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RESEARCH PAPER

In Vitro Release Kinetics of Gentamycin from a Sodium Hyaluronate Gel Delivery System Suitable for the Treatment of Peripheral Vestibular Disease

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ABSTRACT

For certain patients who experience intense vertigo arising from unilateral vestibular lesions, the primary therapy is a vestibular nerve section, an intracranial surgical procedure. One alternative to this treatment is therapeutic ablation of vestibular function on the unaffected side using an ototoxic agent. We prepared a biodegradable sustained-release gel delivery system using sodium hyaluronate that can be administered into the middle ear using only a local anesthetic. The gel contains gentamycin sulfate, the ototoxic agent of choice for treatment of unilateral vestibulopathy, and it exhibits diffusion-controlled release of the drug over a period of hours. The released gentamycin could then diffuse into the inner ear through the round membrane. This represents an important advance over previous formulations, which used only gentamycin sulfate solutions, in that it should allow more careful

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control of the dose, it should reduce loss of the drug from the middle ear site, and it should maintain intimate contact with the round membrane. By carefully controlling the dose, it should be possible to inhibit vestibular function while minimizing hearing loss. Herein we describe the in vitro release kinetics of gentamycin sulfate from sodium hyaluronate gels and find that the system obeys Fickian behavior.
Key Words: *Controlled release; Osteomyleitis; Sodium hyaluronate.*

INTRODUCTION

Patients with a unilateral functional lesion at the level of the vestibular labyrinth component of the inner ear experience intense episodes of vertigo, usually with nausea and vomiting. This condition is often referred to as Ménière's disease. They may also experience continuous disorientation in space, all of which can be incapacitating. This affliction is caused by a variety of histopathological states, all of which can be termed as vestibulopathies. The current primary therapy for these patients, if change in diet and administration of antibiotics does not alleviate these symptoms, is a vestibular nerve section. By sectioning the vestibular nerve, vestibular function is irreversibly lost on one side and allows the patient to reorient over a period of time. The success rate is high, yet some significant risk is involved. This is a major intracranial operation, with hospital stays averaging 5 days, and even though the procedure is straightforward, it presents the risk of intracranial infection, cerebrospinal fluid (CSF) leak, and complications due to general anesthetics. In addition, there is severe postoperative vertigo for 2–3 days.

One possible alternative therapy is to inhibit vestibular function with an ototoxic therapeutic agent. Aminoglycosides, such as gentamycin sulfate (GS), are known to be ototoxic, causing both loss of hearing and disruption of vestibular function (1–6). However, with the appropriate dose, it should be possible to inhibit vestibular function without affecting hearing. If the drug is placed in the middle ear, which is accessible from the outside by surgically circumventing the eardrum, the drug could diffuse through the round membrane and into the inner ear, which is inaccessible from without. It is essential that the drug remain in intimate contact with the round membrane for all of the dose to be delivered. Clinical studies have been performed using GS to treat Ménière's disease, with some success (1–6). However, all previous studies used only gentamycin solutions, which cannot reliably remain in the middle ear either because of leakage around the eardrum (via percolation back through the eardrum, if administered intertympanically) or down the patient's throat (6). Many patients report tasting the drug as it overflows the middle ear (3) because it appears that 0.8 ml is approximately the maximum capacity of the middle ear crevice. Each of these events leads to a variation in dose, often necessitating additional treatments (3), which increases the chance of hearing loss. Consequently, the procedure has met with limited acceptance. Development of a dosage form that could reliably deliver a certain dose and remain in contact with the round membrane would be highly advantageous.

Consequently, we developed an injectable gel system that would be suitable for delivering GS to the inner ear in a more controlled fashion. Considering the problems associated with using solutions (repeated injections, drug percolation back into the ear canal, loss down the eustachian tube, difficulty in maintaining continuous contact with the round membrane, which is the route the drug takes to reach the inner ear), an alternative is clearly indicated. Furthermore, the gel must be able to carry high loading factors to minimize the volume administered and to allow a single dose to be administered. Finally, the dosage form needs to be absorbed into the surrounding tissues after delivery of the drug is accomplished. The best solution appears to be a viscous gel, which would control the rate of release and ensure the drug did not leave the site of administration.

