Rifampicin loading of vascular grafts

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Antibiotic-bound arterial prostheses may contribute to the management of prosthetic infections. The in-vitro absorption and release of rifampicin was measured in four different polyester arterial prostheses, including unsealed VP 1200, gelatin-sealed Gelsoft, collagen-impregnated Hemashield and gelatin-sealed Unigraft. Gelsoft grafts bound more rifampicin than unsealed VP 1200. Rifampicin concentrations with the gelatin sealed to Gelsoft grafts reached a threshold when the rifampicin concentrations of the soaking solution were 10 mg/mL or more. Rifampicin adsorption plateaued after 15 min of soaking VP 1200, and peaked after 25 min of soaking Gelsoft. pH variations did not significantly influence antibiotic binding. Prosthesis-bound rifampicin concentrations decreased rapidly after soaking, but a significant portion of the antibiotic remained associated with the material after 7 days. Release was slower with Gelsoft than with VP 1200 during the first 3 h, but after 24 h, the amounts of rifampicin released were similar in the two materials. Other experiments were performed in dogs receiving sealed grafts as infrarenal aortic bypass after soaking the material in a 1 mg/mL solution of rifampicin in normal saline. After 3 days of implantation, the amount of rifampicin in explanted grafts was higher in Gelsoft and Hemashield than in Unigraft. This study confirms that rifampicin bonding to prosthetic material occurs and is enhanced by sealed collagen or gelatin.

Introduction

Vascular graft infection is a rare but feared complication of arterial reconstructive surgery. Despite systemic perioperative antimicrobial prophylaxis, the average incidence of graft infection has been reported to be 1–5%. (Szilagyi et al., 1972; Goldstone & Moore, 1974; Liekweg & Greenfield, 1977; Bunt, 1983; Lorentzen et al., 1985; O'Hara et al., 1986). Once a prosthetic graft is infected, the treatment almost always requires its excision and a new prosthetic bypass through uninfected fields. Morbidity and mortality from these cases are still very high, especially when the aorta is involved. The development of infection-resistant vascular prostheses may therefore contribute to the prevention and the treatment of this complication.

With the advent of protein-sealed polyester grafts, recent publications have discussed the ability of these grafts to retain antimicrobials (Chervu et al., 1991a,b; Goëau-Brissonnière et al., 1991, 1994). In our laboratory, a system involving soaking...
a gelatin-sealed knitted polyester graft in a solution of rifampicin, 1 g/L, has been shown to assure a full protection against a stringent bacteremic staphylococcal challenge in dogs (Goéau-Brissonnière et al., 1991). Further study demonstrated that rifampicin-bonded gelatin-sealed polyester grafts were resistant to infection when used for in-situ replacement of an infected graft in the dog (Goéau-Brissonnière et al., 1994). In these experiments grafts were soaked in a 60 g/L solution of rifampicin.

However, the kinetics of absorption and desorption of rifampicin in protein-sealed materials remain unknown, even though such parameters are important for evaluating the contribution to the bonding of the proteins sealed to the prosthesis, and for optimising the practical conditions of usage. The purpose of this study was to measure in-vitro absorption and release of rifampicin in four different arterial polyester prosthetic materials. Additionally, we assayed rifampicin in arterial prostheses after implantation in dogs.

**Materials and methods**

**Graft materials**

The prosthetic grafts chosen for in-vitro experiments included unsealed VP 1200 grafts and gelatin-sealed Gelsoft grafts (Vascutek Ltd., Inchinnan, Scotland), collagen-impregnated Hemashield Microvel Double Velour grafts (Meadox, Oakland, NJ, USA), and gelatin-sealed Unigraft grafts (B. Braun, Melsungen, Germany). All prostheses were prepared as circular discs 2 cm in diameter. The prostheses tested after implantation in dogs were commercially available six millimetre-diameter gelatin-sealed grafts (Gelsoft and Unigraft) and collagen-impregnated grafts (Hemashield Microvel Double Velour).

**Rifampicin**

For in-vitro experiments, rifampicin was used as sterile powder of known potency provided by Ciba-Geigy, Basel, Switzerland and by Lepetit, Milano, Italy. Rifampicin solutions were freshly prepared, taking potency into account, by dilution into ethanol then into phosphate buffer at various pH or into human plasma. For in-vivo experiments, rifampicin solutions were prepared by dilution of a commercially-available vial of rifampicin (Rifadin, Marion Merrel Dow, Levallois-Perret, France) into normal saline.

**Rifampicin assays**

Rifampicin was first extracted from the prosthetic discs and the explanted prostheses by soaking overnight in methanol. We checked that this treatment removed all detectable rifampicin from the graft material by putting the discs or a piece of the explanted grafts after extraction onto Antibiotic Medium 1 (Difco, USA) containing *Bacillus subtilis*. No growth inhibition appeared. Levels of extracted rifampicin were determined using two methods. The microbiological method used *Bacillus subtilis* as the test organism, and Antibiotic Medium 1 in a conventional plate diffusion technique. This method was applied for measuring rifampicin levels of 0.02–10 mg/L, but appeared inappropriate for higher concentrations because of the non-linearity of the relationship
between zone diameter and drug concentration above 10 mg/L. Therefore, a spectrophotometric method was preferred for rifampicin levels over 10 mg/L. According to the absorption spectrum of rifampicin solution, we used the peak 334 nm for these assays. The microbiological and spectrophotometric methods yielded (±2%) the same results in solutions containing 1–10 mg/L of rifampicin.

