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Original Research Article

In vitro pharmacokinetics of sirolimus-coated stent for tracheal stenosis

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Abstract

Purpose: *To investigate the in vitro pharmacokinetics of sirolimus-coated stent for tracheal stenosis*
Methods: *Naked nickel titanium alloy stent was placed in methylene chloride leaching solution with different ratios of sirolimus/poly(lactic-co-glycolic acid) (PLGA). The morphology, thickness, and pellicles on the surface of the stent were observed by scanning electronic microscopy. Drug release from the stent was determined by enzyme amplification immunoassay.*
Results: *Sirolimus was smoothly and uniformly attached to the stent, with an optimal sirolimus: PLGA coating ratio of 1:10. Further increases in sirolimus: PLGA ratio did not improve stent drug loading. A slow release of sirolimus from the stent was observed in the first week, followed by a rapid release and then much slower release process. Release of sirolimus persisted in the stent throughout the period of 42 days.*
Conclusion: *The sirolimus-coated stent has a good surface morphology, and sustained and effective drug release characteristics. Thus, it may be effective and safe for use in the treatment of tracheal stenosis in vivo.*

Keywords: *Tracheal stenosis, Sirolimus, Drug-coated stents, poly(lactic-co-glycolic acid) PLGA*

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INTRODUCTION

Tracheal stenosis is a congenital or secondary disease caused by infection, cancer, trauma, or endometrial tuberculosis. Clinical manifestations of tracheal stenosis include cough, expectoration, dyspnea and respiratory failure. Stent implantation quickly relieves the dyspnea and improves the quality of patient's life [1]. Under some conditions, stent (a small mesh tube that is used to treat narrow or weak arteries) placement is absolutely necessary, since surgery is contra-indicated [2]. Unfortunately, intra-tracheal stent can damage the airway mucosa, and cause excessive proliferation of fibrous tissue. These lead to stent restenosis, which seriously affects the clinical efficacy of stent implantation [3]. In recent years, clinically-applied drug-eluting stent (DES) has been used in a wide range of patients with cardiopathy, gastroenteropathy or urologic diseases [4,5]. It has also been shown that DES significantly reduces incidence of stent restenosis when compared with bare-metal stent [6]. However research on applications of DES in tracheal stenosis treatment is still limited.

Sirolimus is a new immunosuppressant drug. Klugherz e*t al* [7] found that sirolimus inhibits DNA synthesis in vascular smooth muscle cells and platelet-derived growth factor (PDGF)-induced migration of cells, as well as proliferation and migration of smooth muscle cell and granuloma growth. Thus, sirolimus-coated stents have been widely used in the prevention of restenosis in patients with coronary heart disease needing coronary stent implantation [5]. However studies on the application of sirolimus-eluting stent in intratracheal restenosis are limited. A drug-coated stent consists of bare-metal stent, drugs, and a carrier. PLGA is a degradable, organic functional polymer approved by the US Food and Drug Administration (FDA). It can be used in the manufacture of medical devices, and has been applied in rat or rabbit model research on biological coating materials [8,9]. Sirolimus is a novel anti-tumor and immunosuppressive agent, which can suppress the growth of granuloma by inhibiting local inflammatory cascade [7,8,10,11]. Charytan *et al* [12-9] confirmed that m-TOR inhibitor sirolimus-coated stent was better than bare-metal stent in the inhibition of coronary stent restenosis. However, the effects of sirolimus-coated stent or bare-metal stent on tracheal restenosis are still unknown.

In this present study, we investigated the preparation of sirolimus-coated trachea stent and its pharmacokinetics *in vitro*. The duration of drug release, drug loading, optimum concentration ratio of drug and PLGA were determined, with a view to determining efficacy and safety of sirolimus-coated stent in the treatment of tracheal stenosis *in vivo*.

