Experimental Studies and Preliminary Clinical Trial of Vinorelbine-loaded Polymeric Bioresorbable Implants for the Local Treatment of Solid Tumors

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ABSTRACT

Vinorelbine is a new 5'-nor Vinca alkaloid, active by i.v. route, in various types of cancer disease such as non-small cell lung cancer and advanced breast cancer. In order to evaluate the possibility of using this drug for local treatment of cancer, Vinorelbine-loaded bioresorbable polymeric implants were prepared using a copolymer of D,L-lactic and glycolic acids (PLA 37.5 GA 25). According to the manufacturing process, the 1.2-mm-diameter cylindrical rods obtained had a drug content of 1, 5, or 20% (w/w) and released half of their content within about 6 days in vitro. In vivo release in rats was slower, half of the drug being released after about 14 days. A dose-dependent antitumoral effect was observed in mice (solid P388 leukemia model) when implants were administered into or in contact with the tumor. At highest drug loads and when administered soon after tumor implantation, Vinorelbine implants were more effective than i.v. administration (median survival time of treated animals relative to untreated controls, >360 versus 188). In dogs, results of toxicity experiments revealed that administration of implants in vital organs must be avoided. On the contrary, s.c. administration was well tolerated. A transient local necrosis was observed in the days following implantation, but normal skin was recovered after about 10 weeks. Thus, a clinical trial was conducted on patients with head and neck cancer; implantation of 20% loaded polymeric implants into the tumor sites succeeded in 8 of 9 patients. The sole failure was attributed to the unusual hardness of the tumor tissue. Except for a local transient inflammatory reaction (easily treated with nonsteroidal antiinflammatory agents), no other sign of toxicity was detected, and patients tolerated the device well. Fourteen days after implantation, patients underwent their planned surgery, and the implants were recovered. Residual drug content varied from 24 to 55%. In all cases, there was a clearly delimited necrotic area around the implant, ranging from 0.5 to 3.5 cm in diameter. In the smallest tumors, necrosis was also observed in the normal tissue inside this area. These results invite further studies to evaluate such drug-loaded polymeric implants.

INTRODUCTION

Two main categories of devices are currently being developed in order to administer sustained-release drugs. The first one concerns the intravascular injection of drug-loaded microparticles such as microcapsules or microspheres, with the aim of realizing chemoembolization (1–5). The second approach consists in direct implantation of a sustained-release device in the organ or tissue to be treated. Such systems have been developed for the long-term delivery of hormonal steroids and peptides (6–7). Their usefulness in experimental cancer chemotherapy has been under investigation for several years (8–14), despite the fact that local treatment of tumors is generally accomplished by surgery or radiotherapy.

The properties of natural or synthetic polymers are such that the choice of a product as an effective drug delivery system is critical, as has been discussed by many authors (15–19). Indeed, efficient local treatment of cancer by drug-containing polymeric implants requires both safe matrix components and an active antitumor drug. The chosen drug must also be compatible with the polymer. Among the numerous polymers available, PLA-GA is one of the best studied, and its biocompatible and biodegradable properties have been well documented (20–21). Several studies have used this copolymer to produce unloaded or drug-loaded microspheres (22–24). According to the physicochemical properties of the entrapped drug, the release can be controlled by varying the ratio between lactide and glycolide units in the copolymer, as was verified in a previous study with the anticancer drug cisplatin (9). Thus PLA-GA copolymers are good candidates for further investigation as implants in cancer treatment.

In our previous work (9), which was limited to the study of the in vitro and in vivo release characteristics of two PLA implants, i.e., PLA 37.5 GA 25 and PLA 75, cisplatin was chosen because of its X-ray opacity, which greatly facilitated the evaluation of the in vivo behavior of implants. Cisplatin was not selected for further development, because the molecule could be hydrolyzed in the aqueous medium diffusing in the polymeric matrix (25). Indeed, it would then be possible that the diffusing species differ from the initial ones. Therefore, we chose a more stable antitancer agent, namely Vinorelbine, a new Vinca alkaloid anticancer drug (26–28), which has physicochemical properties which might be associated with interesting release characteristics. Vinorelbine is presently used in treatments of non-small cell lung cancer and advanced breast cancer by systemic chemotherapy (29–33) and is under clinical trial for other cancer localization (34).

