Turning Low-Molecular-Weight Drugs into Prolonged Acting Prodrugs by Reversible Pegylation: A Study with Gentamicin

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Pegylation is a powerful technology to prolong the action of proteins in vivo, but it is impractical for low-molecular-weight (LMW) drugs, which are usually inactivated upon such modification. Here, we have applied a recently developed strategy of reversible pegylation to gentamicin, a LMW antibiotic. Variable length polyethyleneglycol (PEG-SH) chains were covalently linked to gentamicin using two heterobifunctional agents, each containing a spontaneously hydrolyzable bond. The inactive derivatives regained full antibacterial potency upon incubation under physiological conditions in vitro, and following systemic administration to rats, they released native active gentamicin with half-lives 7- to 15-fold greater than those of systemically administered nonderivatized gentamicin. In conclusion, reversibly pegylated prodrug derivatives of gentamicin were found to be capable of releasing gentamicin for prolonged periods in vivo. Most importantly, the major drawback of conventional pegylation, namely, the loss of pharmacological potency following irreversible derivatization, has been overcome.

Introduction

Most low-molecular-weight (LMW) drugs are short-lived species in the circulatory system, being rapidly eliminated by glomerular filtration in the kidney.[1] This therapeutic drawback is even more severe when such drugs cannot be absorbed orally and therefore must be introduced directly into the circulatory system. It is also a major obstacle for drugs having high indices of toxicity, which therefore should be present in the circulation at low but therapeutic levels for long durations.

Pegylated therapeutic proteins often exhibit clinical properties superior to those of their respective unmodified proteins. Pegylated proteins resist proteolysis, show increased solubility, and are shielded from the immune system.[2–4] Most notably, pegylated proteins turn into long-lived species in vivo, a feature attributed predominantly to decreased clearance rate of the conjugates from the circulatory system by kidney filtration.[3–6] However, substantial prolongation in vivo requires the conjugation of several LMW PEG[a] chains or a single high-molecular-weight (HMW) PEG. This covalent introduction of PEG chains to protein drugs often results in a marked reduction in biological/pharmacological potency. For example, a currently used 40 kDa PEG-interferon-α2A exhibits only 7% of the activity of the native cytokine, thus necessitating the administration of high doses.[7]

We have recently developed an approach, termed reversible-pegylation, and demonstrated its ability to prolong the actions of peptide and protein drugs, all of which undergo substantial inactivation by conventional pegylation (refs 8–11, reviewed in ref 12). Here, we investigated whether this approach could be extended to protract the action of LMW drugs in vivo. We, a priori, assumed that with regard to this drug category, conventional pegylation would, more often than not, result in an inactive product, thereby limiting the usefulness of this approach.

As a prototype for this drug category, we selected gentamicin, a LMW nonorally absorbed agent used for the treatment of many serious Gram-negative bacterial infections.[13] Since a major fraction of systemically injected gentamicin reaches the urine within a short period after administration (i.e., this study), we have developed noninvasive procedures that enabled us to investigate several pharmacokinetic parameters, including the time in which the reversibly pegylated gentamicin derivatives release active gentamicin.

Experimental Section

Materials. Gentamicin sulfate, cystamine-di-HCl, dithiothreitol, and 5,5′-dithiobis (2-nitrobenzoic acid) were purchased from Sigma. PEG_{40}-OSu (Y-shape PEG-NHS ester, MW 40 kDa, Y-NHS-40K) was the product of Jenkem Technology Co. (Allen, TX). PEG_{20}-SH (CH_3O-PEG_{20}-SH) was purchased from RAPP Polymere GmbH (Tubingen, Germany), and PEG_5-MAL (mPEG-maleimide, MW 5000) was from Shearwater Group Inc. (Ra'anana, Israel). All other materials used in this study were of analytical grade.

Chemical Procedures. The syntheses of 9-hydroxymethyl-2(amino-3-maleimidopropionate)fluorene-*N*-hydroxysuccinimide ester (MAL-Fmoc-OSu) and of 9-hydroxymethyl-7-(amino-3-maleimidopropionate)-2-sulfo-*N*-hydroxysuccinimide (MAL-FMS-OSu) are described in detail in ref 8. Both syntheses were initiated from 9-hydroxymethyl-2-aminofluorene, following three and four steps, respectively, with overall yields of 84% and 65%, respectively.

