Investigation of a rifampin, fusidic-acid and mupirocin releasing silicone catheter

J.M. Schierholz*, G. Pulverer

Institute of Medical Microbiology and Hygiene, University of Cologne, Goldenfelsstr. 19-21, 50935 Cologne, Germany

Received 15 December 1997; accepted 21 April 1998

Abstract

After strict hygienical measures have been exhausted the use of plastic materials with antibacterial activity may reduce catheter related-bacterial colonization. An antimicrobial silicone catheter was investigated by HPLC-measurement, SEM, antimicrobial assays and standard biocompatibility tests. The modified catheter was highly biocompatible and the antimicrobial leaching non-toxic. The initially release rate was governed by the drug solubility in the ‘sink’ and surface loading (‘burst effect’). The second continuous period depended on the drug velocity in the silicone matrix and was extended up to 100 days with a proportionality to $J_t$ for each drug. Diffusion exponents were in range of $2 \times 10^{-8}$ to $1 \times 10^{-9}$ (cm$^2$ sec$^{-1}$). The lower diffusion exponent of mupirocin was explained by its higher cohesion energy and lower physico-chemical compatibility with the embedding silicone. The antimicrobial drugs were in a molecular-dispersed state with the silicone-matrix, whereas superficially located crystals of the antibiotics covering the catheter surface could be demonstrated by SEM. © 1998 Published by Elsevier Science Ltd. All rights reserved

Keywords: Controlled release; Hemocompatibility; Silicone catheter; Diffusion coefficients; Antibiotics; Biocompatibility; Cohesion energy

1. Introduction

Polymer-associated infections are a major problem in modern medicine [1–5], especially CSF-infections in neurosurgery with an incidence reported of 1–39% [5–9]. Preoperative, postoperative and preventive topical application of antibiotics to the wound and to the valve of prosthetic devices minimized but did not prevent bacterial colonization of the shunt systems [7]. Therefore, eradication of the microorganisms (mostly coagulase negative staphylococci (CNS)) is seldom achieved without removal of the infected device, long-term antimicrobial chemotherapy, transient external drainage, and implantation of a new shunt system [5, 7]. Various attempts to prevent shunt infection have been made, including the use of prophylactic antibiotics and modifications of technique [7–9], but infectious complications remain, causing rising morbidity and mortality. Attempts have been made to make implants resistant to infections by various methods involving different adsorption techniques of antimicrobials onto the polymeric surfaces [10–21]. One major disadvantage of such a plastic material coated with currently available antibiotics is that these complexes are unstable with rapid desorption or leaching of the antibiotics [21–23].

Prevention of colonization of polymeric surfaces by continuous release of bactericidal, highly biocompatible antimicrobial substances incorporated into polymers, has been tried as a new promising approach [11, 24–30]. However, the safety of chlorhexidine–silver–sulfadiazine coated catheters remain contradictory due to the local toxic effects of chlorhexidin in blood and nervous tissues. Silver is a highly biocompatible metal but free silver ion concentrations being bactericidal are toxic to human cell cultures [31]. Detergent agents such as TDMAC (tri-dodecyl-methyl-ammonium-chloride) and benzalkonium-chloride used for catheter-coating induce hemolysis and are deleterious to nervous tissue.

As an improvement of the rifampin-loaded CSF-shunt, which has been shown to be absolutely infection-resistant to staphylococci in vitro and in vivo [24, 32], we incorporated a highly effective and biocompatible combination of antibiotics up to 10% (wt/wt) into the silicone material.
2. Materials and methods

2.1. Materials

Rifampicin ($M_w = 823$, MMD, Darmstadt, Germany) is a competitive inhibitor of bacterial RNA-polymerase with a very good activity against Gram-positive microorganisms and a high physicochemical compatibility with the hydrophobic polydimethylsiloxane.

