept. Exp. Chemotherapy, Theagenion Cancer Hospital*
54007, Thessaloniki, Greece

[*]*Corresponding author*

Summary: Hydroxyapatite (HAP) is the main inorganic component of human skeleton. The last years a lot of interest is focused on its use as drug carrier. In this work the in vitro adsorption of the anti-cancer drug oxaliplatin, by HAP, from its aqueous solution was studied. Various initial concentrations of oxaliplatin aqueous solutions were used in order to determine the maximum adsorption capacity of HAP. Oxaliplatin's concentrations were determined through Pt determinations by atomic absorption spectrometry with flame technique, in the equilibrated solutions after shaking for 48 hours and filtering the HAP-oxaliplatin slurries. The maximum adsorption capacity was found to be 49.1 mg oxaliplatin/g HAP. In order to determine the time needed for the maximum adsorption to be achieved, six oxaliplatin – HAP slurries were prepared. The slurries had initial oxaliplatin concentrations the one that corresponds to the maximum adsorption capacity of the HAP added. The oxaliplatin determination was carried out after 0, 10, 20, 30, 40 and 48 hours in each different slurry. The maximum adsorption capacity was achieved after 20 hours. The adsorption of oxaliplatin by HAP was found to follow the Freundlich equation.

Keywords: Hydroxyapatite, Oxaliplatin, Drug Carrier, Adsorption, Cancer.

1. INTRODUCTION

The development of new drug delivery systems using novel materials such as ceramics and polymers for the treatment of several diseases presents high scientific interest. Major purpose is to achieve a controlled and slow release rate of the drug in order to insure a constant in vivo drug concentration for a longer period of time and to prevent harmful side-effects [1-7].

Many forms of ceramic drug delivery systems have been used to carry various types of drugs such as proteins, steroids, hormones, amino acids, phenolics, vaccines, antibiotics and anti-cancer drugs.

One of the most commonly used ceramic form is HAP $[Ca_{10}(OH)_2(PO_4)_6]$ which is biocompatible, since it is the essentially hard, inorganic component of human bones. Furthermore physical and chemical properties of HAP as chemical composition, structure, porosity, particle size, surface area and ionic composition of the equilibrating solution are determinant in drugs' binding and release [1, 4, 8-12].

Several studies related to the application of HAP as drug carrier, have been published. Yammamura et. al. [13] investigated the load of the antibiotic Cefotiam in samples of porous HAP for the purpose of prevention and avoidance infections during surgical process placing HAP implant. Otsuka et. al. [14] developed a drug-delivery system for use in patients with chronic arthritis, chronic articular rheumatics and other diseases, after surgery. It consists of a self - setting bioactive calcium phosphate cement containing aspirin. Barroug and Glimcher [1], studied the characteristics of the in vitro binding and release of the anticancer drug cisplatin by slurries of synthetic HAP crystals carried out in aqueous media and found that they depend significantly on the ionic composition of the aqueous media used [1, 13-14].

The purpose of the present work was to study the adsorption of an anticancer drug oxaliplatin by HAP. The next step in our work will be the study of the release of oxaliplatin by HAP. Final target is to develop a HAP impregnated with oxaliplatin implant which will act also as a drug delivery system. Oxaliplatin is an antineoplastic agent belonging to a new class of platinum based compounds in which the platinum atom is complexed with 1,2-diaminocyclohexane (DACH) and an oxalate group (Fig. 1).

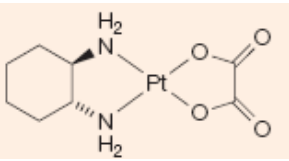

Fig. 1. Molecular structure of oxaliplatin
(cis-[(1R,2R)-1,2-cyclohexane diamine-N,N']) [17]

Oxaliplatin acts as an alkylating cytotoxic agent, inhibiting DNA replication by forming adducts between two adjacent guanine plus

adenine. It is the main drug for the treatment of colorectal cancer and it, also, prevents antitumor activity in the treatments of pancreatic, gastric, ovarian, bladder, breast, small and non-small cell lung, head and neck cancer [15-18].

