A Semisynthetic *Quillaja* Saponin as a Drug Delivery Agent for Aminoglycoside Antibiotics

Joanne Recchia,[1,3] Maria H. A. Lurantos,[1,2] Jennifer A. Amsden,[1] James Storey,[1] and Charlotte R. Kensil[1]

Received July 20, 1995; accepted September 6, 1995

Purpose. The purpose of this study was to investigate the utility of a purified, semisynthetic saponin, DS-1, prepared by deacylation of a naturally occurring saponin from the bark of the *Quillaja saponaria* Molina tree, as a permeation enhancer for mucosal delivery of the aminoglycosides, gentamicin and tobramycin.

Methods. Gentamicin or tobramycin formulations, with and without DS-1, were administered to rats nasally, ocularly, and rectally. Serum aminoglycoside levels following mucosal application were compared with those administered intramuscularly. Gentamicin formulations, with and without DS-1, were administered intranasally to mice 60 minutes after a lethal bacterial challenge. To ascertain nasal irritation potential, DS-1 nosedrops were administered to rats twice daily for 7 days in the right nostril only. Comparison of the left (internal control) and right nostril was made with a control group that received only buffer.

Results. Significant transport across mucous membranes was only observed in formulations containing DS-1. This effect on drug delivery was transient. Administration of an intranasal gentamicin/DS-1 formulation reversed the lethal bacterial challenge in mice, demonstrating that biological activity was retained after absorption. Nasal irritation was not observed in groups receiving DS-1 nosedrops, which were identical to control groups.

Conclusions. DS-1 has potential as a transmucosal delivery agent for the aminoglycoside antibiotics.

KEY WORDS: aminoglycosides; antibiotics; saponin; drug delivery.

INTRODUCTION

The aminoglycosides are an important class of antibacterial agents used for the treatment of serious gram-negative infections such as urinary tract infections, pneumonia, and peritonitis (1). The aminoglycosides are not absorbed after oral administration due to their highly polar cationic nature. Therefore, intravenous (i.v.) or intramuscular (i.m.) injection is required. Hospital stays account for greater than half the cost of i.v. drug delivery, which last an average of 10.4 days (2). Additionally, the i.v. route of administration poses unique problems for pediatric drug delivery (3). The use of an alternative route of administration, such as through the nasal mucosa, would substantially reduce treatment costs by allowing outpatient or home treatment. However, these antibiotics also have poor transmucosal bioavailability.

Permeation enhancers, such as the bile salts (4), sodium glycocholate (5), glycyrrhizic acid (6), STDHF (7), and saponins (8,9) have been utilized to enable mucosal transport of drugs with poor bioavailability. Chiou and Chuang (9) demonstrated that a 1% saponin solution, consisting of a partially purified crude extract from Gypsophyla, showed enhancement of insulin absorption through the eye and the greatest reduction in blood glucose levels when compared with other drug delivery agents.

Our laboratory has investigated the utility of a purified saponin, QS-21, and derivatives from the bark of the tree *Quillaja saponaria* Molina, as immunological adjuvants (10, 11, 12, 13). Removal of the fatty acyl portion from the QS-21 molecule abolished the adjuvant activity (11). This product, a semisynthetic saponin, 3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside, DS-1, had been prepared and characterized previously by Higuchi et al. (14). Studies by Pillion et al. showed that DS-1 was more effective in delivery of insulin by nasal and ocular routes than crude saponin mixtures (15).

The aim of this study was to increase the bioavailability of the aminoglycosides, gentamicin and tobramycin, by mucosal application of formulations containing DS-1. Administration of an intranasal gentamicin/DS-1 formulation reversed a lethal bacterial challenge in mice. This paper describes the utility of DS-1 as an agent for the transmucosal delivery (16) of aminoglycoside antibiotics.

MATERIALS AND METHODS

Materials

Gentamicin sulfate solution (50 mg/ml), tobramycin (free base), mucin, 8-anilino-1-naphthalene sulfonic acid (ANS) and sodium glycocholate were obtained from Sigma Chemical Company (St. Louis, Missouri). Glycyrrhizic acid, ammonium salt, was purchased from Fluka BioChemika (Ronkonkoma, New York). Ketamine hydrochloride was purchased from Fort Dodge Laboratories, Inc. (Fort Dodge, Iowa), halothane from J. A. Webster (Sterling, Massachusetts), and xylazine hydrochloride was obtained from Phoenix Pharmaceuticals (St. Joseph, Missouri). Male Sprague-Dawley rats, 4-6 weeks of age, weighing 210-390 g, and female Swiss Webster mice, weighing 23-30 g, were obtained from Taconic Laboratories (Germantown, New York). All other reagents were of the highest quality available.

Preparation of DS-1

The naturally occurring saponin, QS-21, was purified from an aqueous extract of *Quillaja saponaria* bark by adsorption chromatography and reversed-phase high performance liquid chromatography (HPLC) as described previously (10). DS-1 was prepared from QS-21 by modification of the molecule as follows. Sodium hydroxide was added to an aqueous QS-21 solution to increase the pH to 10-11. After a

[1] Cambridge Biotech Corporation, 365 Plantation Street, Worcester, Massachusetts 01605-2376.

[2] Present address: Amicon, Inc., 72 Cherry Hill Drive, Beverly, Massachusetts 01915-1087.

