Studies on target-specificity and biological activity of *Streptococcus* serum antibody and amikacin sulfate conjugates

Abstract

The concentration of the conventional antimicrobial drugs is low in the animal body tissue and body fluids (except the few parts, such as brain) because of the drug metabolism in the liver. Bacteria are mainly distributed in the target organs when they infect animals. Even within the target organ, the combination of drugs and bacteria is also depend entirely on random collisions.

To evaluate the target-specificity and biological activity of streptococcus serum antibody and sulfate amikacin conjugates, we used polyethylene glycol 6000 (PEG6000) as the coupling agent to prepare the conjugates of streptococcus of serum antibody and amikacin sulfate. Here, we analyzed the conjugates specificity, streptococcus serum antibody reactionogenicity and immunogenicity in conjugates. Besides, we also detected sulfate amikacin acute toxicity, antimicrobial activity and bioavailability. As a result, conjugates specially bind with Streptococcus instead of *Escherichia coil*, pasteurella and Staphylococcus aureus using Fluorescence staining. Biological activity results showed that conjugates maintain Streptococcus serum antibody reactionogenicity, decrease Streptococcus serum antibody immunogenicity and increase Streptococcus serum antibody response sensitivity. Simultaneously, the results indicated that conjugates reduce the acute toxicity of amikacin sulfate and improve amikacin sulfate bioavailability and antimicrobial activity.

Key words: PEG; *Streptococcus* serum antibody; sulfate amikacin

Introduction

The concentration of the conventional antimicrobial drugs is low in animal body tissues and body fluids (with a few exceptions, such as brain) because of drug metabolism in the liver. Bacteria are mainly distributed in the target organs when they infect the animals. Even within the target organ, the combination of drugs and bacteria also depends entirely on random collisions. To guarantee the curative effect of drugs, higher drug concentration must be maintained within the bacterial colonies for a prolonged amount of time. Therefore, the

antibiotics were given at a high dose within a certain time period of treatment. As a result, drugs were deposited in tissues, especially in the adipose tissue [Levisky and Bowerman 2000], and formed drug residues. Drug metabolism can cause not only waste but also organ damage [Chua et al. 2014; Le et al. 2015]. Additionally, some bacteria evolve in the presence of the drugs and form drug resistant strains [Ampaire et al. 2015]. Therefore, the development of pathogenic bacteria treatment programs aimed at bacteria-specific molecular targets has become a hot spot of present research.

Because antigens can specifically bind to the antibody, a desired characteristic of antibody targeting drugs [Elgersma et al. 2015; Gaborit et al. 2015; Marquez-Rodas et al. 2015; Shin et al. 2015; Zhou et al. 2015] is that small drug molecules can couple with specific antibodies and then be delivered to particular pathogenic bacterium multiple times without changing the concentration. This would avoid drug waste caused by normal drug distribution, thereby reducing drug consumption and shortening the course of treatment. We prepared targeted antimicrobial agents through antimicrobial coupling to the antibody molecules, which can significantly improve the drug therapeutic effect and eliminate adverse reactions.

In this study, we prepare *Streptococcus* serum antibody-sulfate amikacin conjugates with polyethylene glycol (PEG6000) as the coupling agent and then evaluate the conjugates' specificity and *Streptococcus* serum antibody and sulfate amikacin biological activity. This study will provide a theoretical and experimental basis for bacteria-targeted drug development. In this study, we conjugated small molecule antibiotics and biomolecule antibodies supramolecularly. We evaluated the bioactivity of the small molecule antibiotics and biomolecule antibodies in the supermolecular model. We optimized methods to search for antibiotics and accumulated related data about how to improve the bacterial patterns of antibiotics to provide a solution to resolve the abuse of antibiotics.

Materials and methods

Preparing the sulfate amikacin and *Streptococcus* serum antibody conjugates

A *Streptococcus* oil emulsion inactivated vaccine was prepared with *Streptococcus* strain 01026 and immunized rabbits (Animal experiments were performed following a protocol approved by the Institutional Animal Committee of Hunan Agricultural University.) to produce the rabbit *Streptococcus* antisera. The conjugates were the mixture of *Streptococcus* serum antibodies, sulfate amikacin and PEG6000 (400:2:9) (with PEG60000 as the crosslinking agent). The conjugates were checked by electron microscopy with phosphotungstic acid dye staining.