2. MATERIALS AND METHODS

2.1. Materials

HAP was purchased from Riedel – de Haen in the form of tri - calcium phosphate extra pure [$Ca_5(PO_4)_3OH$]. Oxaliplatin was used as its commercial form, Eloxatin (5 mg/ml). The bottle contains 100 mg oxaliplatin and 900 mg lactose monohydrate as vehicle and was supplied by Theageneion Cancer Hospital of Thessaloniki. Platinum atomic absorption standard solution (1 mg/ml Pt in 10% HCl) was bought from Acros Organics. All the materials were stored at room temperature.

2.2. Methods

2.2.1. Adsorption of oxaliplatin by HAP

For the determination of the maximum amount of oxaliplatin that can be adsorbed by HAP, eloxatin was dissolved in double deionized water (10 ml) by rapid mixing in glass flasks in order to produce solutions of various concentrations ranging from 92 to 30000 mg/l. Twelve samples were prepared by dispersion of HAP (50 mg) in each one of these solutions. The samples were shaked in a shaker for 48 hours and filtered. In the filtrate Pt was determined.

For the study of the kinetic of the adsorption, samples were prepared by the same way as above using initial concentration of oxaliplatin that corresponds to maximum adsorbed amount, determined. Afterwards the samples were shaked and every ten hours one of them was filtered. Pt was determined in the filtrate.

2.2.2. Determination of platinum concentration

Initial and final concentrations of Pt in solutions were determined by atomic absorption spectrometry with flame technique, using a Perkin Elmer 503 spectrophotometer. Pt standards for calibration were prepared by dilution of stock solution. The calibration curve covered the range of 0 to 50 ppm. All the presented values were the mean of three replicates.

3. RESULTS AND DISCUSSION

3.1. Results

The amounts of adsorbed oxaliplatin by HAP as a function of the residual concentration at equilibrium are presented in Table 1. The adsorbed oxaliplatin increases as its initial concentration increases until a maximum amount of 49.1 mg/g is reached. This amount corresponds to the 26.2% of the initially available oxaliplatin. This could be attributed to the fact that oxaliplatin co-exist with the vehicle, lactose monohydrate. As result, a part of HAP's surface is covered by the vehicle preventing the adsorption of oxaliplatin.

Table 1. Adsorption of oxaliplatin by HAP

Concentration		Amount Adsorbed
C_{in} (mg/l)	C_{eq} (mg/l)	Q (mg/g)
4.11	4.04	0.015
6.45	6.36	0.016
7.78	7.63	0.032
12.89	12.70	0.038
14.21	13.99	0.044
24.86	24.63	0.047
64.46	55.72	1.749
174.97	150.47	4.900
526.85	378.16	29.739
576.30	420.30	31.200
939.89	694.39	49.100
1013.16	769.16	48.800

C_{in}: Initial concentration
C_{eq}: Final concentration after 48 hours equilibration
Equilibrium pH = 6.5

The adsorption of oxaliplatin by HAP is well described mathematically by the Freundlich isotherm (Fig. 2), defined by the relationship: $Q = K_F C_{eq}^{1/n}$, where Q is the amount of solute adsorbed per unit weight of adsorbent, C_{eq} is the residual liquid phase concentration at equilibrium, K_F and 1/n are characteristics constants. The equation linearizes in logarithmic form:

$\ln Q = \ln K_F + 1/n\ C_{eq}$

for parameter evaluation. It was found that $K_F = 0.00067$ and $1/n = 1.737$ [19].

The adsorption kinetic of oxaliplatin by HAP is presented in Fig. 3. The maximum adsorption value was achieved in 20 hours and remains the same for the next 28 hours.

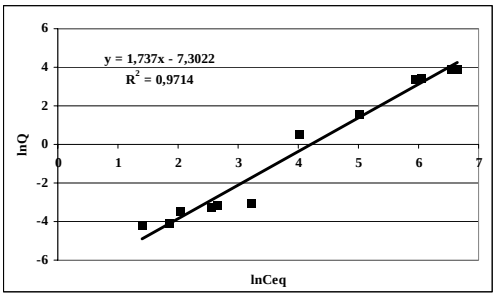

Fig. 2. Freundlich adsorption isotherm of oxaliplatin by HAP: natural logarithm of the amount of oxaliplatin adsorbed per gram HAP (lnQ) vs. natural logarithm of the oxaliplatin equilibrium concentration ($\ln C_{eq}$). (Equilibration time: 48 hours)