Thus, the present study was designed in order to evaluate thoroughly the potential usefulness in cancer treatment of a sustained-release system based on a poly(lactide-co-glycolide) copolymer, namely PLA 37.5 GA 25 (21), loaded with Vinorelbine. Accordingly, it includes the preparation of the device, its in vitro and in vivo characteristics of release, its experimental antitumoral activity in P388-bearing mice, its toxicity in dogs, and, finally, a preliminary clinical trial.

MATERIALS AND METHODS

Polymeric Matrix

The PLA 37.5 GA 25 (21) sample was obtained by ring-opening polymerization of a monomer feed composed of a 75% D,L-lactide/25% glycolide (w/w) mixture. The polymerization was carried out in bulk for 96 h at 140°C using Zn powder as the initiator. After recovering, the crude copolymer was purified twice by dissolution in acetone

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3 The abbreviations used are: PLA-GA, poly(D,L-lactide-co-glycolide) copolymer; T/C%, median survival time of treated animals relative to untreated controls; HPLC, high-performance liquid chromatography.
and precipitated by methanol. The resulting purified copolymer was characterized by size exclusion chromatography in dioxane and showed a monomodal size exclusion chromatogram with a maximum at $M$, 120,000 with respect to polystyrene standards. Molecular weight polydispersity was 1.5, and the glass transition temperature was 44°C.

Preparation of Vinorelbine-containing Implants

The PLA 37.5 GA 25 copolymer ($M$, 120,000) was dissolved at room temperature in a mixed solvent composed of chloroform and n-hexane (80:20, v/v). When the medium was homogeneous, Vinorelbine ditartrate (Pierre Fabre Médicament, Boulogne, France) was added, and the resulting mixture was stirred vigorously for 30 min. Hexane was then added dropwise to the suspension until the copolymer-Vinorelbine combination was completely precipitated. The pasty mass thus obtained (80:20, v/v). When the medium was homogeneous, Vinorelbine ditartrate (Pierre Fabre Médicament, Boulogne. France) was added, and the resulting mixture was stirred vigorously for 30 min. Hexane was then added dropwise to the suspension until the copolymer-Vinorelbine combination was completely precipitated. The pasty mass thus obtained was kneaded in order to eliminate most of the entrapped solvent. Residual solvent was finally evaporated in a vacuum drier for one night at 50°C. The resulting solid matrix was extruded through a 1.2-mm heated die (70–80°C) in order to make cylindrical rods. The polymer-drug combination never remained more than 1 or 2 min in the heated apparatus. Rods were allowed to cool to room temperature, and implants (designated as type E) were cut from the rods according to the desired drug dose. They were finally stored in a light-protected sterilized dessicator in order to avoid any moisture. All manipulations and preparations were done under sterile conditions.

In preliminary studies, cylindrical rods were handmade (since the heated die was not yet available). These devices were designated as type H implants.

HPLC Determination of Drug Load and Stability

Vinorelbine-containing implants were dissolved in 2 ml of dichloromethane. Vindoline (Pierre Fabre Médicament) was added in known amounts to serve as an internal standard. The HPLC apparatus consisted of a constant flow-rate pump (6000A; Waters) a Lichrospher 100-RP-8 (5 μm) column (Merck), and an UV detector (Uvicord; LKB) (λ = 254 nm). The mobile phase was an admixture of 100 mM KCl in diluted HCl (pH 2.5), methanol and tetrahydrofuran (69:19:12, v/v/v), at a flow rate of 1.0 ml/min. In these experimental conditions, retention times were 15 min for Vinorelbine and 10 min for the internal standard Vindoline.

In vitro Release Studies

Type H and type E implants were allowed to diffuse in an aqueous solution of 9 g/liter sodium chloride heated at 37°C. After several periods of incubation (from 1 to 25 days), implants were dried and weighed. Vinorelbine content was determined after dissolution in dichloromethane, according to the HPLC method previously described.

In vivo Release Studies

Type H implants were studied in Wistar male rats (200–300 g), using radioactive Vinorelbine prepared with a trace amount of tritiated Vinorelbine (specific activity, 5 Ci/mmol) (C.E.A., Gif sur Yvette, France). Devices were implanted under general anaesthesia with pentobarbital i.p. (6 mg/100 g). A 10-mm inguinal incision was made to expose the gracilis muscle and, after creation of a small pocket, a 16-gauge needle containing the implant was inserted into the muscle. The implant (2 mm long, 1.2 mm diameter) was expelled from the needle under microscopic control, and the incision was closed with nonresorbable sutures. At different time intervals postoperatively (days 1 to 21), excision of the entire gracilis muscle was performed under anaesthesia, and implants were removed, dried, and weighed. Fragments of muscle were also biopsied at different distances from the implants, i.e., in contact with and at 2, 5, and 7 mm from the implant. For assays, implants were dissolved in dichloromethane as previously described, and muscle samples were digested in a 4 M sodium hydroxide solution for 24 h. Radioactivity of the resulting solutions was then measured using a scintillation spectrometer (Beckman). Portions of the excised muscles were also submitted for histological examination.