Mupirocin (pseudomonic-acid A, $M_w = 500$, Smithkline Beecham, Munich, Germany) has a novel chemical structure exhibiting a spectrum of activity to Gram-positive microorganism and Candida albicans. The mode of action is conferred by the inhibition of bacterial isoleucyl-t-RNA-transferase and the subsequent inhibition of RNA-synthesis. Mupirocin was applied to the insertion sides of the central venous catheters with a reduction of infected tips and in the prophylaxis for CSF-shunt infection [33].

Free fusidic acid ($M_w = 516$, Thomae, Biberach (Riss), Germany), a steroidal antibiotic is active against nearly all strains of *S. aureus* at inhibitory concentrations of 0.015–0.12 µg ml⁻¹.

The cerebrospinal-fluid shunt polymer (2 mm Ø, wall thickness 0.6 mm) was supplied by Fa. CORDIS, Haan, Germany. Its chemical composition is a polydimethylsiloxane network, filled with hydrophobized SiO₂ particles (30% (wt/wt)) and BaSO₄ (10% (wt/wt)) for X-ray detection.

2.2. Methods

2.2.1. Incorporation of the antibiotics into the polymeric materials

The solubility parameter (Hildebrand parameter) of the drugs was used to determine the most useful information for the mixing ability and thus compatibility of polymers with drugs. These parameters were calculated by reduced solubility and increments of molar attraction contributions [32, 34, 35]. Incorporation of the antibiotics into the polydimethylsiloxane matrix (5 wt% rifampicin, 1.7 wt% fusidic acid, 1.4 wt% mupirocin) by a diffusion-controlled process [32, 36] gave an anti-biotic–polymer system having reproducibly defined surface- and matrix-loading relations [32].

The concept of the drug solubility in the macromolecules [36] seems to be useful to describe the physicochemical properties of drugs with respect to the compatibility with the polymeric matrix. We chose the concept of the solubility parameter $\delta$ (cohesion energy, cohesion density, Hansen parameter). The solubility parameter of rifampin, mupirocin and fusidic acid were calculated according to Fedor et al. [32, 34, 35]. With the following equations the cohesion energies of the homomorphs $F_{d,p,h}$ are added increment by increment and divided by the total molecular volume $V$:

$$\delta_d = (\sum F_d)/V.$$  
(1)

The polar density $\delta_p$ was calculated by

$$\delta_p = (\sum F_p)^{0.5}/V.$$  
(2)

The cohesion energies of the hydrogen bonds calculation was performed by

$$\delta_H = (\sum F_x - U_H/V)^{0.5}.$$  
(3)

($F_x$ = increments of hydrogen bonds).

The partial cohesion energies $\delta_d, \delta_p, \delta_H$ were connected to the total cohesion energy:

$$\delta = (\delta_d^2 + \delta_p^2 + \delta_H^2)^{0.5}.$$  
(4)

2.2.2. Measurement of drug release kinetics of the antibiotic-containing silicone shunt

To measure the drug release kinetics, short catheter pieces (1 cm length, 0.6 mm wall thickness, cut in length to get a film-like opened hollow cylinder) were eluted in 0.9% NaCl solution at room temperature (acceptor volume 10 ml, pH 7). Constant removing, replacing fresh solvent (every 3 h in early fractions released, after this every day) and stirring was performed to arrive approximately at the diffusional ‘steady state’ and nearly ‘perfect sink conditions’. The elutions were investigated by HPLC. A high-performance liquid chromatogram two-pump system that could deliver a gradient flow was used (HP 1050). An RP₈ reverse-phase column (Nucleosil ET 200/8/4 5C8, Macherey-Nagel) and a pre-column (Nucleosil ET 200/8/4 C8 720005, Macherey-Nagel) was employed. The mobile phase was monitored with a Diode-Array-variable wavelength-UV-detector (HP 9000 Series 300) and data received by a microprocessor (HP 9000 Series 300). The mobile phase consisted of acetonitrile–MeOH–0.001 M sodium phosphate at 22°C. The solvent gradient started at a 3 : 1 : 6 ratio up to 8 : 1 : 1 with a flow rate of 1.5 ml. Pressure in the column varied starting from 190 to 150 bar. Rifampicin was detected at wavelengths of 254 and 450 nm, mupirocin at 220 nm and fusidic acid at 250 nm.