PEG_{40}-SH. PEG_{40}-SH was prepared by dissolving PEG_{40}-OSu at a concentration of 40 mg/mL in 0.1 M $NaHCO_3$ containing 1 M

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[a] Abbreviations: PEG, polyethylene glycol; IC_{50}, inhibitory concentration (half-maximal) needed to arrest *E. coli* replication; OD, optical density; PBS, phosphate buffered saline; MAL-Fmoc-OSu, 9-hydroxymethyl-2-(amino-3-maleimidopropionate)fluorene-*N*-hydroxysuccinimide; MAL-FMS-OSu, 2-sulfo-9-hydroxymethyl-7-(amino-3-maleimidopropionate)-fluorene-*N*-hydroxysuccinimide; PEG_{40}-SH, a 40 kDa branched polyethylene glycol linked to cystamine; PEG_5-SH, a 5 kDa polyethylene glycol chain containing a sulfhydryl moiety; PEG_{20}-SH, a 20 kDa polyethylene glycol chain containing a sulfhydryl moiety; DTNB, 5,5′-dithiobis(2-ntirobenzoic acid); HSA, human serum albumin; PEG_5-, PEG_{20}-, PEG_{40}-Fmoc-gentamicin, 1:1 conjugates of PEG_5, PEG_{20}, and PEG_{40}-SH linked to gentamicin through MAL-Fmoc-OSu, respectively; PEG_5, PEG_{20}, PEG_{40}-FMS-gentamicin, 1:1 conjugates of PEG_5, PEG_{20}, and PEG_{40}-SH linked to gentamicin through MAL-FMS-OSu, respectively.

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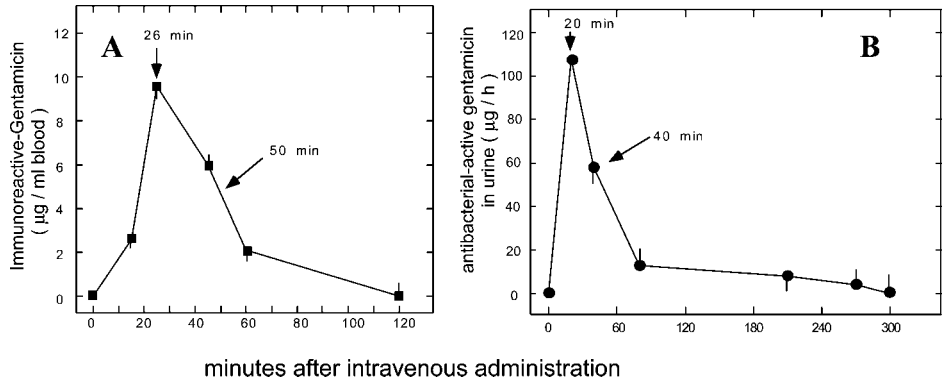

Figure 1. Constructing pharmacokinetic profiles (PK) for intravenously administered gentamicin. Two groups of rats (A and B, $n = 4$ per group) received gentamicin intravenously (0.4 μmol/rat). In group A, blood aliquots were drawn at the indicated time points and assayed for immunoreactive gentamicin. Each point is the arithmetic mean ± SEM from four rats. In group B, rats were placed in metabolic cages and urine fractions were collected immediately after excretion and each quantitated for gentamicin content. The PK profile was constructed according to the protocol described in the Experimental Section.

cystamine-di-HCl. The reaction was carried out for 2 h at 25 °C. The product obtained was dialyzed overnight against 0.1 M $NaHCO_3$, treated with 30 mM dithiothreitol (25 °C, 1 h), and redialyzed against 10 mM HCl−10 mM ascorbic acid. The PEG_{40}-SH obtained contained 1 mol of sulfhydryl moiety per mol of PEG_{40} as determined by reaction with DTNB. The product was lyophilized and kept at 7 °C until used.