Antitumoral Activity in Mice

Generation of P388 Solid Stock Tumor. Subcutaneous solid tumors were obtained by inoculating 0.2 ml of diluted ascitic fluid containing 2 × 10⁶ cells (drawn from a leukemia mouse) into the axilla of a BDF mouse (Iffa Credo, Saint-Germain sur l’Arbresle, France). The mouse was sacrificed after 8 days, when the tumor diameter reached about 1 cm. The excised tumor was then cut into 2-mm cubic fragments. These tumor fragments (median weight, 25 mg; range, 10–37 mg) were introduced, under general anesthesia, into the axilla of BDF mice (median weight, 20.9 g; range, 14.8–25.5 g), by driving the fragment with forceps through an incision made at 1 cm from the axilla. Incisions were closed with nonresorbable sutures. The day of the implantation was designated as day 0.

Implantation of the Polymeric Devices. By using the incision previously made to insert the tumor fragment, implants were placed either in contact with the tumoral tissue in the case of small tumors (early treatment) or in a slot inside the tumor mass for larger tumors.

Quantification of Effect. Results were reported as T/C%. Antitumor efficacy was evaluated until day 45. Tumor areas were calculated using the product of the two largest perpendicular dimensions on the surface of the tumor mass. Histological examination of tumoral tissues was also performed in some cases.

Intrinsic toxicity of nonloaded polymeric implants (types H and E) was evaluated both by survival time of implanted mice and by their daily weight evolution.

In all cases, median and range were specified. Comparison between groups and correlation testing were performed using nonparametric methods (Mann-Whitney U-test, Spearman rank correlation coefficient).

Toxicity Studies in Dogs

Type E Vinorelbine-containing implants (5 mm long, 1 mm diameter) were inserted under general anesthesia (nitrous oxide, pentothal 38 mg/kg, ethane, oxygen) in different organs and tissues of healthy mongrel dogs (25–50 kg): liver, pancreas, kidney, spleen, muscle, bone, perivascular tissues, and skin. Each implant was kept in position using wire which also facilitated subsequent identification. Toxicity was evaluated both by observation of clinical symptoms and by measurements of several blood parameters: urea, creatinine, bilirubin, alkaline phosphatase, and WBC counts. Local toxicity was determined by macroscopic and histological examination of tissues and organs after death or sacrifice of the animals.

Clinical Trial

The patient group consisted of 9 consecutive patients with carcinoma of the head and neck. The median age was 56 years (range, 42–62 years). The criteria for entry into this study were histologically confirmed carcinoma, life expectancy greater than 2 months, performance status between 0 and 3 (WHO classification), and absence of severe hematological or renal impairment. Finally, all patients selected for this treatment had been previously scheduled for surgical excision of their tumor. Written informed consent was obtained from all patients, and the study had been approved by our Ethical Committee.

Of the patients previously treated with systemic chemotherapy, none sustained biological toxicities ≥2 (WHO classification). Five patients had received previous radiotherapy in the region to be implanted; two patients had received no previous treatment.

Two weeks before surgery, implantation of the polymeric device (type E implants containing 20% Vinorelbine) was performed using a needle with an introducer to insert the implant (1.2 mm diameter) into the tumor mass. The length of the implant was adjusted to the tumor size in order to preserve a 4–5 mm margin at each extremity of the tumor mass. Table 1 summarizes the localization for each patient of the tumor mass to be implanted, its volume at the time of implantation, and the depth of the implant site relative to the skin level.