[3] To whom correspondence should be addressed.

0724-8741/95/1200-1917$07.50/0

minimum of 15 min, the reaction was terminated by the addition of acetic acid to a final pH of 4-5. The product was purified to ≥90% homogeneity by reversed-phase HPLC using a Vydac C4 column (5 micron particle size, 300 Å pore size, 1.0 × 25 cm) on a Waters chromatography system consisting of a 600E System Controller, a Lambda-Max Model 481 variable wavelength detector, and a Model 745 Data Module. A 25-45% acetonitrile gradient over 60 min containing 0.15% tri-fluoroacetic acid modifier and detection at 220 nm was used. Pooled fractions containing DS-1 were lyophilized to dryness. Purity of the product was determined by analytical reversed-phase HPLC using a Dynamax 60 Å C8 (0.46 × 25 cm) column (Rainin Instrument Company) on an HPLC system consisting of a Macintosh computer, Rainin Model HPX pumps, Knauer Model 87 variable wavelength detector, and a Gilson Model 231 autosampler. A 20-45% acetonitrile gradient over 35 min containing 0.15% trifluoroacetic acid modifier and detection at 214 nm was used. The identity of the product was confirmed by fast atom bombardment (FAB) mass spectrometry and carbohydrate linkage analysis. Mass spectral analysis was performed on a VG Analytical ZAB 2-SE high field mass spectrometer. Glycosyl linkage analysis was performed using the Hakamori methylation procedure as described by York et al. (17).

Critical Micelle Concentration and Hemolysis Assays

The critical micelle concentration (CMC) of DS-1 was determined by a fluorescent dye binding assay (18) as follows. Various concentrations of DS-1 were mixed with a constant concentration of the fluorescent probe, ANS, in phosphate buffered saline (PBS). After mixing, the fluorescence emission at 490 nm with excitation at 370 nm was determined. A biphasic curve was obtained and the CMC was defined as the DS-1 concentration corresponding to the intersection of best fit lines. Fluorescence emission increases above the CMC due to intercalation of the fluorescent probe into the micelle. A Perkin Elmer LS5 Fluorescence Spectrophotometer was used.

Hemolytic activity was measured in an *in vitro* assay with sheep red blood cells (SRBC). SRBC were washed several times in PBS and collected by centrifugation at 900 × g for 5 min; the final pellet was resuspended in PBS. Serial dilutions of DS-1 in PBS were added to individual wells of a round bottom 96 well assay plate. A fixed aliquot of the SRBC suspension was added to each well, mixed with saponin solution, and incubated at room temperature for 30 min. The plate was centrifuged at 1000 × g for 5 min. A fixed aliquot of the supernatants was transferred to individual wells of a flat bottom 96 well assay plate and the absorbance at 570 nm was measured.

Drug Delivery Experiments

Sprague-Dawley rats (220-350 g) were anesthetized by i.m. injection of 7.5 mg/kg xylazine/50 mg/kg ketamine and were kept asleep for the duration of the experiment with additional anesthetic as needed. Gentamicin (5 mg/kg) and tobramycin (6 mg/kg) formulations were prepared in PBS containing 0-0.4% DS-1 (w/v). Since the known enhancers sodium glycocholate and glycyrrhizic acid have structures similar to the triterpene of DS-1, formulations containing these enhancers equivalent to the 0.2% DS-1 dose (1.32 mM) with 5 mg/kg gentamicin in PBS were included for comparison. Formulations for i.m. administration did not contain DS-1. The transport across nasal, ocular, and rectal mucosal surfaces was compared to parenteral delivery. The following volumes were administered for each route: nasal and ocular (40 μl), rectal and i.m. (250 μl). Blood was withdrawn from the tail vein at 5, 15, 30, and 60 min post-administration and the serum collected by centrifugation at 13,000 × g for 10 minutes at room temperature. Gentamicin and tobramycin serum levels were measured by a competitive binding fluorescence polarization immunoassay (19). The assay was linear from 0.3-10 μg/ml, with a detection limit of 0.3 μg/ml. The trapezoidal rule was used for calculation of the area under the antibiotic serum concentration vs. time curve (AUC). Data are presented as the mean ± standard error (SE), # animals per group = 3. A value of 0.3 μg/ml was used in calculations for all timepoints with antibiotic serum levels below the limit of detection. Differences between experimental groups were assessed by a two-tailed *t* test. P values less than 0.05 were considered significant.

Challenge Experiment

The minimum lethal intraperitoneal (i.p.) dose (MLD) for the microorganisms *Escherichia coli* (*E. coli*) ATCC 25922 in 8% mucin was determined as described previously (20) in female Swiss Webster mice, except that the volume administered i.p. was decreased to 0.2 ml per mouse. The MLD was defined as the minimum infectious dose that caused 100% mortality within 36 h of inoculation. Viable cell counts were determined by serially diluting the 20 h culture in MH-broth to 10^{-6}, 10^{-7}, and 10^{-8} dilutions and plating on LB agar plates. Colony counts were performed on plates incubated at 37°C overnight. The MLD was determined to be 1.4×10^5 colony forming units (CFU)/mouse, within experimental error of the previously published value of 5.5×10^5 CFU/mouse. The *in vitro* minimum inhibitory concentration (MIC) of gentamicin for this *E. coli* strain was determined to be 1 μg/ml. In the challenge, all mice were given a 5 × MLD i.p. injection of *E. coli*. Antibiotic was administered 1 h after the challenge in a single dose of 10 mg/kg. Groups 1 and 2 received intranasally administered formulations (10 μl) containing 0% or 0.2% DS-1 (w/v) in PBS, respectively. Group 3 received a single i.p. dose of gentamicin (200 μl), in a formulation without DS-1. The last group received no antibiotic or DS-1 treatment. Groups of 20-21 animals were used. Animals were monitored regularly for 1 week.