The effect of the conjugates on the biological activity of *Streptococcus* serum antibodies

Comparison of *Streptococcus* serum antibody reactogenicity

Conjugate response efficiency assay

The serum antibody and conjugates response efficiency were detected by an indirect ELISA method [Bertolotti et al. 2015]. *Streptococcus* strain 01026 was embedded by glutaraldehyde and blocked, and the titers of *Streptococcus*, immune rabbit serum antibody and healthy rabbit serum was determined by ELISA. Healthy rabbit serum served as a negative control and physiological saline as the blank control.

Conjugate response sensitivity assay

Streptococcus bacteria and colloidal gold labeled serum antibodies [Byzova et al. 2014] were mixed at a 4:1 ratio. The mixture was harvested at different time points and centrifuged at 2000 rpm/min for 30 min. The precipitation was embedded and sliced. The slices were stained with phosphotungstic acid and examined under the EM.

Conjugate response specificity assay

E. coli strain C44103, *Streptococcus* strain 01026, *Pasteurella multocida* strain 4401 and *Staphylococcus aureus* strain C26112 were mixed with the conjugates (4:1), respectively.

After incubating at room temperature for 30 min, *Streptococcus* serum antibody response specificity was observed by sections after fluorescence staining.

Comparison of *Streptococcus* serum antibody immunogenicity

Preparation of immune serum

Ten healthy rabbits (1.8 ± 0.2 kg) were randomized into two groups (n = 5 animals/group). Control (*Streptococcus*) and conjugates (1 mg/each) were injected into the rabbits every 15 days. After 21 days, the rabbits were starved and were provided drinking water. All rabbits were sacrificed by drawing-out all of the blood in their hearts next day. The serum was isolated, incubated at 56°C for 30 min and then passed through a 0.3 μm pore size filter and stored in -20°C.

Detectionof *Streptococcus* serum antibody response immunogenicity

The response immunogenicity of the *Streptococcus* serum antibody and conjugates were detected by an indirect ELISA method. *Streptococcus* strain 01026 was embedded with the carbonate buffer solution and blocked; the titers of immune rabbit serum antibody and healthy rabbit serum were detected by ELISA. Healthy rabbit serum served as a negative control, and physiological saline served as the blank control.

Conjugates' effect on biological activity of sulfate amikacin

Acute toxicity assay

Twenty mice were randomized into two groups (n = 10 animals/group): sulfate amikacin (125 mg/kg body weight) and conjugates (750 mg/kg body weight) were injected intraperitoneally (i.p.) into the mice. Mice were monitored daily for appearance and behavior, dietary wishes, activity behaviors, defecation, central nervous system symptoms and death.

Antimicrobial activity assay *in vitro*

The determination of minimal inhibitory concentrations (MIC)

Sulfate amikacin and conjugates were diluted into a certain concentration by microdilution method (5 mg/mL, 1 mg/mL, 500 μg/mL, 100 μg/mL, 50 μg/mL, 10 μg/mL, 5 μg/mL, 1 μg/mL and 0.5 μg/mL) and added to 96-well plates. 50 μL diluted bacteria liquid (10^6～10^7/mL) were co-incubated with sulfate amikacin or conjugates at 37°C for 18 h. The lowest drug concentration with no bacterial growth is the minimal inhibitory concentration.

The determination of minimum bactericidal concentration (MBC)

One hundred microliters of the minimal inhibitory concentration were placed into no resistance agar medium and cultured overnight at 37°C. The minimum bactericidal concentration is the highest drug concentration, with less than five bacterial colonies.

Sulfate amikacin activity assay

Determination of *Streptococcus* LD_{50}

Sixty mice (20 ± 2 g) were divided into 10 groups (n = 6 animals/group). The *Streptococcus* 01026 cultures were diluted with broth medium into 10^{-1}-10^{10} by a 10 times dilution method and then injected intraperitoneally (i.p.) into the mice (0.2 mL/rabbit). The LD_{50} was calculated by the Karber method [Shingaki et al. 2015].

***Streptococcus* treatment animal model**

The LD_{50} dose of *Streptococcus* was injected into the muscles of 180 mice (20 ± 2 g), and the mice were divided into six groups (n = 30 animals/group). When symptoms appeared, five groups of mice were injected with serum antibodies (0.2 mg), sulfate amikacin (0.2 mg), conjugates (0.4 mg), conjugates (0.2 mg) and conjugate injection (0.1 mg), respectively. Mice were monitored every 12 h for three days.