Surgical excision was performed 14 days after implantation of the device. The implant was recovered from the tumor mass to measure the amount of residual Vinorelbine, while the tumor was submitted for histological examination.
Preparation and Release Properties of Vinorelbine-containing Implants

Preparation. For both type H and type E implants, three different Vinorelbine loads were tested, starting from solutions containing 1%, 5%, and 20% drug with respect to the polymer (w/w). Preparation resulted in hard cylinders at room temperature, with quantitative incorporation of the drug load in the polymeric matrix [19.6 ± 0.5% (SD) for a 20% load]. HPLC experiments revealed no apparent degradation of the drug due to the preparation methods. Studies of stability with time indicated no apparent loss of drug or polymer degradation until at least 2 months of storage in a light-protected dessicator. On the contrary, implants stored in a humid atmosphere became soft and increased in volume. Higher drug loads could be achieved, but the resulting devices appeared to be unsuitable, because they exhibited important loss of mechanical properties.

In Vitro Profile of Release. In physiological sodium chloride solution at 37°C, 50% of the total amount of Vinorelbine was released within 6 days, and 75% was released within 12–15 days for implants containing 20% (w/w) Vinorelbine. No significant difference in release was detected between type H and type E implants. In both cases, a slight increase in implant volume was observed within the first days of incubation. After 30 days of incubation, the weight loss of the implants corresponded approximately to the initial amount of incorporated drug.

In Vivo Profile of Release. Release of Vinorelbine was also measured in vivo in rats. Type H implants containing 5% Vinorelbine and small amounts of [3H]Vinorelbine as a tracer were introduced into muscle. The median weight of the implants was 2.6 mg. Fig. 1 shows the percentage of residual radioactivity in the implant measured at different times after administration. Experiments show that half of the implant content was released between 10 and 20 days after administration. Extrapolating these results led us to expect a complete drug delivery in 6 or 8 weeks.

Table 2 gives radioactivity measurements from tissue samples located near the implants, at three different time intervals. Despite the spreading of data points, tissue concentration appeared to strongly depend on the distance between the implant and the sample site. At 7 mm from the implant, the radioactivity was negligible. The drug concentration in tissues located close to the implants increased from day 1 to day 10. However, no significant accumulation of drug occurred with time in the tissue surrounding the implant.

Antitumoral Efficacy on the P388 Murine Solid Leukemic Model in Mice

Validation of the Model. All nontreated control mice (n = 55) developed a s.c. solid tumor when inoculated with a freshly excised tumoral fragment. Table 3 shows tumoral evolution in the absence of any treatment, characterized by a median survival time of 12.5 days (range, 11.0–14.0 days). Statistical comparison of weights between subgroups of nontreated mice with different survival times indicates that neither initial animal weight nor initial amount of inoculated tumor influences survival, at least within the considered weight ranges. In the absence of any treatment, the median value of the apparent tumor area, measured at death, was 1.9 cm² (range, 0.8–3.5 cm²). Histological examination of nontreated animals at death revealed the presence of multiple metastatic cells in all the organs studied (liver, spleen, kidney, etc.). Table 3 also summarizes the results of different standard schedules of i.v. treatments with injectable 5-fluorouracil or injectable Vinorelbine taken as references for later experiments with implants. Vinorelbine antitumoral efficacy was comparable to that of 5-fluorouracil when administered i.v. soon after tumor inoculation (T/C% = 152% with 24 mg/kg administered on day 1; T/C% = 148% with the same dose administered on day 5). But Vinorelbine had no effect on survival time when administered...
VINORELBINE POLYMERIC IMPLANTS FOR THE TREATMENT OF TUMORS

Table 3 Description of tumor evolution in the P388 solid murine leukemia model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (days)</th>
<th>T/C (%)</th>
<th>Tumor surface at death (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated control</td>
<td>12.5 (11.0–14.0)</td>
<td>1.9 (0.8–3.5)</td>
<td></td>
</tr>
<tr>
<td>i.v. 5-fluorouracil</td>
<td>19.0 (19.0–20.0)</td>
<td>152 ND</td>
<td></td>
</tr>
<tr>
<td>60 mg/kg/d, D6-10-14</td>
<td>19.5 (19.0–22.0)</td>
<td>156 ND</td>
<td></td>
</tr>
<tr>
<td>i.v. Vinorelbine</td>
<td>19.0 (17.0–21.0)</td>
<td>152 1.1 (1.0–2.4)</td>
<td></td>
</tr>
<tr>
<td>24 mg/kg, D1</td>
<td>18.5 (17.0–24.0)</td>
<td>148 2.0 (0.3–2.3)</td>
<td></td>
</tr>
<tr>
<td>24 mg/kg, D5</td>
<td>12.0 (11.0–13.0)</td>
<td>96 1.8 (1.4–2.7)</td>
<td></td>
</tr>
<tr>
<td>12 mg/kg, D6-10-14</td>
<td>20.5 (17.0–24.0)</td>
<td>164 ND</td>
<td></td>
</tr>
<tr>
<td>12 mg/kg, D1-5-9</td>
<td>23.5 (9.0–29.0)</td>
<td>188 ND</td>
<td></td>
</tr>
</tbody>
</table>

Data points are the median and extrema values.