2.2.3. Calculation of diffusion coefficients

The diffusion coefficients of the diffusant migration were calculated by Eq. (6) [37, 38], when the short time approximation of fractional drug release followed Eq. (5) with $n = 0.5$:

$$\frac{M_t}{M_\infty} = k t^n$$  
(5)

$M_t / M_\infty$ is the relationship between the released amount at time $t$ and the initial loading concentration of the device. $k$ is an individual constant of each polymer/drug system. Equation (2) is a short time approximation of fractional drug release derived from the second Fickian...
law, when $M_i/M_{\infty} < 0.6$:

$$M_i/M_{\infty} = \frac{4(Dt/r^2)^{1/2}}{\Delta}$$  \hspace{1cm} (6)

$M_i$ is the cumulative rate of the released drug and $\Delta$ the thickness of the sample. The diffusion coefficient in Eq. (6) is constant with a constant slope of $M_i/M_{\infty}$; with following times of release ($M_i/M_{\infty} > 0.6$) the diffusivity decreases significantly with decreasing loading concentration [36].

2.2.4. Serial dilution or three-dimensional checker-board technique

The antibiotic concentrations to be tested ranged from 0.25 to twice the MIC. Every combination matching to each of these concentrations was given to the overnight cultures ($10^4$ CFU/ml $S$. epidermidis RP62, KH$_{11}$ and $S$. aureus 5aW 1136) achieving 36 concentrations for the threefold combination. Microtiter plates were incubated overnight and examined for growth pattern. Antimicrobial activity was exhibited at concentrations suppressing visible growth of bacteria during incubation. Each antibiotic in a combination producing the specified effect was expressed as a fraction of the concentration that produces the same effect when the antibiotic was used alone (fractional inhibitory concentration, FIC). Checker-board titration is interpreted by the sum of these fractions. For the sum ($\Sigma$ FIC) near 1, the combination acts indifferent, combinations $< 1$ exhibit synergistic effects and antagonistic cooperation is expected for a sum $> 1$ following Eq. [39, 40]:

$$\frac{[A]}{MIC_A} + \frac{[B]}{MIC_B} + \frac{[C]}{MIC_C} = FIC_A + FIC_B + FIC_C$$  \hspace{1cm} \Sigma FIC  \hspace{1cm} (7)

2.2.5. Measurement of bacterial adhesion to the antibiotic containing polydimethylsiloxanes

Antibiotic-loaded silicone samples and unmodified ones were incubated for 3 h in phosphate-buffered saline (PBS) containing the test strain $S$. aureus 5aW 1136 (isolated from an infected implant). At the equilibrium of bacterial adhesion (2 h), the contaminated samples were transferred into the proliferation medium (Müller Hinton, OXOID UK) and incubated for periods of 24, 48 and 72 h at 37°C. After each incubation period, the bacteria were removed from the polymeric surfaces by ultrasonication (Branson sonifer, Danbury USA, 90 s, 150 W) and the number of detached viable bacteria was determined by a colony count method.

2.2.6. Scanning electron microscopy (SEM)

Observations were performed with a scanning electron microscope Jeol JSM T200 and the objects investigated were gold-sputtered with an SCD 303 (Fa. Balzers Union, Liechtenstein).