Sulfate amikacin pharmacokinetic parameters assay

Twenty rabbits (1.8 ± 0.2 kg) were divided into two groups: i.p injection of the control (sulfate amikacin 10 mg/kg body weight) or i.p injection of the conjugates (10 mg/kg body weight). Blood samples were taken from the ear vein of the rabbits at 0, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min after treatment. The samples were centrifuged, and then the plasma concentrations of the supernatants were determined by a microbiological method. Pharmacokinetic parameters were obtained from the plasma concentration-time data treated with the MCP-KP pharmacokinetic program. A two-sample *t*-test was used to compare sulfate amikacin pharmacokinetic parameters in conjugates versus control [Shingaki et al. 2015].

Results

Preparing the conjugates of the sulfate amikacin and *Streptococcus* serum antibody

To prepare the conjugates of the sulfate amikacin and *Streptococcus* serum antibody, *Streptococcus* serum antibodies, sulfate amikacin and PEG6000 were mixed (400:2:9) and tested by electron microscopy. As shown in Fig 1, all sulfate amikacin were attached to the antibody molecule. To analyze the stability of the conjugates, the conjugates were stored in 4°C for 30 d, 90 d and 180 d and observed under EM. Sulfate amikacin was still attached to the *Streptococcus* serum antibody and no free sulfate amikacin (data not shown) was seen.

Conjugates specifically binding *Streptococcus*

To detect the response specificity of the conjugates, *Streptococcus* strain 01026, *E. coli* strain C44103, *Pasteurella multocida* strain 4401, and *Staphylococcus aureus* strain C26112 were respectively mixed with conjugates (1 mg/mL). Fluorescence staining results indicated that the conjugates only bind with *Streptococcus*, not with *Escherichia coli*, *Pasteurella* and *Staphylococcus aureus* (Fig 3A-B).

Conjugates improve *Streptococcus* serum antibody biological activity

Conjugates maintain *Streptococcus* serum antibody reactogenicity

To compare *Streptococcus* serum antibody response efficiency, Serum antibody and conjugate response efficiency were detected by an indirect ELISA. Fig 2 indicated that the ELISA value of the conjugates is slightly less than *Streptococcus* serum antibody, but the ELISA titer of conjugates is 1:1024, the same as *Streptococcus* serum antibody. Therefore, the conjugates do not affect *Streptococcus* serum antibody response efficiency.

Conjugates increase *Streptococcus* serum antibody response sensitivity

To analyze the *Streptococcus* serum antibody in the conjugates' response sensitivity, conjugates and *Streptococcus* serum antibody were labeled with colloidal gold. According to Fig 3D, we can see that the conjugates combine with *Streptococcus* cell surfaces when conjugates and *Streptococcus* were mixed for 3 min. After mixing for 7 min, conjugates entered into *Streptococcus* bacteria (Fig 3F). The *Streptococcus* serum antibody combined with the *Streptococcus* cell surface after being mixed for 4 min and entered into the *Streptococcus* bacteria after 8 min, Therefore, the conjugate response sensitivity is higher than *Streptococcus* serum antibody response sensitivity.

Conjugates decrease *Streptococcus* serum antibody immunogenicity

Specific antiserum was generated by immunizing rabbits with either *Streptococcus* or the conjugates. *Streptococcus* serum antibody and conjugate immunogenicity were detected by an indirect ELISA method. The results show that the titer of *Streptococcus* serum antibody is 1:64, and the conjugates is only 1:8 (Fig 4). The *Streptococcus* serum antibody immunogenicity is four times the immunogenicity of the conjugates.

Conjugates enhance sulfate amikacin biological activity

Conjugates reduce the acute toxicity of sulfate amikacin

Mice were injected intraperitoneally with sulfate amikacin (125 mg/kg body weight) and conjugates (750 mg/kg body weight). After seven days, five of the mice injected with sulfate amikacin were dead, but no mice injected with the conjugates died. Only 20% of the mice injected with conjugates had reduced ambulation and sleepiness symptoms, but they returned to normal two hours after the injection.