T/C% were calculated as 100 × (median survival time of test animals)/ (median survival time of nontreated animals).

Apparent tumor surfaces were evaluated by the product of the larger axis and the perpendicular axis.

All i.v. treatments were administered in the tail vein.

ND, not determined.

Table 4 Effect of Vinorelbine-containing PLA 37.5 GA 25 implants on P388 solid murine leukemia model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (days)</th>
<th>T/C (%)</th>
<th>Tumor surface at death (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonloaded implants</td>
<td>13.0 (11.0–16.0)</td>
<td>104 1.9 (1.2–3.6)</td>
<td></td>
</tr>
<tr>
<td>1% loaded implants</td>
<td>17.0 (15.0–18.0)</td>
<td>136 ND</td>
<td></td>
</tr>
<tr>
<td>5% loaded implants</td>
<td>&gt;45.0 (17.0–45.0)</td>
<td>&gt;360 ND</td>
<td></td>
</tr>
<tr>
<td>20% loaded implants</td>
<td>&gt;45.0 (16.0–45.0)</td>
<td>&gt;360 0.0 (0.0–1.2)</td>
<td></td>
</tr>
<tr>
<td>Implantation on D1</td>
<td>15.5 (13.0–20.0)</td>
<td>124 0.5 (0.0–0.6)</td>
<td></td>
</tr>
<tr>
<td>Implantation on D6</td>
<td>13.0 (12.0–16.0)</td>
<td>104 2.8 (1.4–3.8)</td>
<td></td>
</tr>
</tbody>
</table>

See Table 3 for explanations.

later (T/C% = 96% after administration on day 10). Multiple injections of Vinorelbine allowed administration of larger cumulative doses and resulted in a slight increase in the survival time (T/C% = 188% with injections of 12 mg/kg/day on days 1, 5, and 9).

Toxicity of Nonloaded Implants. Both unloaded type H and type E implants had no effect on the survival time of P388 leukemic mice (Table 4, T/C% = 104%) or on the apparent tumor area (median, 1.9 cm², range, 1.2–3.6 cm²). In addition, histological examination led to results comparable to those found for nontreated mice. No other apparent toxicity could be assigned to the polymer matrix itself.

Antitumor Efficacy of Vinorelbine-containing Implants on P388 Leukemia. A significant increase in survival time was caused by all the Vinorelbine-containing implants when implantation was performed within the first period of the tumor evolution (Table 4). One % loaded implants were the least active (T/C% = 136%). When implanted on day 1, 5% and 20% loaded implants resulted in T/C% values >360 in each case and cured more than half of the mice. The numbers of long-term survivors were 4 of 6 and 18 of 24, respectively. Type H and type E implants behaved similarly, so that the results have been grouped in Table 4 for the sake of clarity. The antitumoral effect of these implants is also demonstrated on Fig. 2, with reference to a nontreated tumor.
Table 5 Effect of combination of 20% Vinorelbine-containing PLA 37.5 GA 25 implants and i.v. administration of Vinorelbine on P388 solid murine leukemia model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (days)</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant on D6 + i.v. 12 mg/kg on D6</td>
<td>21.5 (18.0-24.5) (n = 6)</td>
<td>172</td>
</tr>
<tr>
<td>Implant on D7 + i.v. 24 mg/kg on D6</td>
<td>7.0 (7.0-27.0) (n = 6)</td>
<td>56</td>
</tr>
<tr>
<td>Implant on D6 + i.v. 12 mg/kg, D6-10-14</td>
<td>20.0 (17.0-24.0) (n = 12)</td>
<td>160</td>
</tr>
<tr>
<td>Implant on D6 + i.v. 12 mg/kg, D1-5-9</td>
<td>10.0 (10.0-12.0) (n = 3)</td>
<td>80</td>
</tr>
</tbody>
</table>

*See Table 3 for explanations.