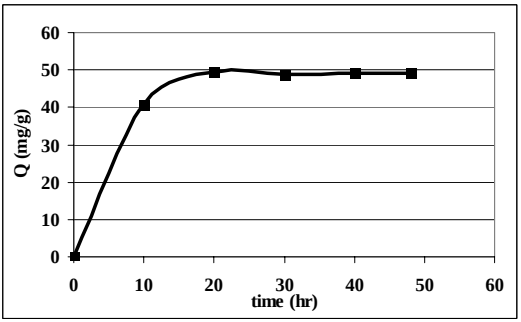

Fig. 3. Kinetic of adsorption of oxaliplatin by HAP (Q: amount of oxaliplatin adsorbed per 1 gram HAP)

3.2. Discussion

This study shows that a reasonable amount (49.1 mg/g) of oxaliplatin can be adsorbed by HAP. This amount could be altered with the addition of different amounts of vehicle (lactose monohydrate).The advised dose of oxaliplatin is 85 mg/m^2 of body-surface area (BSA) intravenously every two weeks [15]. The "normal" BSA is generally taken to be 1.7 m^2 but, in actual fact, the BSA depends on more than just height and weight. Other influential factors include the age and gender of the individual. So average BSA for adult men is 1.9 m^2 and for adult women 1.6 m^2 [20]. According to these, a man patient has to take 161.5 mg oxaliplatin every 2 weeks, which can be delivered by 3.3 g HAP. The equivalent dose for a woman patient is 136 mg oxaliplatin every 2 weeks, which can be delivered by 2.8 g HAP.

Since oxaliplatin is given intravenously only a small amount of a dose is expected to reach to the tumor. The rest creating serious side effects on human organism [21]. The amount of 49.1 mg oxaliplatin per 1g HAP indicates that a porous HAP implant or a paste could be loaded with enough quantity of oxaliplatin possibly to complete required treatments.

The matter that arises and will be investigated in our future work is the kinetics of release of oxaliplatin from HAP in SBF (simulated body fluid). It is expected the release to be completed in some days.

Moschidis A. et. al [22], studied in vivo the anticancer action of oxaliplatin against murine pancreatic adenocarcinoma PAN02. According to these experiments, the constituting dose for a mouse is 6.78 mg/kg [22, 23]. On the basis that the mean weight of a mouse is 25 g, the administered dose is 0.17 mg oxaliplatin / mouse. Considering that the requisite dose of oxaliplatin is similar in the case of bone cancer, this quantity can be carried by 0.02 g HAP.

4. CONCLUSION

HAP was found to adsorb 49.1 mg oxaliplatin per gram, which corresponds to the 26.2% of the initially available oxaliplatin of the oxaliplatin / lactose monohydrate (10/90) system. The maximum oxaliplatin's adsorption by HAP was achieved in 20 hours. The amount adsorbed and the kinetic of adsorption suggest the possible use of the system as drug carrier in the case of implants or paste but further release and in vivo experiments are needed.