Nasal Histopathology Study

The nasal irritation potential of 0.2% (w/v) DS-1 was determined after 7 consecutive days of twice daily administration. As a control, one group received buffer in the absence of enhancer. Locke-Ringer solution and PBS were used as buffers. Male Sprague-Dawley rats (215-295 g) were anesthetized by inhalation of halothane, which does not cause irritation of the nasal mucosa, prior to application of nosedrops (20 μl) to the right nostril only. The left nostril served as an internal control. Groups of two animals were used. On the eighth day, the animals were sacrificed and decapitated. The heads were fixed in alcoholic formalin for

48 hours and sent to Tufts University School of Veterinary Medicine for evaluation. The head of each rat was then demineralized in 5% nitric acid. The nose was sliced in cross section at from 3-5 sites from the tip of the nose to the cribriform plate at the back of the nasal cavity. Sections of 6 μm thickness were made of each cross section, stained with hematoxylin and eosin, and examined microscopically. Tissues were evaluated on a scale of 0 to 4+, with 1+ equivalent to mild inflammation and 4+ as severe inflammation. All animal experimentation adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

RESULTS

Preparation of DS-1

QS-21 was completely converted to DS-1 with the experimental conditions used (data not shown). The FAB mass spectrometry results, m/z $(M+Na)^+$ = 1536, were consistent with the designated structure (14) of DS-1 (Fig. 1). Carbohydrate linkage analysis, (relative area percent = 0.0% t-arabinose, 8.6% t-apiose, 8.9% t-xylose, 30.0% 4-rhamnose, 13.0% t-galactose, 9.9% 2-fucose, 16.0% 3-xylose, 5.8% 2,4-linked rhamnose, 8.3% 2,3-glucuronic acid), confirmed the absence of the sugar arabinose.

Surfactant Properties of DS-1

Saponins from *Quillaja saponaria* exhibit the self-associating properties of surfactants due to their amphipathic structure. The self-association is in the form of micelles. The propensity for DS-1 to form micelles was determined by measure of the CMC. The CMC for DS-1 was determined to be 1.09 mM. The lysis of SRBC in an *in vitro* assay was used as an additional measure of detergent activity. DS-1 caused 50% hemolysis of SRBC at a concentration of 77.9 μM. Removal of the fatty acid acyl portion of QS-21 decreased the surfactant activity of DS-1, but did not abolish it.

Drug Delivery Experiments

Gentamicin was detected in the serum of the rat after intranasal administration of a formulation containing 0.2% DS-1 (Fig. 2). The concentrations were about 60% of that obtained after an i.m. injection of the same dose, and were well in excess of the MIC for many gram negative pathogens. Antibiotic was not detected when DS-1 was omitted from the