Absorption experiments
Discs were totally immersed in rifampicin-phosphate buffer solutions, at room temperature. At the end of soaking time, discs were removed from the solution, vigorously shaken for 1 min and left 1 h at room temperature for drying. Rifampicin extraction and assay immediately followed. Different variables were investigated, including rifampicin concentrations, duration of soaking, pH of the soaking solution and prosthetic material.

Desorption experiments
Discs were allowed to soak in rifampicin-phosphate buffer solution (60 mg/L) for 25 min at room temperature and pH 7, and then were shaken and dried as previously described. After soaking, the discs were placed in large volumes (10 mL per disc) of citrated human plasma (Centre de Transfusion Sanguine, Annemasse, France) at 37°C and pH 7.4 (maintained by CO₂ gas bubbling). At various time intervals, discs were removed from the plasma, shaken, and dried. The rifampicin was extracted with methanol and assayed. At each of these times, the plasma in which remaining discs were kept for further assays was removed and replaced by the same volume of fresh plasma.

Expression of results
Rifampicin concentrations were expressed as μg/cm² of graft. Preliminary studies indicated strict linear correlations between surface, volume and weight in all varieties of discs, and similar buffer absorption by the three varieties of protein-sealed discs (pH 7, room temperature). Reference to surface was preferred because it allowed an expression independent of presence or absence of gelatin. However, since the various grafts tested would be expected to differ in thickness and in knitting density, this expression does not allow the comparison of grafts in their ability to absorb and release rifampicin.

Results
Absorption assays
At all rifampicin concentrations tested, Gelsoft discs bound more antibiotic than did unsealed VP 1200 discs (Figure 1). Rifampicin levels in the gelatin were estimated by subtracting VP 1200 amounts from those found in Gelsoft. These calculations suggested that absorption by gelatin reached saturation when the soaking solution contained 10 mg/mL of rifampicin or more.
Figure 1. Absorption of rifampicin in grafts vs rifampicin concentrations in the soaking solution. Graft materials were Vascutek Gelsoft (●) and Vascutek VP1200 (■). Rifampicin gel concentrations (▲) were determined by subtracting VP1200 values from Gelsoft values. Soaking conditions were: 15 min, room temperature, pH 7.4. Each point represents the mean of three determinations.

Over the course of the soaking time, absorption of rifampicin by the discs increased at the same rate in Gelsoft and VP 1200, i.e. rapidly between the 2nd and the 5th minute, and more slowly between the 5th and the 15th minute (Figure 2). At later times, rifampicin levels plateaued in VP 1200 but still increased in Gelsoft until the 25th minute, after which time we observed a slow decrease. The rifampicin amounts trapped

Figure 2. Absorption of rifampicin in grafts vs duration of soaking. Symbols as Figure 1. Soaking conditions were: room temperature, pH 7.4, rifampicin concentration 1000 mg/L. Each point represents the mean of three determinations.
Figure 3. Absorption of rifampicin in grafts vs pH of the soaking solutions. Symbols as Figure 1. Soaking conditions were: 15 min, room temperature, rifampicin concentration: 1000 mg/L. Each point represents the mean of three determinations.

by gelatin, again estimated by calculation, increased until the 25th minute, and dropped later (Figure 2), possibly because some of the gelatin was dissolved into the soaking solution.

pH variation of the soaking solution did not greatly alter absorption by discs and gelatin (Figure 3). Figure 4 illustrates the amount of rifampicin trapped by Gelsoft,
Figure 5. Desorption of rifampicin in human plasma (37°C, pH 7.4) from Vascutek Gelsoft and Vascutek VP1200. Gelatin rifampicin concentrations were calculated by subtracting VP1200 values from Gelsoft values. Each point represents the mean of three determinations. Symbols as Figure 1.

Unigraft and Hemashield after 15 min of soaking in solutions containing various rifampicin concentrations: differences remained within 20%.

Release experiments

The rifampicin amounts assayed in the four discs tested showed a rapid drop within the first 3 h, followed by a markedly slower release rate (Figures 5 and 6). A more careful analysis of Gelsoft and VP 1200 indicated a less rapid release rate for the former:

Figure 6. Desorption of rifampicin in human plasma (37°C, pH 7.4) from Vascutek Gelsoft, Braun Unigraft and Meadox Hemashield. Symbols as Figure 4.
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Table. In-vivo dog experiments: concentrations of rifampicin 3 days after graft placement

<table>
<thead>
<tr>
<th>Type of prosthesis</th>
<th>Concentration of rifampicin, mean ± S.D. (mcg/cm²)</th>
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<tbody>
<tr>
<td>Unigraft</td>
<td>0.072 ± 0.003</td>
</tr>
<tr>
<td>Hemashield</td>
<td>0.098 ± 0.019</td>
</tr>
<tr>
<td>Gelsoft</td>
<td>0.109 ± 0.016</td>
</tr>
</tbody>
</table>

Student's t test showed a significant difference (P < 0.02) in rifampicin concentration between Gelsoft and Unigraft or Hemashield and Unigraft. No significant difference between Hemashield and Gelsoft.