Table 6 Toxicity in dogs of Vinorelbine-containing PLA 37.5 GA 25 implants

<table>
<thead>
<tr>
<th>Dog</th>
<th>% of Vinorelbine/implant</th>
<th>Total no. of implants</th>
<th>Sites of implantation</th>
<th>Lethal toxicity (survival time in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>7</td>
<td>Liver, pancreas, kidney, bone, skin</td>
<td>Yes (2)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>6</td>
<td>Liver, pancreas, kidney, bone, skin</td>
<td>Yes (5)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4</td>
<td>Kidney, spleen, muscle, bone</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>1</td>
<td>Liver</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>2</td>
<td>Skin</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>5 and 1</td>
<td>2</td>
<td>Skin</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>5 and 1</td>
<td>2</td>
<td>Skin</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 7 Measurements of the necrotic area and of the amount of released Vinorelbine in patients receiving intratumoral 20% Vinorelbine-loaded implants (Type E) for 2 weeks

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Amount of Vinorelbine administered by implant (mg)</th>
<th>% of released Vinorelbine</th>
<th>Diameter of the necrotic area (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.90</td>
<td>76</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>1.10</td>
<td>70</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>57</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>51</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>1.10</td>
<td>46</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>0.83</td>
<td>45</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>1.15</td>
<td>58</td>
<td>3.5</td>
</tr>
<tr>
<td>9</td>
<td>0.85</td>
<td>54</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Evaluated by histological examination.

Twenty % loaded implants placed on day 6 increased the survival time to a lesser extent (T/C% = 124%), although significant reduction of the tumoral mass was still observed (median, 0.5 cm²; range, 0-0.6 cm²). Administration of the same implants on day 10 appeared to be ineffective. The combination of 20% implants on day 6 or day 7 with i.v. Vinorelbine administration led to different results, according to the schedule used (Table 5). An improvement in antitumoral efficacy was observed when i.v. administration was performed at the same time or after implantation (T/C% = 172%, with 12 mg/kg i.v. on day 6), whereas lethal toxicity occurred when i.v. administration preceded implantation (T/C% = 56% for i.v. administration on day 6 and implantation on day 7).

Toxicity in Dogs

Simultaneous administration of 20% loaded type E implants in vital organs of dogs resulted in early death (Table 6, dogs 1 and 2). Blood analyses before death revealed severe increases in WBC counts, urea, creatinine, and alkaline phosphatase. Histological examination of the organs showed massive necrosis in the vicinity of implants.

Lethal toxicity seems to be dose dependent since less heavily loaded implants (5% Vinorelbine) did not lead to death (Table 6, dogs 3 and 4), even when administered in major organs.

Administration s.c. caused no major toxicity regardless of the implant loading (Table 6, dogs 5 to 7). However, local necrosis was observed, especially for 20% loaded implants. Such toxicity was always transient, and previously healthy skin healed after about 10 weeks.

Clinical Trial

Implantations of type E 20% loaded polymeric devices into the tumor mass (epidermoid carcinoma) were readily and easily performed for all patients, except for patient 6, for whom the presence of a postradiotherapeutic neck fibrosis prevented needle introduction into the tumor mass. Lengths of implants ranged from about 7 to 10 mm, which corresponded to a Vinorelbine dose of 0.83 to 1.25 mg (Table 7).

Local Tolerance. The first patient entered in the study developed a significant local inflammatory reaction early in the first days after implantation. This local reaction rapidly improved with treatment by a nonsteroidal antiinflammatory agent (Ketoprofene). Antiinflammatory treatments were thus systemically prescribed for subsequent patients. Despite this treatment, only patient 3 did not reveal local inflammatory reaction.

General Tolerance. No sign of general toxicity was observed for any patient, as indicated both by biological analysis and clinical examination performed each day (data not shown). Only patient 1 had a febrile reaction, probably secondary to local inflammation.

Clinical Response. While patient 2 demonstrated a significant decrease in tumor size at the time of surgical intervention, all other patients had an increase in the tumor volume, associated with a change in the consistency of the tumor mass, which appeared as a fluctuating mass. Macroscopic examination of the tumors after surgical excision revealed the existence of a circular necrotic area centered on the implant. This necrotic area had a diameter ranging from 0.5 to 3.5 cm (Table 7) and consisted of both coagulation and liquefaction types of necrosis, as determined by histological examination of the excised tumors (Fig. 3). There was frequently a transitional zone between necrosis and viable tumor, composed of dystrophic squamous carcinomatous cells. The cytoplasm and nuclei of these cells were enlarged, and some giant tumoral cells were observed. Mitosis figures were less numerous. A peripheral ring of viable tumor was observed in all cases. In some cases, the necrotic zone had reached the surrounding normal tissue, damaging the peripheral healthy cells.