The gel described in this study is a commercial preparation of sodium hyaluronate called Healon™ (Pharmacia, Monrovia, CA). It can be impregnated with a high concentrations of GS (capable of loads equivalent to 200 mg/ml of gentamycin base, corresponding to 314 mg/ml of GS). The upper limit of loading was not investigated. The gel could then be injected behind the eardrum under local anesthetic, whereas general anesthetic is needed for the vestibular nerve section. The gel is fluid enough to be injected through a fine-gauge needle (as small as 26 gauge), ensuring proper placement of the gel, but viscous enough that it will remain in contact with the tissue for an extended period of time. Finally, the gel is biodegradable, that is, after a period of time, the gel decomposes to biocompatible materials or is absorbed into the surrounding tissues, disappearing from the site of administration (7,8). In the laboratory, the gel becomes progressively less viscous and appears to begin to dissolve over

7–10 days with minimal agitation, suggesting it might be easily removed from the injection site. Consequently, the GS/sodium hyaluronate gel delivery system may be a superior dosage form for nonsurgical treatment of unilateral vestibular dysfunction. This report describes the in vitro release kinetics for GS from sodium hyaluronate gels. In general, the system displays classic diffusion-controlled limitation of drug release.

MATERIALS AND METHODS

Materials

GS USP was obtained from Paddock Laboratories (Minneapolis, MN). Sodium hyaluronate (Healon) gels are commercially available from Pharmacia. The sodium hyaluronate used in these studies has an initial concentration of 14 mg/ml. Concentrations of gentamycin are given in terms of GS. This corresponds to a gentamycin base concentration that is 0.63 times the concentration of GS. The actual gel preparations are formed by mixing equal volumes of Healon (14 mg/ml) and a concentrated solution of GS dissolved in phosphate-buffered saline (PBS). For most studies, the final GS concentration in the gel was 314 mg/ml (i.e., 200 mg/ml of gentamycin base).

Release Studies

Samples were incubated at 37°C in a water bath unless otherwise noted. Release studies were measured as cumulative release, with the entire receiver volume replaced with fresh fluid at various times. Amounts are given as the percentage of the total dose of GS. For a typical release study, the loading factor was 200 mg of gentamycin base per 1 ml of gel. A total of 0.2 ml of gel was placed into 0.8 ml of receiver fluid. The receiver volume for all in vitro release studies was 0.8 ml, but the kinetics did not change if the receiver volume was doubled. Therefore, the total dose was 64 mg of GS, which has been found to be effective for inhibition of vestibular function (M.J. Hart, unpublished results). The remaining sodium hyaluronate was found to completely dissolve within 7–10 days, depending on the extent of agitation, suggesting biodegradation should be rapid.

Analysis

Determination of gentamycin levels in the release medium, either PBS or CSF, was performed on an Abbott TDx clinical analyzer. Calibration standards were run each time samples were analyzed. In addition, gentamycin levels were measured by derivatization with *o*-phthaldehyde (OPA), following known procedures (9,10). The two methods were found to be in excellent agreement (see below).

RESULTS AND DISCUSSION

The target dose for these studies was taken to be approximately 64 mg of GS (equivalent to 40 mg of gentamycin base). Because the volume of the middle ear is limited and to allow the administration of the drug to be as convenient as possible, the total dose was loaded into 0.2 ml of sodium hyaluronate gel. The middle ear has been shown to accommodate volumes up to 0.8 ml.

Initial release studies were performed into PBS, a common receiver fluid for controlled release studies. The release kinetics were well controlled and reproducible over the course of the study. There appears to be a small burst effect from drug adsorbed to the surface of the gel, but it is much less than normally seen with GS incorporated into other biodegradable polymers (10–12). Steady-state release is rapidly established (see below). By 4 hr, much of the drug is released (~50 %), and the rate of release begins to slow, with approximately 60% delivered by 24 hr (Fig. 1). The variability is very small, with a relative SD of 2–4% from run to run (Figs. 1 and 2).