PEG_5-SH. PEG_5-SH was prepared by dissolving PEG_5-MAL in water at 20 mg/mL (4 mM). Solid dithiothreitol (15.4 mg) was then added (final concentration 100 mM, 25 molar excess over PEG_5-MAL). The reaction was carried out for 20 min at 25 °C followed by extensive dialysis against 10 mM HCl and lyophilization. PEG_5-SH at a concentration of 5.2 mg/mL (~1 μmol/mL) contained 0.9 ± 0.03 μmol/mL of sulfhydryl moiety as determined by reaction with DTNB. The lyophilized material was stable for weeks at 7 °C.

Preparation of $PEG_{5,20,40}$/Fmoc/FMS Derivatives of Gentamicin. PEG_5-SH, PEG_{20}-SH, or PEG_{40}-SH (1 μmol) was dissolved in 1.0 mL of 0.1 M $NaHCO_3$ containing 50 mg of gentamicin (0.1 mmol/mL, 100 molar excess over PEG-SH). Either MAL-Fmoc-OSu (1.1 μmol, 55 μL from a fresh solution of 10 mg/mL in DMF) or MAL-FMS-OSu (1.1 μmol, 64 μL from a fresh solution of 10 mg/mL in DMF) was then added to the stirred solutions. Reactions were carried out over a period of 3 h at 25 °C. Extensive dialysis against water was followed with several changes over a period of 24 h. All reversibly pegylated gentamicin derivatives prepared by this procedure (namely, $PEG_{5,20,40}$-Fmoc/FMS-gentamicin) were 1:1 PEG−gentamicin conjugates. The concentration of each conjugate was determined both by its absorbance at 280 nm and by amino acid analyses following acid hydrolyses. The conjugates absorb at 280 nm with a molar extinction coefficient of 21 200 L mol^{-1} cm^{-1}. Absorbance is exclusively due to the Fmoc and/or the FMS spacer, as neither gentamicin nor the PEG chains absorb at this wavelength. Covalently linked gentamicin in conjugates was quantitated by hydrolyzing an aliquot in 6 M HCl (110 °C, 22 h) followed by precolumn reaction with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). Acid-hydrolyzed gentamicin produced two peaks in a 1:1 ratio at the positions of proline (retention time, $t_R = 24.94$ min) and leucine ($t_R = 32.965$ min). An amount of 100 nmol of gentamicin yielded 179 nmol of either leucine- or proline-like peaks. By use of peak areas of leucine and proline, the obtained values were divided by 1.79.

Biological Procedures. Using *E. coli* Replication Assay for Quantitating Gentamicin in Urine Fractions. A suspension of *E. coli* in Luria−Bertani (LB) broth (about 10^4 cells/mL) was divided into plastic tubes (0.5 mL/tube) and incubated at 37 °C for ~7 h until the suspension lacking gentamicin reached a value of $OD_{600} = 0.8-0.9$. A standard inhibitory curve for gentamicin was constructed using 10 gentamicin concentrations ranging from 0.05 μg/mL (0.1 μM) to 1.5 μg/mL (3 μM). Aliquots of increasing size

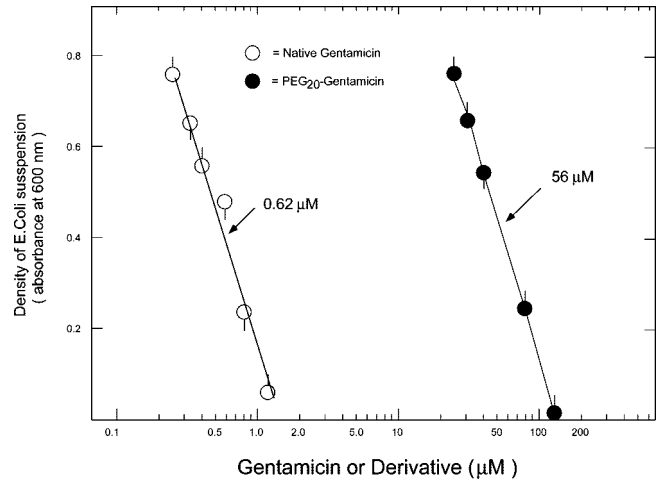

Figure 2. Dose-dependent inhibition of *E. coli* replication by gentamicin and conventionally pegylated gentamicin. Suspensions of *E. coli* (~10^4 cells in LB medium) were incubated for 7 h at 37 °C, in the absence or presence of increasing concentrations of gentamicin (0.2−1.2 μM) or the nonreversible PEG_{20}-gentamicin (20−120 μM). *E. coli* density was then monitored by the absorption at 600 nm. Each point is the arithmetic mean ± SEM of four determinations.