Conjugates improve sulfate amikacin antimicrobial activity

To test sulfate amikacin antimicrobial activity, we measured the MIC and MBC of sulfate amikacin and conjugates. Table 1 data indicate that the *Streptococcus* MIC and MBC of conjugates are 0.5 μg/mL and 1 μg/mL, respectively, which are 20 and 50 times greater than sulfate amikacin, respectively. The bacteriostatic effects of *Staphylococcus aureus* and *E. coli* conjugates are not obvious compared with *Streptococcus*.

To further observe curative effects, serum antibodies (0.2 mg), sulfate amikacin (0.2 mg) and conjugates at three concentrations (0.4 mg, 0.2 mg, 0.1 mg) were injected into a *Streptococcus* animal model, respectively. As shown in Table 2, the effective rate and cure rate of conjugates at 0.4 mg is 100% and 90%, respectively, while the rates for sulfate amikacin are 50% and 10%, respectively. Conjugates have twice the effective rate and nine times the cure rate of sulfate amikacin. We also noticed that conjugates can clearly improve sulfate amikacin antimicrobial activity.

Conjugates improve sulfate amikacin bioavailability

To further study the change of sulfate amikacin metabolic parameters, sulfate amikacin and conjugates were injected intraperitoneally (i.p.) into the rabbits and pharmacokinetic parameters were obtained from plasma concentration-time data treated with the MCP-KP pharmacokinetic program. Table 3 data show that the half-life (T1/2) of sulfate amikacin

terminal elimination extended, the drug-time area under the curve (AUC) increased, and the apparent volume of distribution (VD) and clearance rate (CL) decreased in conjugates. Pharmacokinetic parameters changed significantly ($P<0.01$) in conjugates compared with sulfate amikacin.

Discussion

Streptococcus serum antibodies, sulfate amikacin and PEG6000 were mixed (400:2:9) to form conjugates. Response specificity assays show that conjugates specifically bind *Streptococcus* (Fig 3A-B). The response efficiency assay results indicate that conjugates maintain *Streptococcus* serum antibody reactogenicity (Fig 2). At the same time, conjugates increase *Streptococcus* serum antibody response sensitivity (Fig 3D-F). Conjugates not only improve the antibody targeting but also significantly reduce the body's resistance to antibodies [Taylor and Lindorfer 2010]. Therefore, antibodies can be effective as bacteria-targeted drugs.

The increase in the *Streptococcus* serum antibody response sensitivity in conjugates can be mainly attributed to the mechanism of antibody targeting *in vitro*. The negative charge of an antigen decreases when an antibody binds with antigen, which promotes the negative charged antibody to move to the antigen. The speed of antibody moving to the antigen mainly depends on the binding speed of the antigen and antibody and the antibody moving speed in the solution. The faster the binding speed of the antigen and antibody, the greater the voltage difference is around the antigen and stronger the antibody attraction. Because the conjugates are composed of *Streptococcus* serum antibody, PEG6000 and sulfate amikacin, the conjugates have a larger volume compared to *Streptococcus* serum antibody and can bind faster to *Streptococcus*. The conjugates made with PEG6000 have better water solubility than the *Streptococcus* serum antibody, therefore the conjugates move faster than the *Streptococcus* serum antibody in the electrolyte solution. At the same time, PEG6000 is fat-

soluble and can stimulate the conjugates passing quickly into the cell membrane. Although the conjugates molecular size is bigger than the *Streptococcus* serum antibody, the conjugates entered into *Streptococcus* bacteria faster than the *Streptococcus* serum antibody (Fig 3D-F).

The response immunogenicity results indicated that *Streptococcus* serum antibody immunogenicity is four times higher than the conjugates (Fig 4). PEG6000 is a type of coupling agent often used during conjugate formation [Southern et al. 2009] that allows conjugates to have better water solubility and reduces the conjugate's immunogenicity [Schwenk et al. 2014].

To examine sulfate amikacin biological activity, we analyzed the sulfate amikacin acute toxicity, antimicrobial spectra and pharmacodynamics *in vitro* and *in vivo*. Results show that the conjugates reduced the sulfate amikacin acute toxicity, narrowed the antimicrobial spectra and enhanced the pharmacodynamics (Tables 1-3). The polymers of the sulfate amikacin and *Streptococcus* serum antibody are more safe and effective than sulfate amikacin, which will provide a theoretical and experimental basis for bacteria-targeted drug development.