As a better approximation to the environment of the middle ear, release was measured into CSF. The kinetic

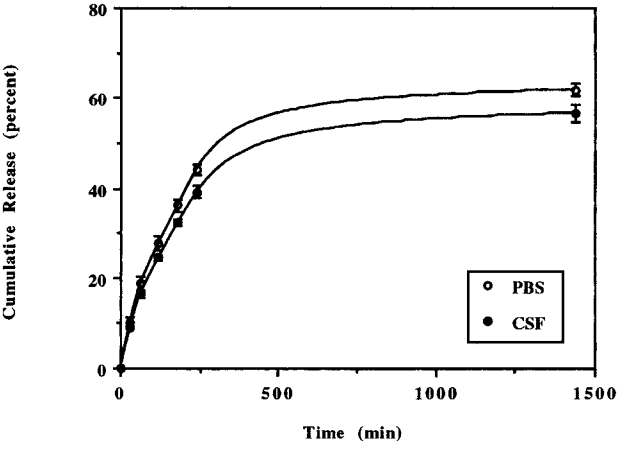

Figure 1. Cumulative release of GS into PBS and CSF from 0.2 ml of sodium hyaluronate gel at 37°C. The loading of GS was 314 mg/ml and the volume of the receiver fluid was 0.8 ml.

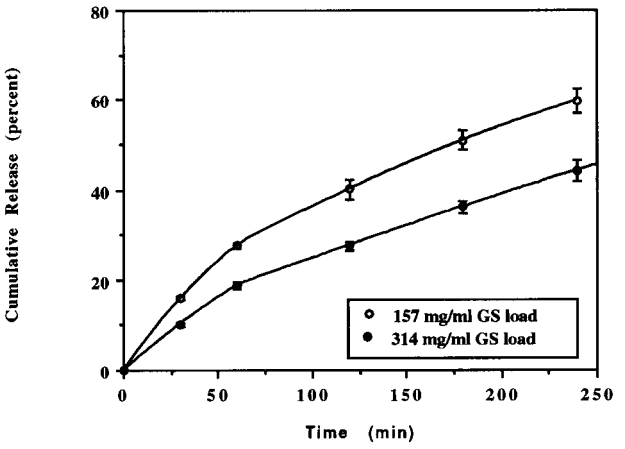

Figure 2. Cumulative release of GS into PBS from 0.2 ml sodium hyaluronate gel at 37°C. The loading of GS was either 157 and 314 mg/ml and the volume of the receiver fluid was 0.8 ml.

profile is approximately the same as in PBS, except that the amount of drug released in a given time is slightly lower (~90% of the level for release into PBS at a given time) (Fig. 1). The correlation between release into PBS and CSF is very high ($r^2 = 0.999$) (Fig. 3). Therefore, it appears the presence of serum components does lead to small diminution in the release from the gel but does not significantly alter the release profile, suggesting that variation in the exact composition of the middle ear fluid should not limit its utility in vivo.

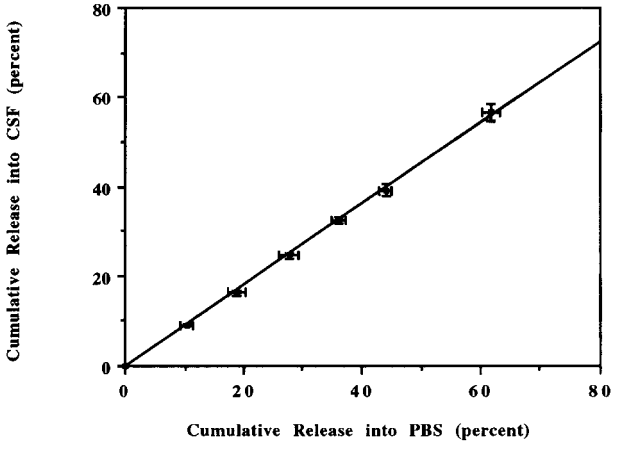

Figure 3. Correlation of the release kinetics for GS from a sodium hyaluronate gel into either PBS or CSF.