EXPERIMENTAL

Preparation of sirolimus-coated trachea stents

Bare nitinol stent was soaked in dichloromethane (Tianjin Tianli Chemicals Ltd) for 2 h and then washed with distilled water before it was placed in ventilation cabinet for 2 h to remove residual water. The stent was further washed with distilled water in ultrasonic cleaner for 20 min to remove surface impurities, and dried in the oven at r 50^{o}. Sirolimus (Dalian Meilun Biotechnology Co., Ltd, Dalian, Liaoning, China) and PLGA (molecular weight: 1000, LA/GA 75:25, final concentration 2 %) were dissolved in 20 mL dichloromethane in different ratios of 1:5, 1:10, 1:20, 1:30 or 1:40. The washed nitinol stent was then soaked in the above drug solutions for 2 h and was incubated in an oven at 37 ^{o}C 2 h. This procedure was repeated twice.

Scanning electronic microscopy (SEM) of coated stent

The morphology, thickness and drug pellicles of the prepared stent surface were observed with SEM (× 100). The coated stent was then put in phosphate buffered saline for 42 days, and changes in morphology and structure of the stent surface were observed with SEM.

Evaluation of pharmacokinetics of DES *in vitro*

The prepared stents were separately placed in glass tubes; 10 mL phosphate buffer (0.15mol/L, pH 7.4) were added as dissolution medium and all tubes were incubated at 37 ^{o}C in a shaker box stirred at 75 rev/min continuously for 48 h. The dissolution medium was then collected for determination of drug concentration, and was replaced with fresh phosphate buffer. This process was repeated consecutively for six weeks. Finally, the stent was put in 10 mL dichloromethane solution overnight at room temperature. The dichloromethane was then transferred into a vial to analyze its drug residue content. Concentrations of sirolimus in dissolution medium were determined every two days by enzyme amplification immunoassay for a consecutive period of 42 days [13]. Drug release, cumulative release and residual amount of drugs in DES were determined. Total drug loading and drug release time from DES were calculated and the pharmacokinetic profiles of stent and optimal coated ratio of drug and PLGA were also evaluated.

Statistical analysis

Data were analysis using descriptive statistics (percentages, mean ± standard deviation) as appropriate and one-way analysis of variance (ANOVA) was used for data comparison. $P < 0.05$ was considered significant.

RESULTS

Morphological characteristics of drug-eluting stents

As shown in Figure 1a, drug pellicles were smoothly attached to the stent after the drug-coated stent preparation procedures. After soaking in phosphate buffer for 42 days, the coated structure of stent surface changed with the degradation of PLGA, as was evident from the numerous holes on its surface (Figure 1b).