(from 1 to 40 μL) from each urine fraction were added to the assay, and the concentration was determined with values intercepting the standard inhibitory curve. Native gentamicin inhibits *E. coli* replication with $IC_{50} = 0.3$ μg/mL (0.6 ± 0.03 μM). A urine fraction in which 5 μL was added to the assay (1:100 dilution), facilitating 50% inhibition of *E. coli* replication, contains 30 μg/mL gentamicin. Rat urine alone (up to 40 μL per tube) neither arrested nor supported *E. coli* replication.

Constructing a Pharmacokinetic (PK) Profile for Intravenously Administered Gentamicin in Rats Based on Glomerular Ultrafiltration. Male Wistar rats weighting 170 ± 10 g, having received 0.2 mL of saline containing 200 μg of gentamicin (iv), were placed in metabolic cages. Urine fractions were collected immediately following excretion and their volume measured, and gentamicin concentrations were quantitated by their ability to inhibit *E. coli* replication (previous paragraph). For constructing the PK profile for intravenously administered gentamicin in rats (Figure 1B), results were expressed as micrograms of gentamicin excreted over a period of 1 h prior to collecting the fraction. Thus, the amounts of gentamicin found in five consecutive urine fractions collected 15, 30, 60, 80, and 110 min following administration were multiplied by 4, 4, 2, 3, and 2, respectively. In construction of the curve, the sequence between the two urine fractions containing the

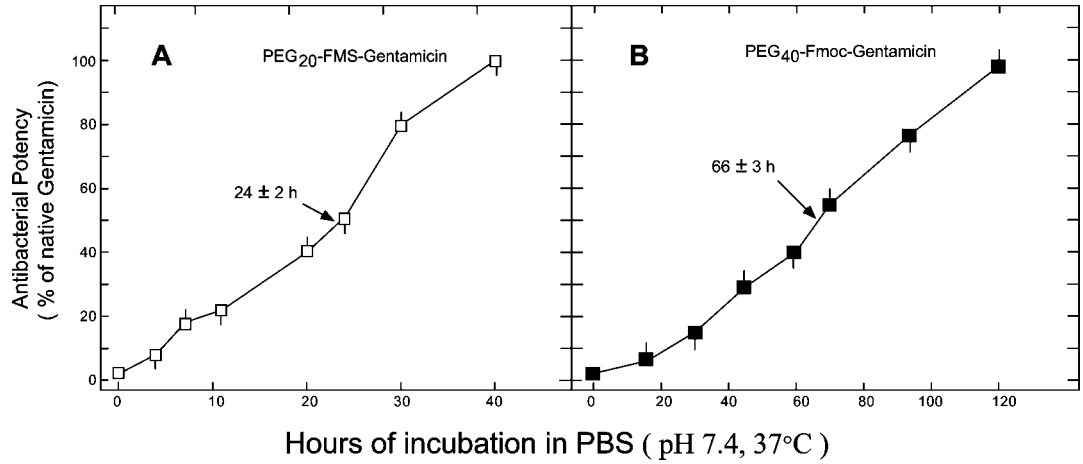

Figure 3. Time course of in vitro reactivation of reversibly pegylated gentamicin derivatives. PEG_{20}-FMS-gentamicin (A) and PEG_{40}-Fmoc-gentamicin (B) (0.1 μmol/mL of each) were incubated in PBS, pH 7.4, containing 2% (w/v) HSA at 37 °C. Aliquots were withdrawn at the indicated time points and analyzed at several concentrations in the antibacterial assay. Gentamicin inhibits *E. coli* replication with $IC_{50} = 0.62 \pm 0.02$ μM (Figure 2). An aliquot with $IC_{50} = 6.2 \pm 0.2$ μM is considered as having 10% of the native gentamicin antibacterial potency.