2.3. Biocompatibility testing

2.3.1. C$_{3A}$-Des-Arg-ELISA

The activation of the complement system was measured by a C$_{3A}$-ELISA (ProGen, Heidelberg, FRG). 1 cm silicone catheter, 1 cm modified silicone catheter and 1 cm sterilized, modified silicone catheter ($\gamma$-irradiation) were incubated in 1 ml fresh Na$_2$EDTA plasma for 5, 15, 30 and 60 min. A standard curve was employed by 1100, 560, 337 and 150 ng ml$^{-1}$ C$_{3A}$. All samples were incubated for 1 h and o-nitrophenylidiamine was added. The reaction was stopped after 5 min with acetic acid and the substrate was measured photometrically at 496 nm.

2.3.2. Hemocompatibility (Hemolytic activity)

Silicone catheters (1 cm, silicone pure, modified silicone, modified silicone sterilized ($\gamma$)) were incubated in CH$_{50}$-Test-Tubes (Noviment, CH$_{50}$-Test, FRG) containing heparinized serum and the supernatant fluid was measured photometrically at 405 nm.

3. Results

3.1. Three-dimensional isobologram

Figure 1 shows the idealized isobolograms depicting the possible interactions between antibiotics in combination. The theoretical definition of synergy requires the FIC index to be $< 1.0$ (figure in the middle), the geometric definition is that the isobologram formed falls below the additive plane (left figure). Antagonistic combinations require FIC indices significantly higher than one.

In Fig. 2, the lowest concentration suppressing bacterial growth for each antibiotic in the combinations was expressed as the fractional inhibitory concentration (FIC). Most of the sums of inhibitory concentrations $\Sigma$FIC were smaller than one indicating synergistic action (points of the plane in the isobologram). The computer-generated three-dimensional isobologram for the isolate $S$. aureus SaW1136 sinks toward the origin (i.e. the fractional inhibitory concentration (FIC) for each drug approaches nearly 0) [40]. Antagonistic effects as shown by fractional sums significantly higher than one were not detected.

Similar results were achieved with the strains $S$. epidermidis RP62, $S$. epidermidis KH$_{11}$, and $S$. epidermidis KH$_{6}$. Mutants of resistant strains to rifampin in combination did not emerge in either the checker-board or kill-rate experiments (not shown) indicating resistance prevention [40] by this combination.
Fig. 1. Idealized isobolograms depicting three possible interactions (left: additive, middle: synergistic, right: antagonistic) between the FICs of three antibiotics (x, y, z) in combination.

Fig. 2. The computer-generated three-dimensional isobologram of the checker-board titration. Fractional inhibitory concentrations (FICs) of rifampicin, fusidic acid and mupirocin do not significantly exceed 0.5 FIC showing synergistic cooperation in vitro.

3.2. Controlled release of rifampicin, mupirocin and fusidic acid from the silicone

Perfect sink conditions were used to determine controlled release of these antibiotics (Fig. 3). The release rate of each of these antibiotics showed two significant periods. The initial release rate, the so-called ‘burst effect’, depended on the mass, distribution and solubility of antibiotic crystals on the surface of the loaded polymer as revealed by a proportionality to log t in the first 24 h [41]. The second continuous period occurred after one day and was diffusion-controlled by the structure of the
Fig. 3. Dynamic release of the antibiotic-loaded shunt reaching nearly 'perfect sink conditions' measured per day. Release pattern of each antimicrobial out of the silicone matrix is governed by Fickian diffusion processes after the 'burst effect'.

Fig. 4. Proportionality of cumulative release of rifampin to $t^{1/2}$ in the matrix-controlled period of the antibiotic-loaded silicone.