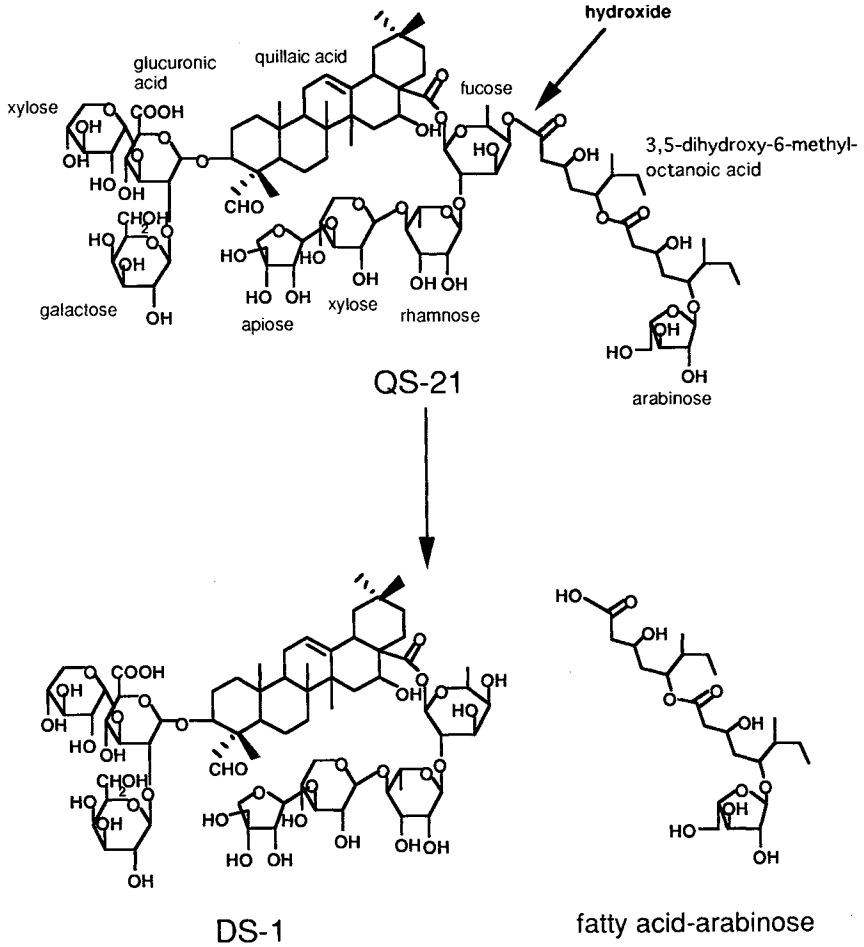

Fig. 1. Structure of the deacylation products, DS-1 and the fatty acid-arabinose molecule, produced after alkaline-catalyzed aqueous hydrolysis of the starting material, QS-21.

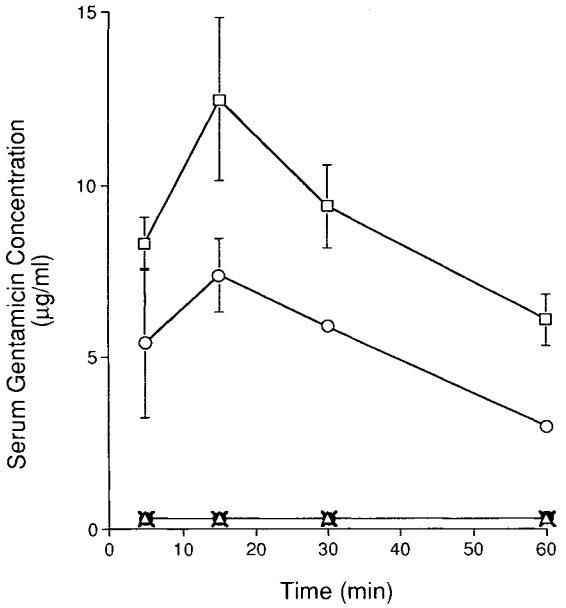

Fig. 2. Gentamicin concentration in serum as a function of time post-administration. Formulations delivered intranasally contained 5 mg/kg gentamicin with 0% DS-1 (△); 0.2% (1.32 mM) DS-1 (○); 1.32 mM sodium glycocholate (●); or 1.32 mM glycyrrhizic acid (×). For comparison, gentamicin levels after i.m. injection are also included (□). Gentamicin levels below the detection limit of 0.3 μg/ml were plotted as 0.3 μg/ml. Data represent the mean ± SE (n = 3).

intranasally administered formulation. The permeation enhancers, sodium glycocholate and glycyrrhizic acid, did not transport detectable levels of gentamicin in serum when used at an equivalent molar concentration to this DS-1 dose (1.32 mM).

To determine the effect of the DS-1 concentration on gentamicin transport, 5 mg/kg gentamicin was administered intranasally to rats with varying concentrations of DS-1 (Fig. 3). The AUC increased linearly up to 0.2% DS-1, but the amount of gentamicin transported across the nasal mucosa did not increase further at a higher DS-1 concentration. The transport was not dependent upon micelle formation in the formulation, as significant levels of gentamicin were obtained in the serum when the DS-1 concentration was below the CMC (0.16% w/v).

To determine if the intranasal transport of antibiotic in the presence of DS-1 was applicable to other aminoglycosides, the effect on tobramycin absorption was also examined (Fig. 4). Serum tobramycin levels were low, but detectable, when the antibiotic was administered alone intranasally. A significant increase in serum levels were obtained when the formulation contained 0.2% DS-1. Peak levels were well in excess of the MIC for many gram negative pathogens.

Other mucosal routes of administration were tested using a constant aminoglycoside dose and DS-1 concentration. Gentamicin was not detected in serum after ocular or rectal administration without DS-1. However, the drug was detected by these routes after administration of formulations containing 0.2% DS-1 (Table I). Transport by these routes was not as efficient as with intranasal delivery as reflected by the lower AUC values. Gentamicin levels were barely above detectable limits when the drug was delivered conjunctivally with DS-1. When tobramycin was tested for transport via other mucosal routes, significant levels of the drug were detected when DS-1 formulations were used for both ocular and rectal administration (Table II). Again, these routes of administration were not as efficient as the intranasal route.

To determine the duration of the DS-1 effect on transmucosal delivery, gentamicin was administered intranasally 1 h after 0.2% DS-1 was administered by the same route (Fig. 5). The serum levels of gentamicin were significantly decreased when the drug was administered after the enhancer.

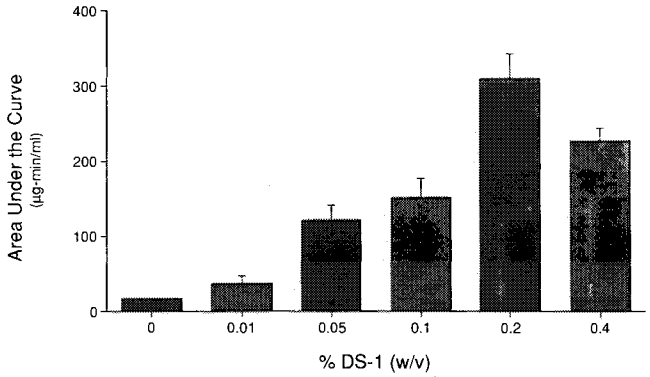

Fig. 3. Area under the gentamicin serum concentration - time curve as a function of % DS-1 in the formulation. All formulations contained 5 mg/kg gentamicin and were administered intranasally. The AUC calculation for gentamicin serum concentrations below the detection limit of 0.3 μg/ml were estimated using a value of 0.3 μg/ml for that timepoint. Data represent the mean ± SE (n = 3).