To determine the nature of the controlled release properties of this system, the rate of initial release was examined carefully. Presumably, the control of the rate of drug release would be limited by the diffusion of GS through the sodium hyaluronate gel. Controlled release delivery systems that operate by this mechanism exhibit two phases of drug release (13). During the initial phase, the depletion layer around the polymer is small and the flux of drug released into the medium increases linearly with time. However, this depletion zone increases in thickness as the surface of the polymer erodes, whereas the diffusion layer (over which a concentration gradient occurs) shrinks. At this point, the system reaches steady state, and the release of the drug (as measured by the concentration of drug in the surrounding medium) increases in proportion to the square root of time (Fig. 4). This behavior was first reported by Higuchi (14) and has been observed for a number of other controlled-release systems (13). In contrast, membrane-limited release of a drug from a device leads to a rate of release that is linear with time (13). A plot of the amount released versus time was distinctly nonlinear, indicating that release was not zero-order. Another possibility is that the drug is released by non-Fickian diffusion. Korsmeyer et al. (15) demonstrated that such behavior leads to time dependence that can be represented by a power law (t^n, $0.5 < n < 1.0$). Least-squares fitting allows an estimation of the extent of non-Fickian behavior (16). Such an analysis indicated that the best fit occurred for n values of 0.5–0.55, indicating that the release kinetics of gentamycin from hyaluronate gels are an almost completely Fickian diffusion-controlled process.

The time it takes to achieve steady state is referred to as the transition time. Monitoring the release rate over the first half hour, one observes that steady state is achieved within 10 min. From that time and over the next 24 hr, the amount of GS released is linear with the square root of time, with almost no variation. Also, notice that the sodium hyaluronate/GS gel displays very little burst effect, even at loads of 200 mg/ml of GS, indicating that very little of the drug is adsorbed to or exposed on the surface of the gel. These release properties (high loading capacity, well-behaved release kinetics, rapid equilibration, and lack of a significant burst effect) are ideally suited for delivering a high concentration locally, as is needed for this application.

While determining release profiles, it was found that the rate of release is much faster as the system is sampled more often. The sodium hyaluronate gel is always somewhat fluid, unlike solid implants, polymeric microspheres, or other controlled-release dosage forms. This

mobility means that agitation of the gel surface will be more disruptive than in those solid preparations, leading to an increased rate of release. In addition, all release studies are cumulative determinations, meaning receiver fluid is replaced by fresh solution. This will have the effect of increasing the concentration gradient that existed just before sampling. The more frequently this is done, the faster the release rate. For example, compare the amount released at 30 min with one sampling (Fig. 1) and with six samples taken (Fig. 4). In the first case, approximately 10% of the drug had been released, whereas 30% was released in the latter case.

The release of GS from sodium hyaluronate was measured for loading factors of 78, 157, and 315 mg/ml of GS (corresponding to 50, 100, and 200 mg/ml of gentamycin base). The cumulative percent release into PBS was more rapid for the lower loading factor. However, the total flux decreased with loading factor, as would be expected for diffusion-limited delivery systems (Fig. 5) (13).

Two different methods were used to quantitate GS release from the sodium hyaluronate gel: spectrophotometric determination of gentamycin after chemical modification with OPA and an automated clinical chemistry method using antibody binding and fluorescence detection. The instrument is marketed by Abbott Laboratories under the name TDx analyzer. Both methods are well established for their ability to measure GS at microgram per milliliter concentrations (9). Each method has advantages and drawbacks. Although automated, the TDx method is relatively expensive. On the other hand, the OPA method is straightforward and inexpensive but time consuming. Furthermore, OPA derivatization does not work well for samples containing biological fluids, whereas TDx can accommodate most any kind of solution. Both analytical methods exhibited linearity across the concentration range used in these studies. All samples were diluted 500- to 1000-fold before being assayed. The variability of the OPA assay was greater than for TDx (Fig. 6), with the OPA method giving slightly higher val-

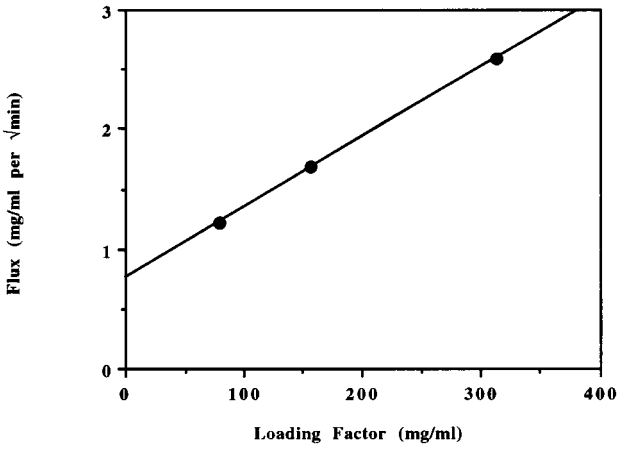

Figure 5. Effect of loading factor on the flux of GS from a sodium hyaluronate gel.