The antibacterial effects of conjugates may be due to adsorption, the release on contact and membrane fusion. First, conjugates attach on the surface of bacterial cells through the serum antibody. Second, the PEG6000 in the conjugates and bacteria experience a contact release effect and the conjugates enter into the bacterium by increasing the membrane permeability or membrane fusion [Lason et al. 2013] because PEG6000 is easily dissolved in lipids and induces membrane fusion. Once fusion of the conjugates and bacterial cell membrane occurs, the wall and the membrane of the bacterial cells are damaged, which increases membrane permeability. Finally, the balance of osmotic pressure is broken, and the loss of intracellular material results in cell death [Liu et al. 2010]. Additionally, the antibody carrying conjugates can directly enter into the cell cytoplasm and exert antibacterial activities. These reasons

enable the conjugates to significantly outperform the sulfate amikacin in terms of antibacterial activity.

Sulfate amikacin was protected by PEG6000 and *Streptococcus* serum antibody through coupling. The retention time of was longer, and the amount of sulfate amikacin removed was less than these before coupling. At the same time, conjugates can target pathogens by antibody binding [Sanchez-Barcelo and Mediavilla 2014]. These reasons result in the increase in the drug-time area under the curve (AUC) and bioavailability.

The application of antibody-based drugs for targeting bacteria is beneficial to humans and animals. The diagnosis of bacterial disease can not only be qualitative but also quantitative [Schulte et al. 2014]. According to the number of the pathogenic bacteria in the body, bacterial diseases may be cured by antibody-targeted drugs. In general, antibody-targeted drugs can eliminate the adverse reactions and organ damage caused by drug residues, reduce or stop the formation the drug resistant strains, prolong the life of antibiotics and give obsolete antibacterial drug new life.

References

Ampaire LM, Katawera V, Nyehangane D, Boum Y, Bazira J. 2015. Epidemiology of Carbapenem Resistance among Multi-drug Resistant Enterobacteriaceae in Uganda. Br Microbiol Res J 8:418-423.

Bertolotti L, Muratore E, Nogarol C, Caruso C, Lucchese L, Profiti M, Anfossi L, Masoero L, Nardelli S, Rosati S. 2015. Development and validation of an indirect ELISA as a confirmatory test for surveillance of infectious bovine rhinotracheitis in vaccinated herds. BMC Vet Res 11:300.

Byzova NA, Smirnova NI, Zherdev AV, Eremin SA, Shanin IA, Lei HT, Sun Y, Dzantiev BB. 2014. Rapid immunochromatographic assay for ofloxacin in animal original foodstuffs using native antisera labeled by colloidal gold. Talanta 119:125-32.

Chua NG, Zhou YP, Lingegowda PB, Kwa AL, Lee W. 2014. Echinocandin-induced eosinophilia: a case report. Scand J Infect Dis 46:809-12.

Elgersma RC, Coumans RG, Huijbregts T, Menge WM, Joosten JA, Spijker HJ, de Groot FM, van der Lee MM, Ubink R, van den Dobbelsteen DJ and others. 2015. Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody-Drug Conjugate SYD985. Mol Pharm 12:1813-35.

Gaborit N, Lindzen M, Yarden Y. 2015. Emerging anti-cancer antibodies and combination therapies targeting HER3/ERBB3. Hum Vaccin Immunother:1-17.

Lason W, Chlebicka M, Rejdak K. 2013. Research advances in basic mechanisms of seizures and antiepileptic drug action. Pharmacol Rep 65:787-801.

Le XY, Luo P, Gu YP, Tao YX, Liu HZ. 2015. Interventional effects of squid ink polysaccharides on cyclophosphamide-associated testicular damage in mice. Bratisl Lek Listy 116:334-9.