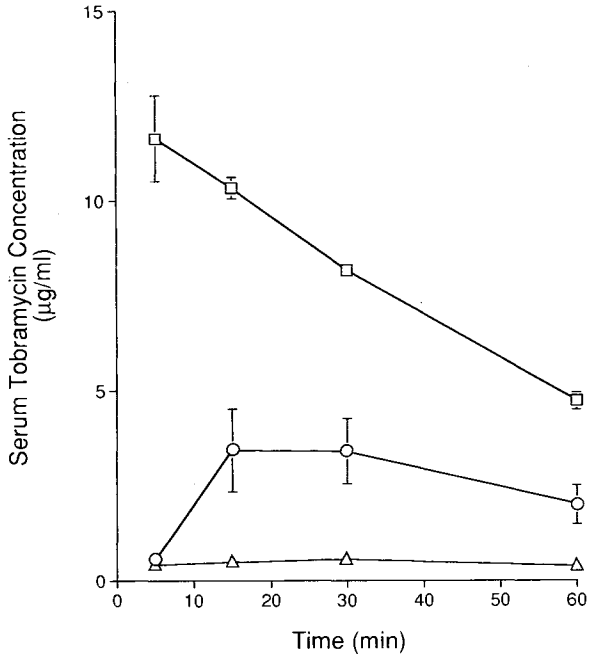

Fig. 4. Tobramycin concentration in serum as a function of time post-administration. Formulations delivered intranasally contained 6 mg/kg tobramycin and either 0% (△) or 0.2% (○) DS-1. For comparison, tobramycin levels after i.m. injection are also included (□). Tobramycin levels below the detection limit of 0.3 μg/ml were plotted as 0.3 μg/ml. Data represent the mean ± SE (n = 3).

Table I. Gentamicin Area Under the Curve and Relative % Efficiency of Transport vs. the Intramuscular Route

Route	Conc. DS-1[a] (%)	$AUC_{0\rightarrow60}$[b] (μg-min/ml)	% Efficiency[c]
Intramuscular	0	519.6 ± 74.1	100.0
Nasal	0	<17.2[d]	<3.3[d,e]
Nasal	0.2	310.3 ± 32.8	59.7
Rectal	0.2	78.5 ± 44.0	15.1[e]
Ocular	0.2	21.8 ± 1.9	4.2[e]

[a] Formulations were in PBS and contained 5 mg/kg gentamicin.
[b] $AUC_{0\rightarrow60}$ = area under the serum concentration − time curve measured up to 60 minutes post-administration. Data are presented as mean ± SE, n = 3.
[c] % Efficiency of Transport is calculated as the ratio of the AUC to the AUC for the intramuscular route times 100%.
[d] The serum concentrations obtained for all timepoints were below the assay detection limit of 0.3 μg/ml. Since the $AUC_{0\rightarrow60}$ was based on a gentamicin level of 0.3 μg/ml for each timepoint, the data represents the maximum possible $AUC_{0\rightarrow60}$.
[e] $P < 0.05$ compared with the intramuscular route.

The AUC decreased by 85% to 46.2 μg-min/ml ($P < 0.05$). Therefore, the duration of the permeation enhancement effect of DS-1 was limited.

Challenge Experiment

The biological activity of gentamicin after transmucosal delivery was assessed in a challenge experiment. The actual number of *E. coli* organisms administered was 6.7×10^5 CFU/mouse i.p. All of the animals that did not receive antibiotic treatment died within 2 days after administration of the *E. coli* (Fig. 6). All but 1 animal in the group that received gentamicin intranasally in the absence of DS-1 also died within 2 days. One animal died in the group that received an i.p. injection of gentamicin. Intranasal administration of gentamicin with a formulation containing DS-1 increased animal survival to the same degree as the group that received gentamicin i.p. Animals were monitored for 7 days post-challenge/treatment. Clinical observations of the survivors were indistinguishable from unchallenged animals after the first 2 days. Therefore, DS-1 enabled the intranasal absorption of gentamicin to therapeutic levels in mice.

Table II. Tobramycin Area Under the Curve and Relative % Efficiency of Transport vs. the Intramuscular Route

Route	Conc. DS-1[a] (%)	$AUC_{0\rightarrow60}$[b] (μg-min/ml)	% Efficiency[c]
Intramuscular	0	471.2 ± 5.3	100.0
Nasal	0	28.2 ± 5.6	6.0[d]
Nasal	0.2	140.3 ± 21.1	29.8[d]
Rectal	0.2	77.2 ± 38.6	16.4[d]
Ocular	0.2	40.3 ± 10.9	8.6[d]

[a] Formulations were in PBS and contained 6 mg/kg tobramycin.
[b] $AUC_{0\rightarrow60}$ = area under the serum concentration − time curve measured up to 60 minutes post-administration. Data are presented as mean ± SE, n = 3.
[c] % Efficiency of Transport is calculated as the ratio of the AUC to the AUC for the intramuscular route times 100%.
[d] $P < 0.05$ compared with the intramuscular route.

