ATLANTIK Genta, A New Concept Of Gentamicin Loaded HAP/TCP Bone Substitute For Prophylactic Action - In Vitro Releasing Mechanisms Study

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Abstract. Despite systemic prophylaxis, infection rates after orthopedic surgery can reach more than 1%. A new HAP/TCP bone substitute loaded with 125 mg of gentamicin was designed for prophylactic use. Its aim was to enhance the efficacy of systemic prophylactic treatments by increasing the local antibiotic concentration. For prophylactic applications, release had to take place within 48 hours not to select antibiotic-resistant bacterial strains. The purpose of this study was to investigate the releasing mechanisms of gentamicin from the porous HAP/TCP matrix. The release rate of gentamicin through the porosities of the bone substitute was investigated in vitro, in 0.9% sodium chloride solution. The rate appeared to be related to the bone substitute volume and fit classical diffusion laws. All the gentamicin was released in less than 48 hours: this rate corresponds to the recommendations for the prophylactic use of antibiotics.

Introduction

Despite advances in prophylaxis against infection, post-operative osteomyelitis remains a considerable problem in orthopaedic surgery. In order to reduce the risk, systemic prophylaxis is carried out 1 hour before surgery. However, bone is poorly vascularised and surgery disturbs the vascularisation of the operated site. As the antibiotic is driven by blood after injection, the local concentration in bone is low and bacteria can proliferate. Systemic antibiotics should be administrated in high concentrations for prolonged periods of time to yield adequate concentrations within bone tissues, but such high concentrations of drugs in blood for a long period may induce toxicity [1]. Furthermore, for long treatments, antibiotic-resistant microbial strains can emerge because of selective pressure induced by antibiotics.

Local delivery of antibiotics has the advantage of achieving high local levels of the drug with low risk of systemic toxicity. For bone repair surgery, a new gentamicin loaded hydroxyapatite and tricalcium phosphate (HAP/TCP) bone substitute was developed for prophylactic use. The release
rate was investigated *in vitro*, in NaCl solution. The release duration should be less than 48 hours, not to select antibiotic resistant bacterial strains.

**Materials and Methods**

A new commercial bone substitute composed of 70% hydroxyapatite and 30% β-tricalcium phosphate containing 125 +/- 25 mg of gentamicin (ATLANTIK Genta, Medical Biomat, France) was used in this study. This bone substitute had a high ratio of porosity (70%) composed of macroporosities (300-600 μm in size) and microporosities (1-2 μm in size). The porosities were interconnected with macro-interconnections of 10-15 μm [2]. Its compressive strength was 10 MPa. Gentamicin was incorporated in the porosities by impregnation with a solution of gentamicin sulphate and sterile water (sterilized water for injection, Aguettant, France) after machining and cleaning of the HAP/TCP matrix. After impregnation, the implants were dried, packaged and sterilized (gamma sterilization between 25 and 40 kGy, Ionisos, France). As different sizes of bone substitutes are used in orthopaedic surgery, the gentamicin release rate was studied on blocks of different shapes: parallelepipeds of minimum size 10x5x5 mm or maximum size 50x30x15 mm and cylinders of medium size Ø10H15 mm.

SEM observations were performed with a Jeol 840 ALGS scanning electron microscope (SEM).

*In Vitro* Characterisation Of The Release Rate: The bone substitutes were introduced in 100 ml of 0.9% sodium chloride solution (Aguettant) at 37°C +/- 1°C and placed on plate agitator at 80 rpm. As the solution was not renewed, and as the gentamicin concentration in the solution can influence the release rate, the volume of solution was chosen high enough for the final gentamicin concentration being negligible. 500 μl of the release medium were collected at predetermined time intervals (0, 1h, 4h, 8h, 12h, 24h, 48h, 96h). The gentamicin concentration of each sample was determined after dilution by immunoassay.

**Results**

Physicochemical characteristics of HAP/TCP/Genta bone substitutes: Previous studies on the bone substitute without gentamicin [2] demonstrated that the biomaterial contained two types of porosities: macroporosities (300-600 μm size) and microporosities (1-2 μm size). In the gentamicin loaded bone substitutes, macroporosities are observed but very little microporosity appear between the ceramic grains (Fig. 1). It has been assumed that those later are filled with gentamicin. It has been confirmed by comparing the observations at the same magnification of the same implant after releasing for 48 hours in a NaCl solution (Fig. 2): the gentamicin present in the microporosities has been eluted and the microporosities appear empty.