Levisky JA, Bowerman DL. 2000. Drug deposition in adipose tissue and skin: evidence for an alternative source of positive sweat patch tests. Forensic Sci Int 110:35-46.
Liu Z, Ma Z, Guo X, Shao H, Cui Q, Song W. 2010. Changes of cytosolic Ca(2+) fluorescence intensity and plasma membrane calcium channels of maize root tip cells under osmotic stress. Plant Physiol Biochem 48:860-5.
Marquez-Rodas I, Cerezuela P, Soria A, Berrocal A, Riso A, Gonzalez-Cao M, Martin-Algarra S. 2015. Immune checkpoint inhibitors: therapeutic advances in melanoma. Ann Transl Med 3:267.
Sanchez-Barcelo EJ, Mediavilla MD. 2014. Recent patents on light based therapies: photodynamic therapy, photothermal therapy and photoimmunotherapy. Recent Pat Endocr Metab Immune Drug Discov 8:1-8.
Schulte B, Eickmeyer H, Heininger A, Juretzek S, Karrasch M, Denis O, Roisin S, Pletz MW, Klein M, Barth S and others. 2014. Detection of pneumonia associated pathogens using a prototype multiplexed pneumonia test in hospitalized patients with severe pneumonia. PLoS One 9:e110566.
Schwenk R, DeBot M, Porter M, Nikki J, Rein L, Spaccapelo R, Crisanti A, Wightman PD, Ockenhouse CF, Dutta S. 2014. IgG2 antibodies against a clinical grade Plasmodium falciparum CSP vaccine antigen associate with protection against transgenic sporozoite challenge in mice. PLoS One 9:e111020.
Shin DH, Lee SJ, Kim JS, Ryu JH. 2015. Synergistic Effect of Immunoliposomal Gemcitabine and Bevacizumab in Glioblastoma Stem Cell-Targeted Therapy. J Biomed Nanotechnol 11:1989-2002.
Shingaki T, Katayama Y, Nakaoka T, Irie S, Onoe K, Okauchi T, Hayashinaka E, Yamaguchi M, Tanki N, Ose T and others. 2015. Visualization of drug translocation in the nasal cavity and pharmacokinetic analysis on nasal drug absorption using positron emission tomography in the rat. Eur J Pharm Biopharm 99:45-53.
Southern J, Borrow R, Andrews N, Morris R, Waight P, Hudson M, Balmer P, Findlow H, Findlow J, Miller E. 2009. Immunogenicity of a reduced schedule of meningococcal group C conjugate vaccine given concomitantly with the Prevenar and Pediacel vaccines in healthy infants in the United Kingdom. Clin Vaccine Immunol 16:194-9.
Taylor RP, Lindorfer MA. 2010. Antigenic modulation and rituximab resistance. Semin Hematol 47:124-32.
Zhou Y, Gu H, Xu Y, Li F, Kuang S, Wang Z, Zhou X, Ma H, Li P, Zheng Y and others. 2015. Targeted antiangiogenesis gene therapy using targeted cationic microbubbles conjugated with CD105 antibody compared with untargeted cationic and neutral microbubbles. Theranostics 5:399-417.