Characteristics of Removed Implants. The implants recovered from the tumor mass after surgical intervention exhibited an increase in volume, with a diameter of about 1.5 mm, but were not dramatically altered with regard to their external aspect. Measurements of residual Vinorelbine in the implants indicated that total drug release after 2 weeks ranged from 45 to 76% of the total amount administered (Table 7).

DISCUSSION

The PLA 37.5 GA 25 copolymer selected to serve as the matrix in the investigated sustained release system is a member of the family of biocompatible and bioresorbable aliphatic polyesters derived from lactic and glycolic acids. The selection was made on the basis of its specific physical, thermal, and biological properties in order to achieve the best compromise with prerequisites of the application. PLA 37.5 GA 25 is a glassy and thus rigid polymer at room temperature, since its glass transition temperature is in the 40–45°C range. However, it is a viscous
Fig. 3. Histological examination of the antitumoral effect of Vinorelbine-loaded PLA 37.5 GA 25 implants on human head and neck tumors. Examination was performed on samples taken at the time of tumor exeresis (14 days after implant administration) and stained with hematoxylin and eosin. A, macroscopic photograph of a tumor sample, performed just after exeresis. The excised tumor was cut into two parts relative to the implantation site. B, same tumor sample. The implant cavity is surrounded by a circular necrotic area. Peripheral viable tumor is visible on the right (arrow). H&E, ×6. C, same tumor sample. Left to right, implant cavity necrosis, fibrotic and dystrophic zone, and viable tumor. H&E, ×25. D and E, the last two areas at higher magnitude. D, Left, necrosis with cellular remains; right, transitional zone with altered, dystrophic squamous carcinomatous cells with cytoplasmic and nuclear enlargement. H&E, ×250. E, viable squamous cell carcinoma. Solid clusters of moderately differentiated nonkeratinizing squamous cell carcinoma are well preserved. H&E, ×250.
liquid at low temperatures. This property allows easy and drug-respecting processing. As Vinorelbine is not soluble in the polymer, the glass transition temperature of the drug/matrix mixture is unchanged, and thus the drug delivery needle-like system is also rigid at room temperature and can be easily implanted in the solid tumor masses. Furthermore, PLA 37.5 GA 25 is known to degrade rather rapidly. Generally, no solid residue is detectable after 6–8 weeks (21). Another advantage is that the system softens at body temperature, which is close to the glass transition. This allows fast absorption of water, a factor which is significant for the release of the drug.

The combination of Vinorelbine with the PLA 37.5 GA 25 copolymer, obtained by mixing a solution of the polymer with the solid drug, results in easily produced and reproducible monolithic systems with an averaged dispersion of the solid drug particles in the polymeric matrix. The load of drug can be set by the relative proportion of polymer and drug in the initial solution. The maximum loading that allows practical use is 20% (w/w), since higher contents of drug lead to some decreases in mechanical properties of the devices. The solid-drug-containing polymeric matrix can be shaped in cylindrical implants either manually (type H) or by using an extrusion mold (type E), without significant modification in the Vinorelbine release properties. Both types of implants release one-half of their drug content within about 6 days in vitro.

In vivo release in rat muscle is slower than in in vitro experiments, as demonstrated in Fig. 1, which depicts results obtained from titrated Vinorelbine in type H implants. In this case, one-half of the drug content is released within about 14 days. The distribution of Vinorelbine in tissues around the implant indicates an increase of tissue exposure to the drug, rather than an effect of peak concentration. This is evidenced by results in Table 2 which show no drug accumulation in the surrounding tissues, although almost all the initial drug load has been released from the implant. Vinorelbine probably diffuses continuously from the implant to the whole body, but the slow release rate avoids any enhancement of local drug concentration.