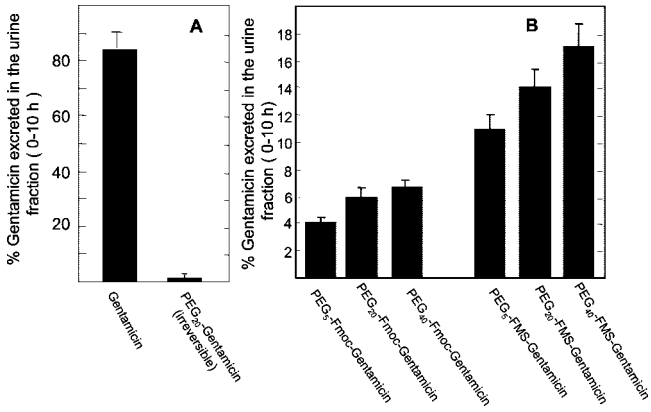

Figure 4. Quantitation of active gentamicin in urine following intraperitoneal administration of pegylated gentamicin derivatives in rats. Gentamicin and the indicated pegylated derivatives of gentamicin were administered intraperitoneally, each at a concentration of 0.4 μmol/rat ($n = 4$ per group). The urine pool of each rat was collected and quantitated for active gentamicin. Results are expressed as percentage of the administered amount that reached the urine over a period of 10 h (arithmetic mean ± SEM from four rats treated similarly).

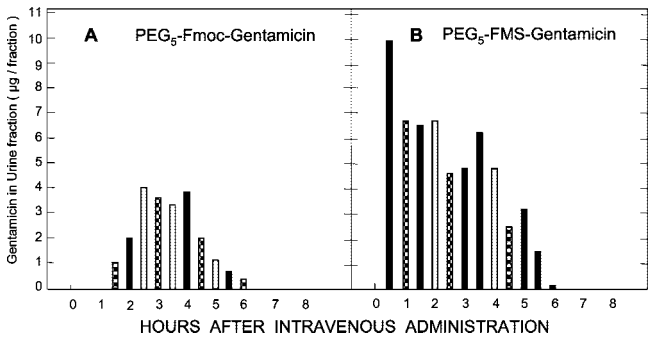

Figure 5. Pattern of distribution of PEG_5-Fmoc/FMS-gentamicin following intravenous administration to rats. PEG_5-Fmoc-gentamicin (A) and PEG_5-FMS-gentamicin (B) were administered intravenously to rats (0.4 μmol/rat, $n = 3$ per group). Rats were then placed in metabolic cages. Urine fractions were collected immediately when excreted and quantitated for active gentamicin. Each figure is composed of urine fractions obtained from three similarly treated rats (marked as white bar, black bar, and checkered bar in the figure).

"turning point" at the maximal peak height deviated significantly and therefore was not included.

Immunoreactive Gentamicin. Immunoreactive gentamicin in blood aliquots was quantitated using fluorescence polarization immunoassay procedure.[14]

All protocols of animal treatment complied with the recommendations of The Weizmann Institute—Institutional Animal Care and Use Committee (IACUC).

Results

PK Profile for Intravenously Administered Gentamicin Based on Glomerular Ultrafiltration. In preliminary experiments in rats we found that a major gentamicin fraction, amounting to over 80% of the intraperitoneally or the intravenously administered dose, reaches the urine within a period of 10 h (i.e., Figure 4A). Our initial intention therefore was to construct a convenient, noninvasive, in vivo procedure that reflects closely the PK profile of gentamicin based on this clearance pathway. In designing such a profile, one must consider the phenomenon that takes place, as gentamicin arriving from the whole circulating volume is being concentrated into considerably smaller volumes of the urine. Figure 1A shows the typical PK profile constructed by quantitating immunoreactive gentamicin in serum fractions taken at several time points following intravenous administration in rats; Figure 1B shows the PK profile constructed from quantitating gentamicin in urine fractions. Both procedures yielded remarkably close PK profiles with regard to time dependency. With both procedures, intravenously administered gentamicin peaked at 23 ± 3 min and declined with $t_{1/2}$ value of 45 ± 5 min. Thus, by use of our calculating protocol, the time course of gentamicin excretion appears similar to the time course of serum gentamicin concentration (Figure 1A versus Figure 1B).

Conventional Pegylation Inactivates Gentamicin. Pegylation of gentamicin with PEG_{20}-OSu was carried out under experimental conditions in which a single PEG_{20} chain is linked in a nonreversible fashion to one of the three available amino groups of gentamicin. The procedure yielded a conjugate nearly fully devoid of antibacterial activity ($IC_{50} = 56$ μM versus 0.62 μM for the native aminoglycoside (1.19% antibacterial potency, Figure 2). Thus, conventional pegylation, in which the PEG remains attached to the drug, appears to be an unsuitable approach for prolonging the action of aminoglycosides such as gentamicin.