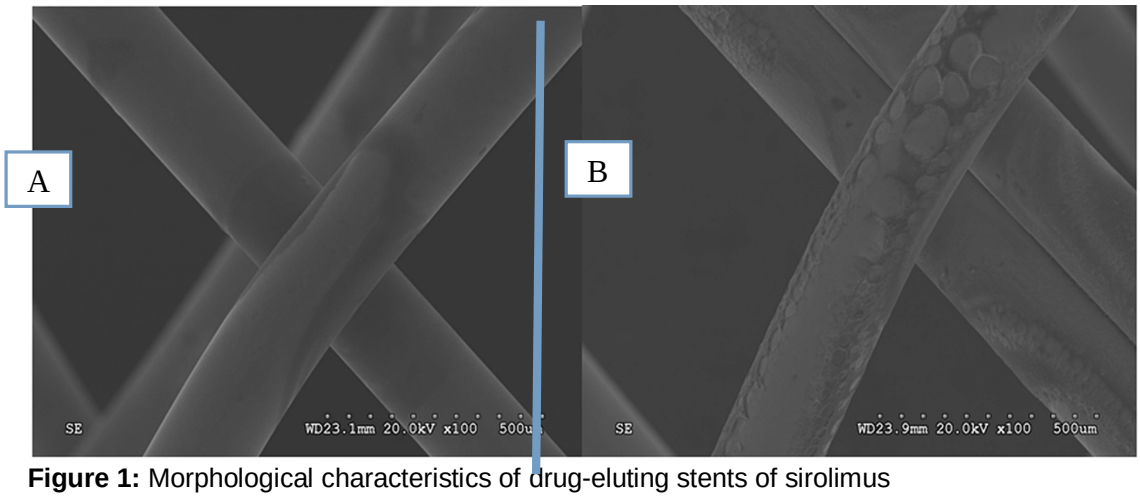

Figure 1: Morphological characteristics of drug-eluting stents of sirolimus

Optimal coating ratio of sirolimus and PLGA

Drug-coated stents prepared at different sirolimus/PLGA ratios presented different drug loading profiles (Table 1). Maximum drug loading of stent was obtained at sirolimus/PLGA ratio of 1:10. Stent drug loading did not increase with further increases in sirolimus/PLGA ratios.

***In vitro* pharmacokinetics of sirolimus release**

The release profiles of sirolimus in DES over time are shown in Figure 2. From Figure 2, it can be seen that about 02-0.5 mcg of the drug was released in the first 5 days (in which case there was slow release of drug during that period) and rapid release occurred about the 5^{th} – 9^{th} day (during which about 60 %) of the drug was released) and thereafter a slower sustained release occurred.

About 50 % stent-carried drug was released in first two weeks; there was nearly 70 % drug release in 28 days; while about 80 % was released in 42 days (Figure 3).

Table 1: Drug loading and release at different drug-eluting stent ratios

Drug-eluting stent	**Sirolimus/PLGA ratio**				
	1:5	***1:10***	***1:20***	***1:30***	***1:40***
Sirolimus:PLGA	0.08g: 0.4g	0.04g: 0.4g	0.02g: 0.4g	0.0013g: 0.4g	0.01g: 0.4g
Drug release (µg)	39.98	80.07	69.98	32.93	27.52