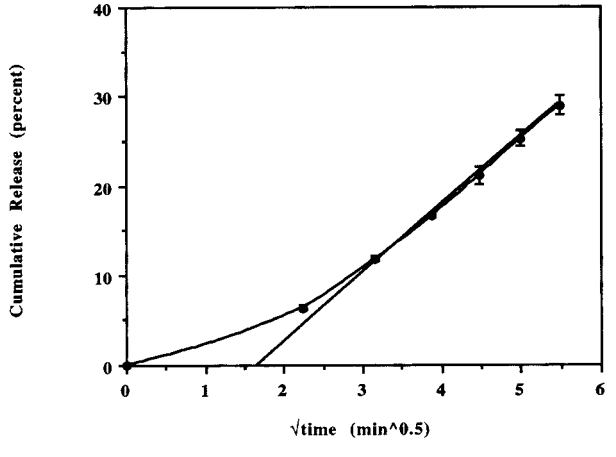

Figure 4. Release of GS from a sodium hyaluronate gel as a function of the square root of time. The loading of GS was 314 mg/ml and the volume of the receiver fluid was 0.8 ml.

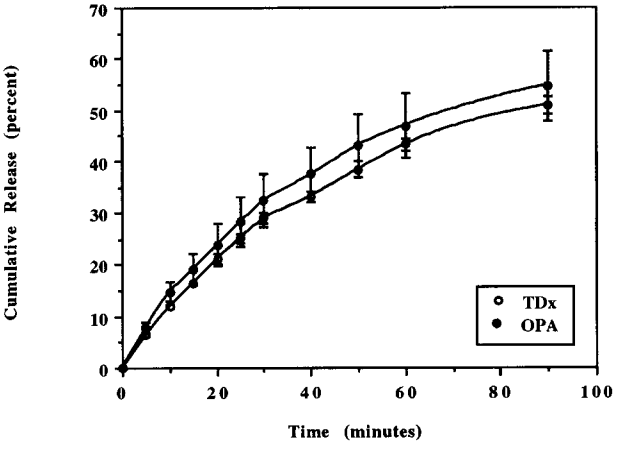

Figure 6. Cumulative release of GS from 0.2 ml of sodium hyaluronate gel at 37°C as determined by the OPA and TDx methods. The loading of GS was 200 mg/ml and the volume of the receiver fluid was 0.8 ml.

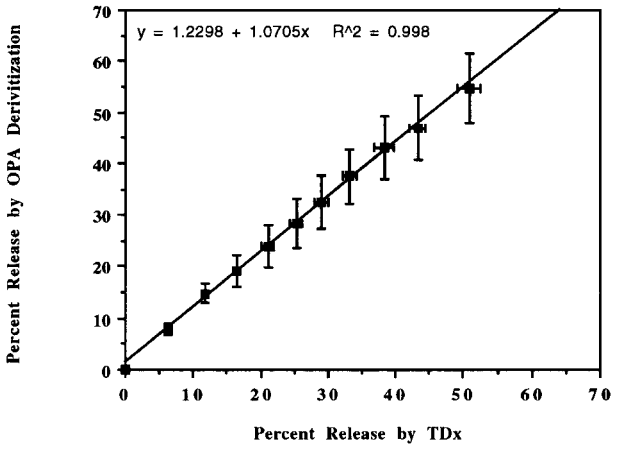

Figure 7. Correlation of GS levels as measured by TDx or OPA methods.

ues for the concentration of GS in the received fluid. Yet there was a very good correlation between the two methods (Fig. 7).

CONCLUSIONS

The in vitro studies demonstrate that the sodium hyaluronate gel delivery system should work well for GS administration to the middle ear. These release properties (high loading capacity, well-behaved release kinetics, rapid equilibration, and lack of a significant burst effect) are ideally suited for delivering a high concentration locally, as is needed for this application. Nearly two-thirds of the dose is delivered within 24 hr with a high level of precision. The gel itself then biodegrades over a period of approximately 2 weeks. It appears this could be an extremely beneficial alternative to major surgery and a lengthy hospital stay (average of 5 days).

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