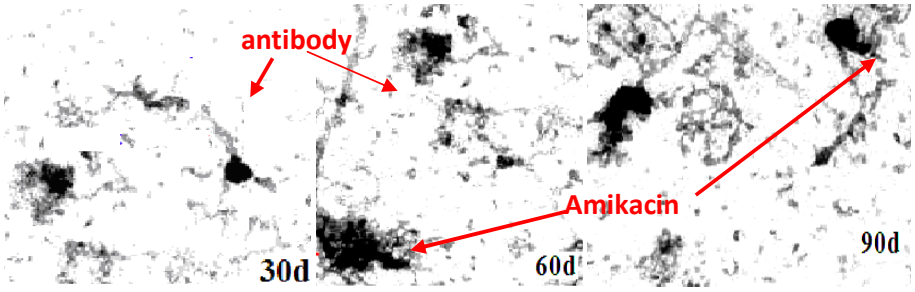
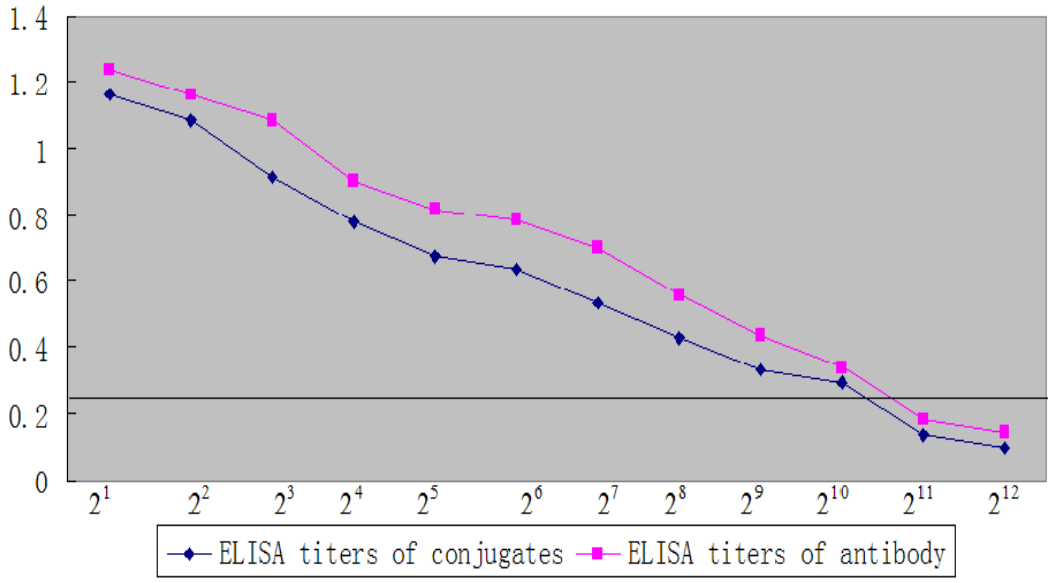
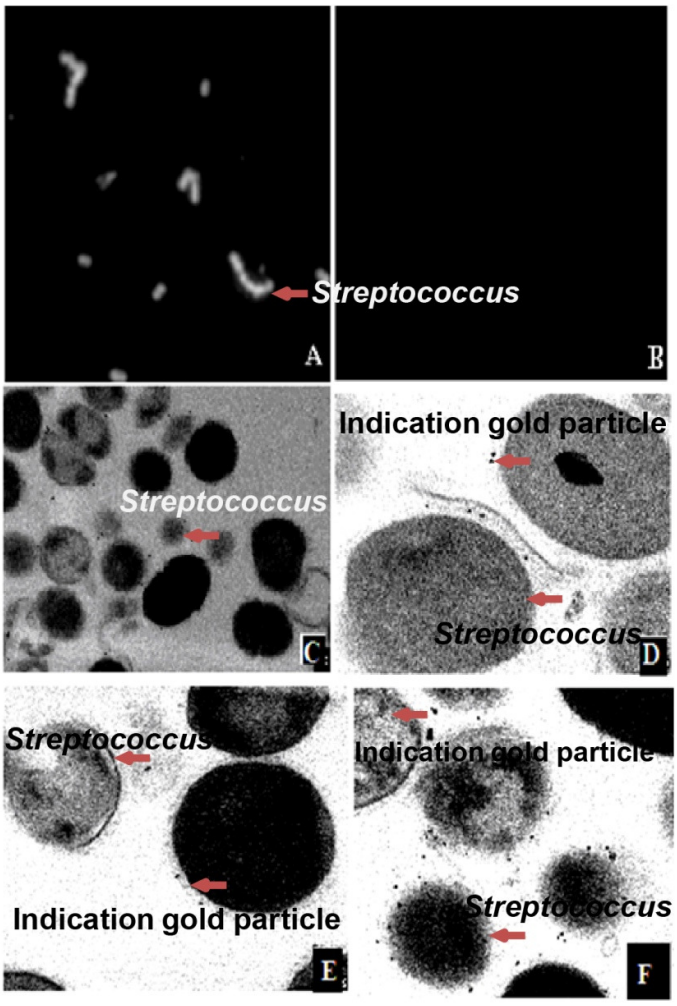


Fig. 1 Scanning electron microscopy (SEM) images of the conjugates



329

330 Fig. 2 Conjugates do not affect Streptococcus serum antibody response efficiency



331

Fig. 3 Conjugates effect on biological activity of Streptococcus serum antibodies

(A) Immunofluorescence microscopy image of the mixture of the conjugates and Streptococcus incubated for 3 min (100x). (B) Immunofluorescence microscopy image of the mixture of the conjugates and Streptococcus incubated for 2 min (100x). (C) Streptococcus without the immunogold-labeled conjugate (50,000x). (D) Immunogold-labeled conjugates and Streptococcus incubated for 2 min (50,000x). (E) Immunogold-labeled conjugates and Streptococcus incubated for 3 min (50,000x). (F) Immunogold-labeled conjugates and Streptococcus incubated for 7 min (50,000x).