3.3. Solubility parameter (cohesion energy)

A higher cohesion energy was calculated [1–4] for mupirocin ($\delta = 25.55 \text{ MPa}^{1/2}$) in comparison to rifampin ($\delta = 20.76 \text{ MPa}^{1/2}$) [36] and fusidic acid ($\delta = 15.71 \text{ MPa}^{1/2}$). The lower compatibility of the more hydrophilic mupirocin with the lipophilic silicone ($\delta = 19.38 \text{ MPa}^{1/2}$) [36] and the lower solubility in the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Diffusion exponents $D$ (cm$^2$ sec$^{-1}$) for rifampicin, mupirocin and fusidic acid in the polydimethylsiloxan matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>Fusidic acid</td>
</tr>
<tr>
<td>$D$ (cm$^2$ sec$^{-1}$)</td>
<td>$2 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

elastomeric matrix of the filled polydimethylsiloxane [32] and proportional to $\sqrt{t}$ (Fig. 4). Antimicrobial delivery was prolonged up to 100 days in the range 1–10 $\mu$g cm$^{-2}$ d$^{-1}$.

The diffusion exponents calculated by Eq. (6) were in the range $2 \times 10^{-8}$ to $1 \times 10^{-9}$ cm$^2$ s$^{-1}$ (Table 1).
Table 2
Cohesion energies (solubility parameter $\delta$) of mupirocin derived from different homomorphs and their partial parameter $\delta_n$, $\delta_p$, $\delta_H$

<table>
<thead>
<tr>
<th>Structure units</th>
<th>$M$ (g mol$^{-1}$)</th>
<th>$V$ (cm$^3$ mol$^{-1}$)</th>
<th>$F_n$ (J cm$^3$ mol$^{-1}$)</th>
<th>$F_p$ (J cm$^3$ mol$^{-1}$)</th>
<th>$\delta_H$ (J mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$3^*\text{CH}_3$</td>
<td>15</td>
<td>31.7</td>
<td>$3^*\text{420}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$10^*\text{CH}_2$</td>
<td>14</td>
<td>16.6</td>
<td>$10^*\text{270}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$8^*&gt;\text{CH}$</td>
<td>13</td>
<td>$-1$</td>
<td>$8^*\text{80}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$-\text{OH}$</td>
<td>16</td>
<td>3.8</td>
<td>100</td>
<td>401</td>
<td>1467</td>
</tr>
<tr>
<td>$3^*\text{OH}$</td>
<td>17</td>
<td>10.5</td>
<td>$3^*\text{210}$</td>
<td>500</td>
<td>9770</td>
</tr>
<tr>
<td>cyclohexyl-</td>
<td>13</td>
<td>13.5</td>
<td>190</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$=\text{C}&lt;\text{C}$</td>
<td>12</td>
<td>$-5.7$</td>
<td>45</td>
<td>70</td>
<td>143</td>
</tr>
<tr>
<td>$\text{CH}=\text{CH}$</td>
<td>131</td>
<td>2.4</td>
<td>223</td>
<td>70</td>
<td>143</td>
</tr>
<tr>
<td>O-&gt;</td>
<td>28</td>
<td>10</td>
<td>291</td>
<td>769</td>
<td>978</td>
</tr>
<tr>
<td>COOH</td>
<td>45</td>
<td>27.8</td>
<td>530</td>
<td>420</td>
<td>4900</td>
</tr>
<tr>
<td>COO–Ester</td>
<td>44</td>
<td>8.2</td>
<td>670</td>
<td>510</td>
<td>2550</td>
</tr>
<tr>
<td>Divided by mol volume:</td>
<td>$\Sigma$: 351.6</td>
<td>$\Sigma$: 7279</td>
<td>$\Sigma$: 39493</td>
<td>$\Sigma$: 112.32</td>
<td></td>
</tr>
<tr>
<td>With Refs. [1–3]</td>
<td></td>
<td></td>
<td></td>
<td>$\delta_n = 20.68$</td>
<td>$\delta_p = 20.68$</td>
</tr>
<tr>
<td>With Refs. [4]</td>
<td></td>
<td></td>
<td></td>
<td>$\delta_H = 10.59$</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Adhesion assay of not modified and antimicrobial-loaded shunts under static and dynamic conditions (flow 2000 ml Müller-Hinton Bouillon/d)