Reversibly Pegylated Gentamicin Derivatives Are Inactive but Activatable Prodrugs. In Figure 3, PEG_{20}-FMS-gentamicin or PEG_{40}-Fmoc-gentamicin (0.1 μmoL/mL) was incubated at

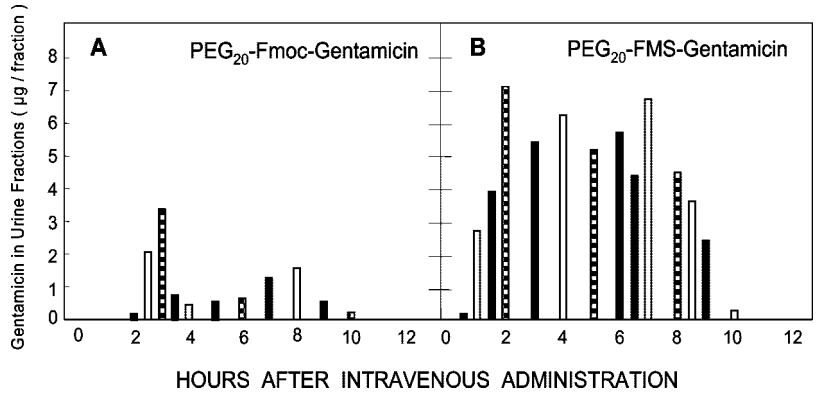

Figure 6. Pattern of distribution of PEG_{20}-Fmoc/FMS-gentamicin following intravenous administration to rats. PEG_{20}-Fmoc-gentamicin (A) and PEG_{20}-FMS-gentamicin (B) were administered intravenously to rats (0.4 μmol/rat, $n = 3$ per each group), which were then placed in metabolic cages. Urine fractions were collected and quantitated for active gentamicin as described in the caption to Figure 5.

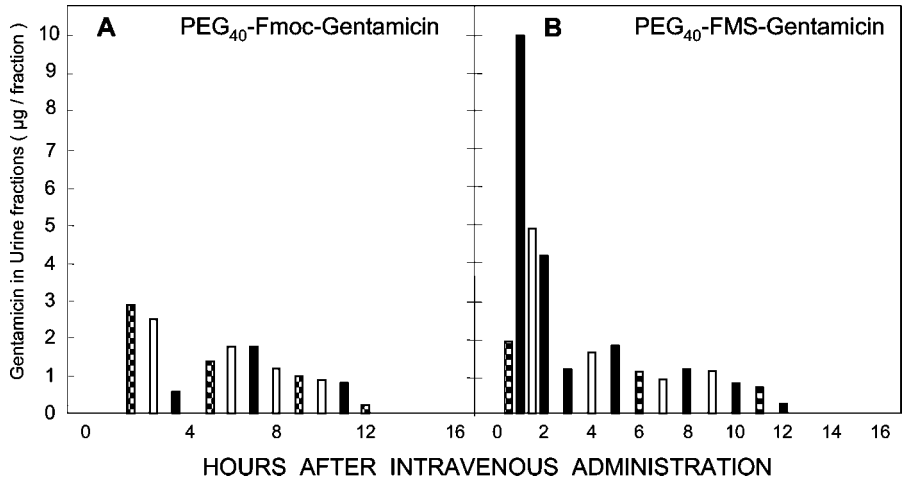

Figure 7. Pattern of distribution of PEG_{40}-Fmoc/FMS-gentamicin following intravenous administration to rats. PEG_{40}-Fmoc-gentamicin (A) and PEG_{40}-FMS-gentamicin (B) were administered intravenously to rats (0.4 μmol/rat, $n = 3$ per each group), which were then placed in metabolic cages. Urine fractions were collected and quantitated for active gentamicin as described in the caption to Figure 5.