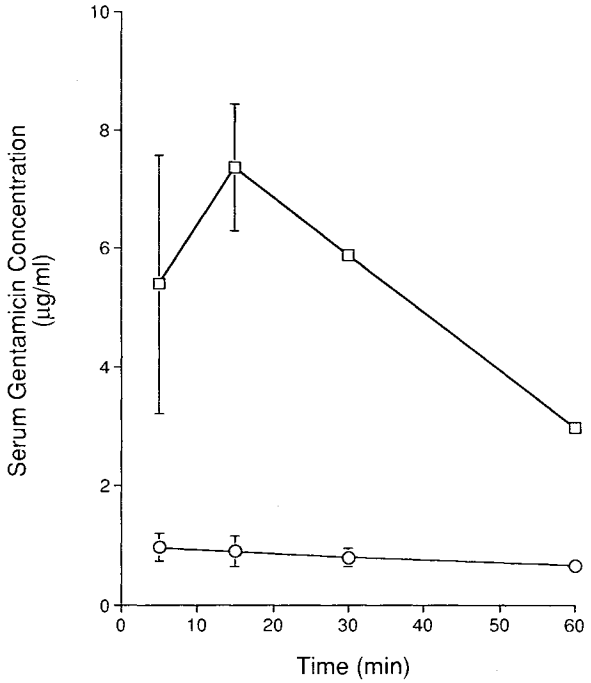

Fig. 5. Comparison of gentamicin concentration in serum when 5 mg/kg of gentamicin was administered 1 hour after 0.2% DS-1 (○), or administered simultaneously (□). Data represent the mean ± SE (n = 3).

Nasal Histopathology Study

The nasal tissue from the right nostril of animals in which 0.2% DS-1 was administered was indistinguishable from the untreated left nostril in all animals (evaluation = 0 on a scale of 0 to 4+). Furthermore, the nasal tissue from the

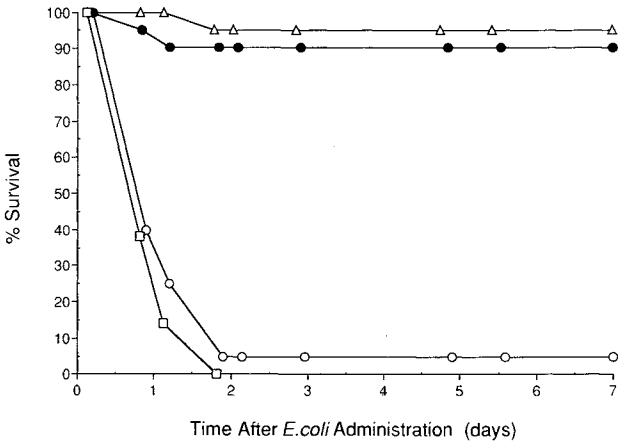

Fig. 6. Percent survival of mice in the days following administration of a 5 × MLD *E. coli* challenge. Gentamicin (10 mg/kg) was administered 1 hour after the *E. coli*. One group of animals received i.p. gentamicin treatment (△), other groups received intranasal gentamicin in the presence (●) or absence (○) of 0.2% DS-1. A control group (□) received no gentamicin or DS-1 treatment. (n = 20-21).

DS-1 treated group was equivalent to that of the buffer control group (evaluation = 0 on a scale of 0 to 4+). Hence no microscopic sign of irritation, such as macrophage or neutrophilic infiltration of the nasal mucosa, was observed. All animals appeared healthy and gained weight during the course of the study.

DISCUSSION

DS-1 facilitates the transmucosal delivery of aminoglycosides in mice and rats. A linear dose-response relationship was observed by increased DS-1 concentrations up to 0.2% (w/v). The mucosal surfaces with decreasing efficacy for aminoglycoside transport were nasal, rectal and ocular, respectively, with the nasal route 30-60% as efficient as the i.m. route. Further optimization of the DS-1 formulation may increase this efficiency, since the effect of variables such as pH were not investigated. Regardless, peak antibiotic levels were well in excess of the MIC for many gram negative pathogens. The effect DS-1 had on transport of aminoglycosides was transient and significantly decreased after 1 h. The addition of DS-1 to nasal formulations of gentamicin was shown to protect animals challenged with a lethal bacterial infection. Negligible amounts of gentamicin or tobramycin were detected in serum when administered intranasally, ocularly, or rectally in the absence of DS-1.

The recommended i.m. or i.v. dose of gentamicin for adults is 3 to 5 mg/kg/day (in divided doses every eight hours). The solubility of gentamicin (about 500 mg/ml in PBS) should be sufficient to achieve this dose level. Therefore, nosedrop formulations consisting of DS-1 and aminoglycosides may be a viable alternative to i.m. or i.v. administration. The observed transport through rectal surfaces also suggests that suppositories of aminoglycoside/DS-1 may be useful. However, drug solubility could limit the scope of applications available for alternate routes of delivery. The feasibility of DS-1 to promote permeability of other drug classes and polypeptide drugs through mucosal surfaces is under investigation.