| (CFU/cm$^2$) | $t$ (d) |  |  |  |  |
|--------------|---------|  |  |  |  |
|               | 1       | 2    | 3    | 4    | 14   |
| Static assay  |         |      |      |      |      |
| Not modified  | $2 \times 10^5$ | $3.1 \times 10^7$ | $5 \times 10^9$ | $8 \times 10^9$ | |
| Modified      | $1 \times 10^5$ | $8 \times 10^5$ | $<1$ | $<1$ | |
| Dynamic assay |         |      |      |      |      |
| Not modified  | $1 \times 10^5$ | $3 \times 10^7$ | $5 \times 10^8$ | $1 \times 10^9$ | $1 \times 10^9$ |
| Modified      | $5 \times 10^5$ | $2 \times 10^2$ | $15$ | $<1$ | $<1$ |

silicone matrix as well is a possible explanation for the lower diffusivity and the narrow loading.

3.4. Adhesion assay

Table 3 depicts the effectiveness of modified silicone devices containing high amounts of rifampicin (5% wt/wt), fusidate (2.5% wt/wt), mupirocin (2.5% wt/wt) in the stationary adhesion assay. Sterility was achieved after 24 h in contrast to the conventional shunts. In a second perfusion experiment (2.000 ml flow day$^{-1}$), sterility of antimicrobial shunts was achieved for 14 days, respectively (Table 3).

3.5. Biocompatibility testing

C$_{3A}^\text{A}$-Des-Arg-ELISA: In all samples a slight increase could be shown after 1 h incubation within a physiological range of 200–270 ng ml$^{-1}$ C$_{3A}$. The coated shunt induced no significant activation of the complement system.

3.6. Hemolysis test

C$_9$-induced hemolysis of erythrocytes is directly proportional to the activity of the overall complement. The hemolysis rate for modified catheters as well as for unmodified silicone shunts ranged from 88 to 115% of the initial hemolysis rate of the serum. The modified shunts do not induce hemolysis.

3.7. Scanning electron microscopy

In contrast to the mixture of drug crystals located on the surface (Fig. 5a), a monolithic, molecular dispersed distribution in the polymeric bulk could be demonstrated (Fig. 5b). After 24 h controlled delivery in the sink, the polymer surface appears smooth as the conventional silicone material (Fig. 5c).

4. Discussion

4.1. Choice of antimicrobial substances

Several criteria were taken into consideration to select the antimicrobials used in this study. The first was that they should be capable of molecular migration through cross-linked silicone elastomer. An index of this was their cohesion energies [32, 34, 35]. Similarity of cohesion energies is a prerequisite for incorporation of fine dispersed drugs into a polymeric matrix and for a high drug rate of diffusion out of the matrix during implantation [36]. Antimicrobials as aminoglycosides and ciprofloxacin
were excluded being not compatible with the shunt material. The second criterion was that they should be active against most strains of staphylococci, enterobacteriaceae and candida. Neither clindamycin, nor β-lactam antibiotics or fluoroquinolones as ciprofloxacin or sparfloxacin possess adequate activity against methicillin-resistant staphylococci, a major cause of catheter-associated infections. Rifampicin, highly compatible with polydimethylsiloxanes, offers advantages in treatment of staphylococcal abscesses [42], osteomyelitis [43, 44], foreign-body infection [45], endocarditis and disseminated infections [39, 46–47]. Most coagulase negative staphylococci isolated from colonized shunts are susceptible to rifampicin, and the majority are susceptible to mupirocin and/or fusidic acid. Enterobacteriaceae in general are susceptible to rifampicin and mupirocin has been shown to be active against candida in burns [49]. The third reason was that they should not have been known for a significant risk of hypersensitivity or toxicity when administered systemically in humans. Penicillin derivates, chlorhexidine and silver-sulfadiazine [31, 50, 51] were excluded for these reasons. The fourth criterion was that the antibiotics should be sufficiently stable to allow sterilization by γ-irradiation or treatment with ethylenoxide. After γ-irradiation, no drug degradation could be determined by HPLC (unpublished data). In general, antibiotics as β-lactams, aminoglycosides and gyrase inhibitors, used for therapy of bloodstream infections should be preserved for systemic application.