Rifampicin concentrations (µg/disc) at 1 and 3 h were 715 and 229 for Gelsoft, against 191 and 151 for VP 1200, respectively. However at 24 h and later, amounts of rifampicin were similar in Gelsoft and VP 1200 (Figure 5). Rifampicin amounts per surface unit were found to be higher in Gelsoft than in Hemashield and Unigraft during the first 24 h, but later, the three materials behave similarly (Figure 6).

Rifampicin assays in explanted grafts

After 3 days of implantation, rifampicin was detected in all the grafts (Table). The concentrations of rifampicin measured in Gelsoft and Hemashield grafts were significantly higher than those in Unigraft prostheses (P < 0.02).

Discussion

In the earliest report of protection of a prosthetic vascular graft by soaking in an antibiotic solution (Richardson et al., 1970), control and antibiotic-protected 0.5 by 0.5 cm pieces of Dacron grafts were implanted into previously-infected subcutaneous wounds on the backs of guinea pigs. The antibiotic-treated experimental grafts were pre-soaked for 15 min in a 10% solution of either cephalothin or cephaloridine. After eight days of implantation, the grafts were removed and cultured for bacteria on appropriate media. The results demonstrated that only 15% of the treated grafts were infected compared with 95% of controls. This early report suggested the possibility of passively bonding antibiotics to polyester vascular grafts.

The present work confirms that an antibiotic can be absorbed by an unsealed polyester graft in a passive system. Nevertheless, the long term duration of this type of protection remains questionable. In order to increase the persistence of antimicrobial activity in polyester grafts, Powell, Burnham & Johnson (1983) proposed adding the antibiotic to an aliquot of the blood used to preclot the prostheses. In an in-vivo canine elution model, these authors compared the absorption onto Dacron grafts of oxacillin, cephalosporins, tobramycin and amikacin, tetracycline, and rifampicin. In this model, only those grafts preclotted with blood containing rifampicin disclosed a measurable activity after 24 h of implantation into canine infrarenal aortas. The advent of protein-sealed polyester grafts then suggested the possibility of using the sealant to increase the capacity of polyester to bind rifampicin, in terms of antibiotic loading.
Comparison of Gelsoft and VP 1200, which differ only by the presence or absence of such sealant, indicated that gelatin allowed about 20% more rifampicin to be bound to the prosthesis, and delayed desorption for a few hours. The contribution of gelatin might involve van der Waals forces, since the Gelsoft sealant is rich in negatively charged carboxyl groups, while rifampicin molecules are positively charged. Rifampicin possesses two ionisation constants (pKa), respectively at pH 1.7 (proton lost on the hydroxyl group at position C8) and 7.9 (proton gained on the piperazine moiety at position N4) (Malabarba et al., 1990). In aqueous solution rifampicin exists as a zwitterion, with an isoelectric point at 4.8. The presence of the two ionisation constants may account for the apparent absence of pH effect between 5 and 9 in the soaking solution. Hence our experiences do not rule out the contribution of an ionic bonding of rifampicin to gelatin which, in turn, is suggested by an increased binding at pH 11 (Ashton et al., 1990).

One unexpected result of our experiences was the prolonged presence of significant amounts of rifampicin both in the in-vitro experiments (after 168 h of plasma soaking) and in the dog experiments (after 3 days of implantation). Since it appeared that the contribution of the gelatin to the binding was trifling after 24 h (Figure 5), we assume that rifampicin persistence in the prosthetic material was due to direct binding of the antibiotic to the polyester material. However this raises the question of antibacterial activity of this firmly bound antibiotic. Previous in-vivo experiments showed that rifampicin bonding totally protected gelatin-sealed Gelsoft grafts from a bacteraemic staphylococcal challenge produced 2 days after graft implantation (Goéau-Brissonnière et al., 1991). The mode of action of very firmly bound antibiotic against bacteria remains unknown, but the possibility exists that rifampicin alters bacterial adherence to the prosthesis without being released as free molecules.

Our finding of improved antibiotic binding with Gelsoft agrees with that of Gahtan et al. (1994) who compared the bioactivity of rifampicin-bonded plain, gelatin-sealed and collagen-impregnated polyester grafts. In their experiments, gelatin-sealed grafts had the highest activity. There are several practical implications of this study. It appears that the presence of a sealant such as the gelatin used in the Gelsoft material promotes the binding and retention of rifampicin. The gelatin-sealed prosthesis also seems to be saturable, and there is probably no need to use soaking solutions containing more than 10 mg/L of rifampicin. Also, the duration of the soaking process should last no less than 15 to 25 min in order to achieve maximum absorption. Further soaking will not increase the concentration.

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