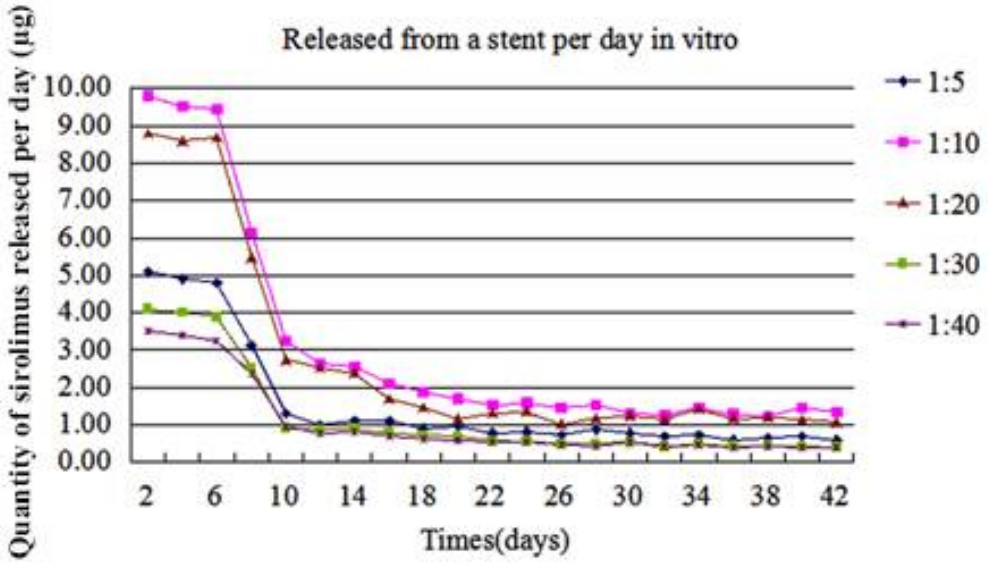

Figure 2: In vitro dailyrelease of sirolimus from sirolimus-coated stent

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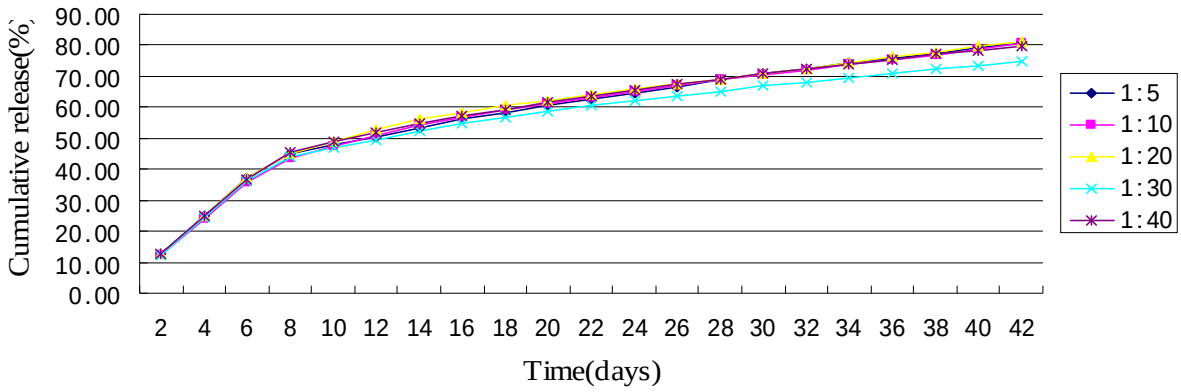

Figure 3: Cumulative drug release from sirolimus-coated stents

DISCUSSION

In this study, a sustained and effective role of sirolimus-coated stent on drug release has been found. Zhu *et al* [14] demonstrated for the first time that drug-eluting stent prepared from mitomycin C-coated tracheal stent is an effective and most promising treatment for stent restenosis. The drugs in coated stent can be released directly and continuously to a specific site without interacting with other tissues. This reduces incidence of restenosis. In this study, we developed the sirolimus-coated stent with favorable surface morphology and good drug release, which was evaluated as safe, effective in the treatment of tracheal stenosis *in vivo*. It has been demonstrated that the performance of DES depends on drug loading, drug release rate and duration. Drug/coating ratio plays an important role in this process [15]. The results of our study suggest that the best ratio for sirolimus : PLGA coating is 1:10, which enables the stent carry the highest amount of drug. Drug loading did not increase with increase the concentration of sirolimus in solution, suggesting a saturation effect. Kong *et al* [16] reported that the optimal paclitaxel : stent coating ratio was 1:20, while Yin-Kai Chao *et al* [17] determined that the optimal cisplatin : coating ratio was 1:9. These differences may be related to differences in solubilities of drugs.

Soaking is a relatively simple and important method for preparing coated stents [18]. Drug pellicles were attached to the newly prepared stent uniformly and smoothly. However, after soaking in phosphate buffer for 42 days, many holes were observed in the surface of drug-coated stent with the degradation of PLGA, indicating that sirolimus might diffuse through these gaps or holes.

The drug release curve of drug-coated stents is uncertain [19]. In sirolimus-eluting stents, drug release lasted for more than 42 days while about 50 % stent carried drugs was released in first two weeks. Although different drug : coating ratios had different drug concentrations, the processes of drug release were roughly the same *in vitro*. Therefore, suitable coating materials and appropriate drug : coating ratios are key factors for maximizing the effects of DES.

Previous studies suggested that the mechanisms of *in vitro* drug release in DES were mainly based on drug diffusion and degradation of the stent polymer [20,21]. Pires *et al* [22] found that there was a quick release period and a subsequent slow release period of drugs from DES *in vitro*. As shown in present study, the sirolimus dissolved rapidly and the stent polymer was eroded in the quick release period. Thereafter, the drug release profile entered a slow phase until the end based on gradual diffusion through porous regions when the polymer materials were degraded or flaked.

The profiles of prepared DES need to be further confirmed *in vivo*, even though some researchers have suggested that sirolimus metabolism *in vitro* is similar to that *in vivo* [23] Drug release process *in vitro* may be actually different from that *in vivo* due to changes in environment and acid-base metabolism [23,24].

CONCLUSION

We have successfully produced a trachea sirolimus-coated stents and evaluated their drug release pharmacokinetics *in vitro*. The results show that the DES has a good surface morphology, as well as sustained and effective drug release characteristics.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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