4.2. Controlled release pattern

The long period of slow release was chosen in order to ensure protection over the operative and immediate post-operative period. Controlled release from this matrix reservoir was typical for monolithically distributed drugs [41, 52] as shown previously for rifampicin [32]. Drug release through these intermolecular spaces between the macromolecules opened by relaxation processes is known to be governed by a Fickian diffusion mechanism with a square-root-of-time dependence at early times [37, 38] (Fig. 4). Properties such as molecular weight of the drugs were not the most influencing factors in controlling release from the silicone. Proportionality of the diffusants flux to molecular weight is in the range \( \sqrt{[\text{g mol}^{-1}]} \) and \( 1/\sqrt{[\text{g mol}^{-1}]} \). The rate-limiting step of diffusion was assumed to be the solubility of the antibiotics with the polymer [37, 38, 41, 52]. More than 50% of residual antibiotics were found after a three months release in the examined drug-polymer-alloy.

A large part of the burst effect could be removed simply by cleaning the catheter surface. However, a major part of catheter-associated infection in early times is due to contamination of skin bacteria from the host or physician during the insertion procedure. It might be assumed that most of the initially adherent bacteria are
detached and killed by the initial high delivery of the superficially located antibiotics.

4.3. Pharmacokinetical and pharmacodynamical considerations

The total concentration of antimicrobials in the modified shunts has been found to be orders of magnitudes less than a single therapeutic dose, and this is released slowly over several weeks. For this reason, major organ toxicity such as hepatic dysfunction is very unlikely. A further possible complication is the local accumulation of antimicrobials in the tissues immediately adjacent to the catheter with local toxicity or irritation. Previous animal toxicological studies have not shown any such local or systemic irritation or toxicity caused by rifampin [13]. Attempts to decrease the concentration of the most potent antimicrobial (rifampicin) led to a failure of protection for the target period [31]. Regarding the superior antistaphylococcal activity of rifampin to mupirocin and fusidic acid, most of the antimicrobial efficacity and the major part of the antimicrobial loading was performed with rifampin. The loading capacity with mupirocin (1.4% wt/wt) was limited by its lower solubility (higher hydrophilicity: \( \delta = 25.55 \text{ MPa}^{1/2} \), \( \delta_p = 20.68 \text{ MPa}^{1/2} \), \( \delta_H = 10.59 \text{ MPa}^{1/2} \)) with the silicone matrix (\( \delta = 19.38 \text{ MPa}^{1/2} \)).

4.4. Drug-dependent adverse reactions

Any coated catheter may elicit an allergic/immune response. The use of muscle relaxants in the ICU is believed to be the main source of the anaphylactoid reaction. Cross reactions between quaternary ammonium groups containing benzalkonium-coated catheters and sensitized patients should be considered. Patients sensitized to penicillin (IgE-mediated) can trigger anaphylactoid reactions (1%). The risk of severe circulation collapse is calculated to be 1:50 000. Chlorhexidine sensitization is a well-known phenomenon due to the frequent use of this disinfectant in urology. Following the growing number of anaphylactic reactions in Japan, benzalkonium groups containing benzalkonium-coated catheters were banned in 1997. Detergent agents such as TDMAC (tri-dodecyl-methyl-ammonium-chloride) and benzalkonium-chloride work by diffusing into cytoplasma membranes and have hemolytic activity. Silver is a highly biocompatible metal but free silver ion concentrations acting as bactericidal are toxic to human cell cultures [31]. We checked different concentrations of rifampin, mupirocin and fusidic acid up to 300 \( \mu \text{g mL}^{-1} \) in a mouse-fibroblast agar layer test and observed no significant proliferation inhibition (unpublished data). The substances are unlikely to induce mutagenicity or sensibilization reactions. For use of this catheter modification as a long-term venous access (Hickman- or Broviac-catheter), the hemocompatibility and device safety has to be evaluated more intensively in animal experiments.