DS-1 was more effective at drug transport than other permeation enhancers. Sodium glycocholate and glycyrrhizic acid have structural similarity with the triterpene of DS-1, however, transport of gentamicin was not detected when these permeation enhancers were used at the same molar concentration as DS-1 (1.32 mM). This concentration of DS-1 is more than 10-fold less on a molar basis than the concentration of bile salts needed to exert a similar effect in rabbits (21) and 15-fold less than the concentration of glycocholate needed to exert a similar effect in humans (22).

The mechanism of action for DS-1 is unknown at present. Sodium tauro-dihydro-fusidate (STDHF), which is also structurally similar to the triterpene of DS-1, required concentrations above its CMC to enhance uptake of insulin in sheep (23). DS-1 does not function by the same mechanism, since DS-1 promotes drug delivery at concentrations below the CMC of 1.1 mM. At DS-1 concentrations greater than 0.2%, the efficiency of transport does not continue to increase.

The concentration of DS-1 that causes 50% hemolysis of sheep red blood cells (77.9 μM) is well below the concentration of DS-1 needed for aminoglycoside drug delivery (0.6-1.3 mM). Therefore, hemolytic activity may be a property that is necessary for the permeation enhancement effect. However, the surfactant strength, as determined by critical micelle concentration and hemolytic activity, is not a predictive indicator of the efficiency of permeation enhancement between various *Quillaja* saponin derivatives (24). Further investigation is necessary to determine if DS-1 interaction with, or perturbation of, cell membranes contributes to the permeation enhancement effect and if the transport mechanism is transcellular or paracellular.

The development of any successful drug delivery agent will require a thorough analysis of local and systemic toxicity. Chronic exposure of the mucous membrane to permeation enhancers such as certain bile salts could result in inflammation and deterioration of normal nasal functions (25). Other absorption enhancers, such as nonionic polyethylene ether and STDHF, have caused unacceptable irritation of the nasal mucosa (26). However, DS-1 (0.2% w/v) did not show signs of inflammation after twice daily administration over 7 days. Toxicity is a potential problem when a crude mixture of saponins is used (27, 28) due to variable and complex profiles of toxic and nontoxic components. In contrast, DS-1, which is a highly purified, semisynthetic saponin, should have a reproducible toxicology and efficacy profile. We have initiated further toxicology studies to balance potential side effects with the benefit of more convenient drug administration. Preliminary data from an eye irritation study in rabbits demonstrated a lack of irritation during a 72 hour observation period with formulations containing DS-1 in concentrations up to 1% (6.6 mM) in isotonic saline (data not shown). Additional toxicology studies are on-going.

ACKNOWLEDGMENTS

We thank Complex Carbohydrate Research Center, Athens, Georgia, for the carbohydrate analysis, which is supported in part by the Department of Energy-funded (DE-FG09-93ER-20097) Center for Plant and Microbial Complex Carbohydrates; MScan Corporation, West Chester, Pennsylvania for fast atom bombardment mass spectrometry; the Drug Concentration Laboratory at the University of Massachusetts Medical Center for analysis of gentamicin and tobramycin in serum; R. T. Bronson, DVM (Tufts University School of Veterinary Medicine) for histopathological evaluation; Dr. D. J. Pillion (University of Alabama at Birmingham) for the training of M.H.A.L.; Drs. G. A. Beltz and R. B. Kammer for helpful discussions; and P. Cloutier for technical assistance and care of the animals. This work was conducted according to the principles outlined in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council.

REFERENCES

1. A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, New York, 1990.
2. D. M. Poretz. Outpatient Use of Intravenous Antibiotics. *Amer. J. Med.* 97 (suppl. 2A):1-2 (1994).
3. R. J. Roberts. Issues and Problems Associated with Drug Delivery in Pediatric Patients. *J. Clin. Pharmacol.* 34:723-724 (1994).
4. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier, and M. C.