REFERENCES

1. Barroug A., M. J. Glimcher, Hydroxyapatite Crystals as a Local Drug Delivery System for Cisplatin: Adsorption and Release of Cisplatin in Vitro, *Appl. Journal of Orthopaedic Research*, 2002, 20, 274-280.
2. Brannon-Peppas L., Polymers in Controlled Drug Delivery, *Appl. Medical Plastics and Biomaterials*, 1997, available at: http://www.devicelink.com/mpb/archive/97/11/003.html.
3. Kiparissides C., Alexandridou S., Kotti K., Chatidou S., Recent Advances in Novel Drug Delivery Systems, *Journal of Nanotechnology Online*, 2006, 2, 1-11.
4. Bajpai P. K., H. A. Benghuzzi, Ceramic Systems for Long-term Delivery of Chemicals and Biologicals, *Journal of Biomedical Materials Research*, 1988, 22, 1245-1266
5. Backles R., Biomaterials for Drug Delivery Systems, *Journal of Biomedical Materials Research*, 1983, 17, 109-128.
6. Verma R. K., S. Garg, Current Status of Drug Delivery Technologies and Future Directions, 2001, 25(2), 1-14, available at: www.pharmanet.com.br/pdf/drugdelivery.pdf.
7. Henry C. M., Drug Delivery, *C&EN Washington, Cover Story*, 2002, 80, 39-47.
8. Dash A. K., G. C. Cudworth, Therapeutic Applications of Implantable Drug Delivery Systems, *Appl. Journal of Pharmacological and Toxicological Methods*, 1998, 40, 1-12.
9. Guicheux J., G. Grimandi, M. Trecant, A. Faivre, G. Daculsi, S. Takahashi, Apatite as Carrier for Growth Hormone: In Vitro Characterization of Loading and Release, *Journal of biomedical materials research*, 1997, 34, 165-170.
10. Hamanishi C., K. Kitamoto, S. Tanaka, M. Otusuka, T. Kitahashhi, Y. Doi, A Self-setting TTCP-DCPD Apatite Cement for Release of Vancomycin, *Journal of Biomedical Materials Research*, 1996, 33, 139-143.
11. Dubok V. A., Bioceramics – Yesterday, Today, Tomorrow, *Powder Metallurgy and Metal Ceramics*, 2000, 39, 381-394.
12. Netz D. J. A., P. Sepulveda, V. C. Pandolfelli, A. C. C. Spadaro, J. B. Alencastre, M. V. L. B. Bentley, J. M. Marchetti, Potential use of Hydroxyapatite Porous Ceramic as an Drug Delivery System, *International Journal of Pharmaceutics*, 2001, 213, 117-125.

13. Yammamura K., H. Iwata, T. Yotsuyanagi, Synthesis of Antibiotic-loaded Hydroxyapatite Beads and in Vitro Drug Release Testing, *Journal of Biomedical Materials Research*, 1992, 26, 1053-1064.
14. Otsuka M., Y. Matsuda, Y. Suwa, J. L. Fox, W. Hitguchi, A Novel Skeletal Drug-delivery System using Self-setting Calcium Phosphate Cement. 4. Effects of the Mixing Solution Volume on the Drug-release Rate of Heterogeneous Aspirin-loaded Cemen, *Appl. Journal of Pharmaceutical Sciences*, 1994, 83, 259-263.
15. Eloxatin, Summary of Product Characteristics, Sanofi Winthrop Ltd, 1999, available at: www.medicines.ie/eMC/assets/c/html/DisplayDoc.asp?DocumentID=10947.
16. Graham J., M. Muhsin, P. Kirkpatrick, Fresh from the Pipeline Oxaliplatin, *Appl. Nature Reviews / Drug Discovery*, 2004, 3, 11-12.
17. Becouarn Y., C. Agostini, N. Trufflandier, V. Boulanger, Oxaliplatin: Available Data in Non-colorectal Gastrointestinal Malignacies, *Critical Reviews in Oncology / Hematology*, 2001, 40, 265-272.
18. Carrato A., J. Gallego, E. Diaz-Rubio, Oxaliplatin: Results in Colorectal Carcinoma, *Appl. Critical Reviews in Oncology / Hematology*, 2002, 42, 29-44.
19. Slejko F. L., Adsorption Technology. A Step-by-step Approach to Process Evaluation and Application, *Chemical Industries*, 1985, 19, Markel Dekker, 13-14.
20. Mosteller R. D., Simplified Calculation of Body-surface Area, *The New England journal of medicine*, 1987, 317, 1098.
21. http://www.melook.net/article.asp?item_id=2046, Chemotherapy: The Basic Points. (in greek)
22. Moschidis A., A. Papageorgiou, D. Tsavdaridis, K. Atmatzidis, K. Tsalis, E. Chrysogelou, P. Oustabasidis, G. Geromichalos, N. Charlaftis, Gemcitabine in Combination with Oxaliplatin in Pancreatic (PAN-02) Tumor Bearing Mice, *Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings*, 22, 41-53.
23. Moschidis A., A. Papageorgiou, K. Atmatzidis, K. Tsalis, E. Moschidis, J. Livanis, E. Chrysogelou, D. Mourelatos, D. Tsavdaridis, N. Harlaftis, Synergistic Antitumor Activity of Oxaliplatin in Combination with Gemcitabine in Pancreatic Tumor Bearing Mice, *Chemotherapy*, 2005, 53, 153-159.