4.5. Topical use of antimicrobial combinations—emergence of bacterial resistance?

Development of resistance in the clinical use of the modified shunt might become a problem. The internal hydrocephalus-shunt is not exposed continuously to skin flora, the external drainage is exposed to the skin. Resistance could develop at the entry site of all types of silicone catheters. Since mutations occur with a frequency of about \( 10^{-6} \) to \( 10^{-9} \), one can predict that drug resistance is most likely to emerge with high bacteria inocula. In contrast, it is extremely rare to retrieve more than \( 10^4 \) cfu from infected catheters. The fear for inducing mycobacterial resistance to rifampin-coated shunts is unlikely to materialize. In case of topical delivery from a coated catheter to blood and subcutaneous tissue, maximum amounts of release can be calculated to be <0.1% as compared to systemic administration. Resistance pressure by antimicrobial doses with magnitudes of the order below sub-inhibitory concentrations is not very probable. Resistance transfer from staphylococci to mycobacteria has not been observed clinically. Attempts to develop bacterial mutations on gradient plates with RFM (rifampicin, fusidic-acid, mupirocin)-combination failed (results not shown), resistant strains could neither be determined in the checker-board experiment nor in the killing experiment, indicating the ability of the combination to prevent the development of resistance [40]. However, combination of rifampin with trimethoprim failed in several killing experiments due to the detection of resistant staphylococci (unpublished data). During a clinical trial with a rifampin–minocyclin-coated short-term central venous catheter, no resistant staphylococci could be determined [53], supporting our suggestion, that carefully developed antimicrobial combinations incorporated in such devices as CVCs may prevent occurrence of resistant bacteria.

4.6. Previous attemps of catheter modification

Various antibiotics were bonded to catheters using benzalkonium chloride or tridodecylmethylammonium chloride (TDMAC) [16–21, 45]. This method has the disadvantage of short duration of activity, not exceeding two days [22, 23], leading to clinical failure [21]. Benzalkonium chloride, TDMAC and chlorhexidine cannot be used in contact with sensitive nervous tissue because of their high toxicity and deleterious effects to cells [50, 51]. The incorporation of the disinfectant trichlorohydroxyphenylether (Irgasan DP 300, Ciba Geigy) mixed into thermoplastics has similar adverse effects [24].

Bayston et al. developed an impregnation method claiming prolonged activity of the processed shunts [10].
They chose very small amounts of antibiotics incorporated in superfluous layers of the material. In an in vitro experiment, shunts with a discrete superficial antimicrobial coating did not withstand considerable microbial challenge [32]. Chlorhexidine–silver–sulfadiazine coating of central venous catheters has been considered as the most efficacious coating despite the high toxicity of the disinfectant. However, recent clinical study results of such coated catheters did not show significant differences between coated and noncoated catheters [55–59]. A minocycline–rifampin coating has been shown to be efficacious in vitro and in vivo [26, 53]. However, the TDMAC spacer-group for the antibiotics does not bind efficiently to hydrophobic polymers such as silicone because of its amphiphilic nature (higher cohesion energy).

Previously we could show in a bacterial infection model that implantation of rifampin-loaded contaminated devices (9% Rifampin in silicone) in the cerebrospinal ventricles of rabbits withstand successfully bacterial colonization without any clinical sign of foreign-body infection [24, 32]. Further animal studies with this broad-spectrum of antimicrobial shunts are warranted for assessing device safety and efficacy.

Acknowledgements

This study was granted by the Fritz-Thyssen-Foundation (Cologne, FRG, 1993–1994).

References