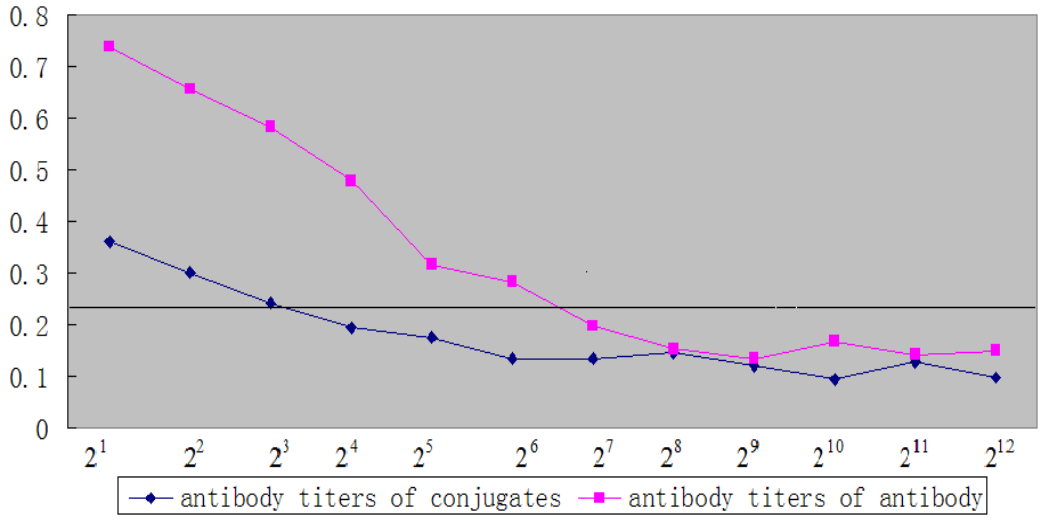


Fig. 4 Conjugates decreased Streptococcus serum antibody immunogenicity

Table 1 The comparison of in vitro antimicrobial activity of sulfate amikacin and conjugates

	conjugates		sulfate amikacin	
strain	**MIC**	**MBC**	**MIC**	**MBC**
Streptococcus	0.5 μg/ mL	1 μg/mL	10 μg/mL	50 μg/mL
Staphylococcus	>5 mg/ mL	>5 mg/mL	10 μg/mL	50 μg/mL
Escherichia coli	>5 mg/mL	>5 mg/mL	5 μg/mL	10 μg/mL
salmonella	>5 mg/mL	>5 mg/mL	1 μg/mL	5 μg/mL

Table 2 The comparison of curative effect of sulfate amikacin and conjugates in *Streptococcus* animal model

Category and the total number of treatment	effective number	cure number	effective rate (%)	cute rate (%)
Serum antibody 30	0	0	0%	0%
Sulfate amikacin 30	15	3	50%	10%
conjugate (0.4 mg) 30	30	27	100%	90%
conjugate (0.2 mg) 30	30	22	100%	75%
conjugate (0.1 mg) 30	30	18	100%	65%

Table 3 The comparison of pharmacokinetics parameters of sulfate amikacin and conjugates

	T1/2(h)		V_d(L/kg)		CL(L/kg.h)		AUC(mg.h/L)	
	Ⅰ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ	Ⅰ	Ⅱ
1	0.986	3.9564	0.290	0.0524	0.1882	0.0092	53.12	204.74
2	0.962	4.0576	0.161	0.0533	0.1158	0.0091	86.33	206.55
3	0.771	3.6430	0.229	0.0543	0.204	0.0103	48.99	181.81
4	0.884	3.7814	0.205	0.0542	0.1604	0.0100	62.33	181.89
5	1.073	3.7200	0.137	0.0549	0.0884	0.0102	113.09	183.77
6	1.317	3.6430	0.218	0.0543	0.1057	0.0103	94.61	181.81
7	1.249	3.8502	0.340	0.0536	0.1741	0.0101	57.45	202.34
8	1.457	3.9319	0.290	0.0537	0.1273	0.0099	78.57	195.72
9	1.437	3.8134	0.304	0.0539	0.1354	0.0097	73.86	187.78
10	1.045	3.7313	0.169	0.0540	0.1121	0.0095	89.20	180.69
$\bar{X}$	1.12	3.8316	0.23	0.0538	0.14	0.0098	76	191.75
±s	0.24	0.1712	0.07	0.001	0.04	0.0006	20	12.72
P	<0.01		<0.01		<0.01		<0.01	

Ⅰ：sulfate amikacinⅡ：conjugates