![Fig. 1. SEM observation of the porosity of the gentamicin loaded bone substitute: macroporosity is free of gentamicin, whereas microporosities are filled with gentamicin](image1)

![Fig. 2. SEM observation of the microporosity of the gentamicin loaded bone substitute after in vitro release during 48 hours: microporosity is empty](image2)
Release rate and microbiological assay of gentamicin: Fig. 3 shows that, for all shapes of bone substitute (50x30x15 mm, Ø10H15 mm and 10x5x5 mm), the concentration in the liquid increases until reaching a plate indicating the end of the release of gentamicin from the bone substitute.

![Graph showing release curves of gentamicin](image)

Fig. 3. In vitro release curves of gentamicin from bone substitute blocks of different shapes. The uncertainty was calculated from the uncertainties of dilution and titration.

The rates depend on the dimension of the bone substitute: the release durations for blocks of large size (50x30x15 mm), medium size (Ø10H15 mm) and small size (10x5x5 mm) are respectively 48 hours, 24 hours and 6 hours. It has been assumed that the release rate is governed by diffusion of the gentamicin through the porosities of the biomaterial and is described by the classical diffusion law Eq. 1 ($X$ being the distance of diffusion, $D$ the diffusion coefficient and $t$ the time). For the samples tested, the release duration ($t_{max}$) corresponds to the diffusion distance of gentamicin from the centre of the sample to the external surface ($X_{max}$). For 50x30x15 mm, Ø10H15 mm and 10x5x5 mm blocks, this distance $X_{max}$ is respectively 7.5 mm, 5 mm and 2.5 mm. Fig. 4, the relation between $X_{max}$ and $t_{max}^{1/2}$ is linear with a regression straight line passing through the origin and a linear regression coefficient $R^2$ close to 1.

$$\frac{1}{t_{DX}} \propto C$$

![Graph showing relation between diffusion distance and square root of release time](image)

Fig. 4. Diffusion distance from the centre of the implants to the surface, versus the square root of the release time. The uncertainty on the evaluation of the release duration from fig.3 was 3 hours.

This result confirms that gentamicin release kinetic is governed by diffusion mechanisms through the porosity of the biomaterials.
Discussion and Conclusion

The majority of the previous published data on antibiotic-loaded bone substitutes focuses on therapeutic applications, i.e. osteomyelitis. The treatment of such infections in a bone site requires large amounts of antibiotic for at least 10 days and sometimes several months. To delay the release of the antibiotic from the bone substitute, different solutions have been presented by authors: embedding the antibiotic in a polymer [3], encapsulating the antibiotic in the porosities of a poorly interconnected ceramic matrix [4], or increasing the specific surface area of the bone substitute [18]. But several problems should be taken into account: i) the addition of polymer should not penalize bone ingrowth by closing porosities or by covering the HAP/TCP with a non-osteoconductive material; ii) the main function of the bone substitute should remain bone healing and porosity should thus be interconnected; iii) the volume of a bone substitute is not sufficient to entrap the dose of antibiotic necessary to reach efficient concentrations during a 10 days treatment \textit{in vivo}. In the present study, the release duration for prophylactic application should be less than 48 hours: the structure of the porosity of the bone substitute and its composition, optimized for bone ingrowth, were not modified to incorporate the antibiotic.

According SEM observations of the bone substitute, gentamicin is stored in the microporosities of the biomaterial, while the macroporosities remain free. This can be explained by the incorporation technique used. During the impregnation of the bone substitute with the gentamicin solution, the solution penetrates by capillarity in macroporosities and microporosities. During the drying step, the liquid evaporates gradually. As the capillary pressure is higher in microporosities, evaporation begins in macroporosities and finishes in microporosities, leaving gentamicin in dry form in the latter.

The release duration was related to the diffusion distance of gentamicin from the centre of the implant to the external surface with a classical diffusion law. The lower the size of the bone substitute, the faster the release rate. From these results, it can be assumed that release proceeds in 3 steps: 1) The liquid diffuses quickly in the bone substitute through the macroporosities which are interconnected and remain free of gentamicin. 2) The most accessible gentamicin, close to the surface of the implant, diffuses out of the ceramic matrix immediately after immersion. As the majority of the gentamicin is close to the surface, the initial release rate is high. 3) The diffusion of gentamicin which is in the centre of the bone substitute is driven by the diffusion rate through the bone substitute porosities. It can be assumed that the diffusion coefficient depends on the characteristics of the porosity, especially the interconnectivity.

The 125 mg of gentamicin incorporated in the HAP/TCP bone substitute in this study were mainly stored in dry form in the micro-porosities of the biomaterial. The rapid release (less than 48 hours) combined with the high gentamicin dose (125 mg) should lead to high local concentrations \textit{in vivo}. Such concentrations should be more effective against bacteria than the usual intravenous injections which generate weak concentrations in bone tissues. The release rate observed is compatible with the recommendations for antibiotic prophylaxis: high dose but limited in time, not to select antibiotic-resistant bacteria.

References