Carey. Nasal Absorption of Insulin: Enhancement by Hydrophobic Bile Salts. *Proc. Natl. Acad. Sci. USA* 82:7419-7423 (1985).
5. A. E. Pontiroli, M. G. Perfetti, B. Fattor, and G. Pozza. Effect of Intranasal Growth Hormone-Releasing Hormone and Corticotropin-Releasing Hormone Administration on Growth Hormone and Cortisol Release: Improved Bioavailability by Means of Sodium-Glycocholate. *J. Clin. Endocrinol. Metab.* 68:821-824 (1989).
6. M. Mishima, Y. Wakita, and M. Nakano. Promotion of Nasal Absorption of Insulin by Glycyrrhetinic Acid Derivatives. *J. Pharmacobio-Dyn.* 12:31-36 (1989).
7. P. A. Baldwin, C. K. Klingbeil, C. J. Grimm, J. P. Longenecker. The Effect of Sodium Tauro-24,25-Dihydrofusidate on the Nasal Absorption of Human Growth Hormone in Three Animal Models. *Pharm. Res.* 7:547-552 (1990).
8. D. J. Pillion, J. D. Bartlett, E. Meezan, M, Yang, R. J. Crain, and W. E. Grizzle. Systemic Absorption of Insulin Delivered Topically to the Rat Eye. *Invest. Ophthalmol. Vis. Sci.* 32:15-21 (1991).
9. G. C. Y. Chiou and C. Y. Chuang. Improvement of Systemic Absorption of Insulin through Eyes with Absorption Enhancers. *J. Pharm. Sci.* 78:815-818 (1989).
10. C. R. Kensil, U. Patel, M. Lennick, and D. Marciani. Separation and Characterization of Saponins with Adjuvant Activity from *Quillaja saponaria* Molina Cortex. *J. Immunol.* 146:431-437 (1991).
11. C. R. Kensil, S. Soltysik, U. Patel, and D. J. Marciani. Structure/Function Relationship in Adjuvants from *Quillaja saponaria* Molina. In: R. M. Chanock, H. S. Ginsberg, F. Brown, R. A. Lerner, (eds.), *Vaccines 92*, Cold Spring Harbor Laboratory Press, Cold Spring, New York, 1992, pp. 35-40.
12. M. J. Newman, J. Y. Wu, B. H. Gardner, K. J. Monroe, D. Leombruno, J. Recchia, C. R. Kensil, and R. T. Coughlin. Saponin Adjuvant Induction of Ovalbumin Specific $CD8^+$ Cytotoxic T-Lymphocyte Responses. *J. Immunol.* 148:2357–2362 (1992).
13. J. Y. Wu, B. H. Gardner, C. I. Murphy, J. Seals, C. R. Kensil, J. Recchia, G. A. Beltz, G. W. Newman, and M. J. Newman. Saponin Adjuvant Enhancement of Antigen-Specific Immune Responses to an Experimental HIV-1 Vaccine. *J. Immunol.* 148:1519-1525 (1992).
14. R. Higuchi, Y. Tokimitsu, T. Fujioka, T. Komori, T. Kawasaki, and D. G. Oakenful. Structure of Deacylsaponins Obtained from the Bark of *Quillaja Saponaria*. *Phytochemistry* 26:229-235 (1987).
15. D. J. Pillion, J. Recchia, P. Wang, D. J. Marciani, and C. R. Kensil. DS-1, A Modified *Quillaja* Saponin, Enhances Ocular and Nasal Absorption of Insulin. *J. Pharm. Sci.* (In press)
16. C. R. Kensil, S. Soltysik, and D. J. Marciani. Methods for Enhancing Drug Delivery with Modified Saponins. U.S. Patent Number 5,273,965 (1993).
17. W. S. York, A. G. Darvil, M. McNeil, T. T. Stevenson, and P. Albersheim. Plant Molecular Biology. In: A. Weissbach and H. Weissbach (eds.), *Methods in Enzymology*, Academic Press, New York, 1986, volume 118, pp. 3-40.
18. E. DeVendittis, G. Palumbo, G. Parlato, and V. Bocchini. A Fluorimetric Method for the Estimation of the Critical Micelle Concentration of Surfactants. *Anal. Biochem.* 115:278-286 (1981).
19. M. E. Jolley, S. D. Stroupe, C. J. Wang, H. N. Panas, C. L. Keegan, R. L. Schmidt, and K. S. Schwenzer. Fluorescence Polarization Immunoassay. I. Monitoring Aminoglycoside Antibiotics in Serum and Plasma. *Clin. Chem.* 27:1190-1197 (1981).
20. C. O. Onyeji, D. P. Nicolau, C. H. Nightingale, R. Quintiliani. Optimal Times above MICs of Ceftibuten and Cefaclor in Experimental Intra-Abdominal Infections. *Antimicrobial Agents and Chemotherapy* 38:1112-1117 (1994).
21. G. S. M. J. E. Duchateau, J. Zuidema, and F. W. H. M. Merkus. Bile Salts and Intranasal Drug Absorption. *Intl. J. Pharmaceutics* 31:193-199 (1986).
22. A. Rubinstein. Intranasal Administration of Gentamicin in Human Subjects. *Antimicrobial Agents and Chemotherapy* 23:778-779 (1983).
23. J. P. Longenecker, A. C. Moses, J. S. Flier, R. D. Silver, M. C. Carey, and E. J. Dubovi. Effects of Sodium Taurodihydrofusidate on Nasal Absorption of Insulin in Sheep. *J. Pharm. Sci.* 76:351-355 (1987).
24. D. J. Pillion, C. R. Kensil, J. A. Amsden, and J. Recchia. A Structure/Function Relationship Among *Quillaja* Saponins Serving as Excipients for Nasal and Ocular Delivery of Insulin. Submitted for publication.
25. F. W. H. M. Merkus, N. G. M. Schipper, W. A. J. J. Hermens, S. G. Romeijn, and J. C. Verhoef. Absorption Enhancers in Nasal Drug Delivery; Efficacy and Safety. *J. Controlled Release* 24:201-208 (1993).
26. S. Gizurarson and E. Bechgaard. Intranasal Administration of Insulin to Humans. *Diabetes Res. Clin. Pract.* 12:71-84 (1991).
27. M. T. Scott, M. Goss-Sampson, and R. Bomford. Adjuvant Activity of Saponin: Antigen Localization Studies. *Int. Archs. Allergy Appl. Immun.* 77:409-412 (1985).
28. R. Strobbe, G. Charlier, A. van Aert, J. Debecq, and J. Leunen. Studies about the Adjuvant Activity of Saponin Fractions in Foot-and-Mouth Disease Vaccine. II Irritation and Adjuvant Activity of Saponin Fractions Obtained by Chromatography on Sephadex G-100. *Arch. Exper. Vet. Med.* 28:385-392 (1974).