37 °C in PBS buffer (pH 7.4) containing 20 mg/mL HSA. Aliquots were withdrawn at different time points and assessed for their antibacterial potencies. Both conjugates were nearly inactive prior to incubation (~2% activity, time 0 in the figure). Upon incubation of PEG_{20}-FMS-gentamicin, antibacterial potency was generated with a $t_{1/2}$ value of 24 ± 2 h (Figure 3A) and with a $t_{1/2}$ value of 66 ± 3 h upon incubation of PEG_{40}-Fmoc-gentamicin (Figure 3B). Both PEG_{20}-FMS-gentamicin and PEG_{40}-Fmoc-gentamicin regained their full (100%) antibacterial potencies following 40 h (Figure 3A) or 130 h (Figure 3B) of incubation, respectively.

Intraperitoneal Administered Reversibly Pegylated Gentamicin Derivatives Release Gentamicin. Quantitation of Antibacterial Potencies in Urine Fractions. Initially, we administered gentamicin and PEG_{20}-gentamicin (0.4 μmol/rat) intraperitoneally and confirmed that a major fraction of the injected gentamicin (84 ± 4%) and essentially none from PEG_{20}-gentamicin (<1%) were excreted in the urine over a period of 10 h (Figure 4A). Thus, an enzymatic system capable of hydrolyzing the bond linking gentamicin to the PEG chain following conventional pegylation is lacking. In Figure 4B the same protocol was applied to a family of reversibly pegylated gentamicin derivatives. Following intraperitoneal administration of PEG_5-Fmoc-, PEG_{20}-Fmoc-, and PEG_{40}-Fmoc-gentamicin, 4 ± 0.3%, 6.0 ± 0.5%, and 6.6 ± 0.3%, respectively, of the injected dose was excreted in the urine. With PEG_5-FMS-, PEG_{20}-FMS-, and PEG_{40}-FMS-gentamicin, the level of gentamicin found in the urine was 11 ± 1%, 14 ± 2%, and 17 ± 3%, respectively, of the injected dose (Figure 4B). Thus, spontaneous hydrolysis with the concomitant release of active gentamicin from the inactive, reversibly pegylated, conjugate takes place in the circulatory system. As expected from the hydrolysis rates in vitro (Figure 3), gentamicin released from the PEG-FMS-gentamicin conjugates in vivo was greater than 2.5 ± 0.2 times that released from the PEG-Fmoc-gentamicin conjugates (Figure 4B).

Distribution Pattern of Reversibly Pegylated Gentamicin Derivatives after Intravenous Administration in Rats. In Figures 5, 6, and 7, PEG_5-, PEG_{20}-, and PEG_{40}-gentamicin derivatives, linked through either the Fmoc or the FMS heterobifunctional spacer, were administered to rats intravenously, each at a concentration of 0.4 μmol/rat. Rats were then placed in metabolic cages, and urine was collected and quantitated for active gentamicin. Each derivative was injected into three rats in order to obtain at least two urine fractions each hour. Administered PEG_5-Fmoc-gentamicin showed a lag period of 1 h, during which no gentamicin was detected. It then reached a value of 3.8 ± 0.4 μg per each excreted urine fraction over a period of 2.5−4 h and declined with a $t_{1/2}$ value of about 5 h. No gentamicin was found in urine fractions taken 7 and 8 h after administration (Figure 5A). With PEG_5-FMS-gentamicin, no lag period was observed; gentamicin was present in the very first urine fraction, taken about 0.5 h following administration. Elevated levels

of gentamicin were maintained in subsequent urine fractions, amounting to 5.8 ± 1 μg gentamicin/fraction over a period of 3 h. The levels then declined with a $t_{1/2}$ value of about 5.5 h (Figure 5B).