Insertion of nonloaded implants adjacent to s.c. tumors (solid P388 murine leukemia) in mice does not influence the survival time (Table 4), the tumor development, or the evolution of animal weight (data not shown). This confirms the absence of acute toxicity due to the polymeric matrix itself. In contrast, Vinorelbine-containing implants reveal potential beneficial effects both on the survival time and on the local evolution of the solid tumor (Table 4). These effects are dose dependent, as revealed by T/C% values for 1% loaded implants (136%) and 5% or 20% loaded implants (>360%) administered on day 1. The delay between tumor implantation and treatment plays a decisive role on efficacy, since 20% loaded implants administered late in the course of treatment (day 6) are unable to cure mice (T/C% = 124%), in contrast to those given on day 1. However, it is of value to note that the tumor size at death in the case of a 20% implant administered on day 6 is significantly smaller than in the case of nontreated mice. This feature is probably related to the well-known metastatic behavior of the P388 model. Thus, the death of animals treated by implants is actually due to metastatic dissemination rather than to local growth of the tumor. When the implant is introduced into the tumor on day 6, the tumor volume does regress; however, the animals still die of metastatic disease. In contrast, when administered on day 1, the implant is able to kill all tumor cells before dissemination begins.

Improvement of the effect of the local treatment beginning on day 6 is obtained by additional i.v. Vinorelbine only when i.v. administration follows or is performed at the same time as implant administration. In this case, both reduction of tumor mass and delay in dissemination are observed (Table 5). When i.v. administration precedes treatment by implant, lethal toxicity is observed and mice die before nontreated animals. This can be explained by the toxic effect of i.v. Vinorelbine which diminishes the ability of animals to survive surgical implantation.

Results in mice more accurately define the potential interest of treatment with drug-loaded implants. They could be used in the treatment of small tumors without any local extension or distant metastases or as adjuvant therapy to decrease large tumor masses.

Additional studies on implant toxicity have revealed a lethal toxicity when highly loaded Vinorelbine implants are administered in the vital organs of healthy dogs (Table 6). Conversely, s.c. administration does not result in severe toxicity. As a result of these observations, clinical trials should not be attempted on tumors located within vital organs.

The best way to evaluate the short-term effects of Vinorelbine-loaded implants in humans, without any therapeutic disadvantage for patients, is to administer the polymeric devices before a planned surgical excision. With this in mind, a clinical trial was designed for patients with carcinoma of the head and neck. This tumor site is well suited to such a clinical trial, since it does not involve any vital organs and since it allows easy access for implant positioning.

The clinical trial revealed the feasibility of this new method in cases where the delivery system can be easily inserted into the tumor. (The only failure was in a patient with a very fibrotic neck secondary to radiotherapy.) No toxicity was observed, except for local inflammatory reactions which were treated by nonsteroidal antiinflammatory agents. Following tumor excision, macroscopic examination of implants revealed an increase in volume (from 1.2 to 1.5 mm in diameter), comparable to that observed during in vitro studies in physiological solution. This phenomenon is typical of such polymeric matrices and corresponds to a swelling process caused by water. Likewise, the total amount of released Vinorelbine after 2 weeks of implantation (45 to 76%; Table 7) is of the same order in humans as that obtained in vitro (70% within 15 days). These results confirm that in vitro release studies of such polymeric devices are of value in predicting in vivo behavior.

Histological examination of freshly excised tumors revealed the existence of a necrotic area around the implant (range of extent, 0.5–3.5 cm; Table 7), showing clearly a cytotoxic effect of Vinorelbine. Also, a relationship between the percentage of drug released and the diameter of the necrotic area might exist, as suggested by the Spearman rank correlation coefficient, which is near the limit of significance (P = 0.08).

Thus, Vinorelbine-loaded implants can induce a marked antitumoral effect, leading to massive necrosis around their implantation site, without serious systemic side effects. Nevertheless, three major characteristics of this treatment should be underlined. First, necrosis appears rapidly in the early days after implantation of the polymeric device, as shown by local inflammation. Second, the necrotic areas are always limited to a spherical zone around the implant. Thus, precise positioning of the implant results in a controlled effect. However, it also implies that several devices must be implanted in the case of large tumors. The third point concerns the apparent nonspeci-
ficity of Vinorelbine action. Indeed, normal tissues surrounding the tumor are also adversely affected by the treatment in cases where implants are located at the periphery of the tumor mass. This phenomenon may be explained by very high local drug concentrations, which are toxic to normal tissues. Consequently, very careful positioning of the implant is required, especially in the case of small tumors.

In conclusion, this study demonstrates a cytotoxic effect of Vinorelbine-loaded polymeric matrices based on poly(lactide-co-glycolide) implants which are directly inserted into a tumor mass. Studies focusing on their long-term effects and use of implantable drugs with different mechanisms of action should help pave the way for a new therapeutic modality in the treatment of cancer.

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Experimental Studies and Preliminary Clinical Trial of Vinorelbine-loaded Polymeric Bioresorbable Implants for the Local Treatment of Solid Tumors

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