The same characteristic features of distribution were obtained for administered PEG_{20}-Fmoc/FMS-gentamicin, with the notable difference of more protracted maintenance ($t_{1/2}$ value of ∼9 h, Figure 6). As with the shorter conjugates, PEG_{20}-Fmoc-gentamicin showed a lag period, amounting in this case to about 2.5 h, followed by the presence of active gentamicin in urine at concentrations in the range of 1.7−2.2 μg/fraction over a period of 6 h before declining. With administered PEG_{20}-FMS-gentamicin, considerably higher levels (3−7 μg gentamicin/fraction) were observed in the first 2 h of administration followed by a concentration of 4.5−5.5 μg/fraction over 2−8 h (Figure 6B). Essentially the same characteristic features of distribution were obtained with PEG_{40}-Fmoc/FMS-gentamicin except that the half-life appeared to extend to a $t_{1/2}$ value of ∼11 h (Figure 7). With administered PEG_{40}-Fmoc-gentamicin, a lag period of ∼1 h was obtained followed by the appearance of gentamicin in urine fractions in the range of 0.8−1.8 μg/fraction over a period of 10 h. With PEG_{40}-FMS-gentamicin, relatively high levels (4−10 μg) of gentamicin were found in urine fractions in the first 2 h following administration. Gentamicin then leveled off to 0.8−1.9 μg/fraction over a period of 10 h following administration. No gentamicin was found in urine fractions taken 14 and 16 h following injection (Figure 7).

Discussion

Conventional pegylation of gentamicin ends up with an inactive product (Figure 2). We therefore engineered and synthesized a family of reversibly pegylated derivatives of gentamicin. PEG-Fmoc- and PEG-FMS-gentamicin derivatives undergo reactivation upon incubation at physiological conditions with $t_{1/2}$ values of 66 ± 3 h ($k = 0.0105\ h^{-1}$) and 24 ± 2 h ($k = 0.0289\ h^{-1}$), respectively (Figure 3). The release of gentamicin was then studied in rats. Active gentamicin was generated from the inactive conjugates in situ (Figure 4) and in agreement with the hydrolysis rates found under physiological conditions in vitro (Figure 3). PEG-FMS-gentamicin conjugates generated 2.5 ± 0.2 times more gentamicin than PEG-Fmoc-gentamicin conjugates (Figure 4B).

We next studied the contribution of incresing the molecular mass of the conjugates to prolong the duration of excreted gentamicin in vivo. This has been evaluated by two different approaches, both of which revealed a residence time of approximately 5, 9, and 11 h following intraperitoneal and/or intravenous administrations of reversibly pegylated PEG_5-, PEG_{20}-, and PEG_{40}- gentamicin, respectively. No significant difference in this parameter was found between the Fmoc-linked and the FMS-linked conjugate for the same length PEG (Figures 4–7). Although PEG_{40}-linked gentamicin conjugates are the longer-lived species in vivo, the residence time for PEG_{20}-linked conjugates is not that different. The usage of PEG chain having a molecular mass of 20 kDa for future application of this strategy appears therefore economical and sufficient.

The administration of our conjugates at a dose of 0.4 μmol/rat (2−2.4 μmol/kg body weight) was required to prolong maintenance levels of gentamicin in microgram concentrations; fortunately with most relevant LMW drugs, the presence of nanogram quantities in the circulatory system is generally of therapeutic benefit.[1] Thus, under these circumstances, administered doses can be reduced by 3 orders of magnitude, namely, to levels of about 2 nmol/kg body weight.

Most importantly, our reversibly pegylated derivatives are inactive but reactivatable compounds (i.e., prodrugs). This feature might be especially relevant for those LMW drugs having a narrow pharmacological window where 2−3 times the therapeutic level is toxic. From our study, Fmoc-linked PEG−drug conjugates appear more suitable in this case, since quite a stable level of gentamicin appeared to be released as a function of time, following a lag period of 1−3 h (Figures 5A, 6A, 7A). For maintaining the desired therapeutic level, the size of the injected dose can be calculated from the rate of drug discharge from the conjugate at physiological conditions.

Finally, we recommend the application of our procedure to construct PK profiles for substances whose major clearance pathway takes place by glomerular filtration, as in the case of gentamicin, although more urine fractions need to be collected and quantitated specifically in the peak period to obtain more accurate PK profiles.

In summary, the conceptual approach termed "reversible-PEGylation" was applied to nonpeptidic LMW substances that otherwise would be inactivated by this technique. A pharmacologically "silent" conjugate that is "trapped" in the circulatory system releases the covalently linked molecule in its active form, by spontaneous chemical hydrolysis, over many hours with desirable pharmacokinetic patterns. This characteristic feature would be a significant asset in the management of those drug candidates having high indices of toxicity.

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Supporting Information Available: MS and HPLC data for reversible-pegylated derivatives of gentamicin. This material is available free of charge via the Internet at http://pubs.acs.org.

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