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Short communication

The release kinetics of drug eluting stents containing sirolimus as coated drug: Role of release media

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ABSTRACT

Prior understanding on the in vitro release profile of the drug from drug eluting devices such as stent (DES) is crucial in designing and optimizing the drug embedded coating or matrices. In fact, assessing in vitro release profile is a mandatory requirement prior to the clinical evaluation of DES. The in vitro release is also employed to estimate parameters such as T1/2. The release profile largely depends on the release medium selected for the studies. Normally PBS with a pH of 7.4 is used for assessing the release kinetics of the drug. Often drug undergoes irreversible changes such as hydrolysis in PBS leading to erroneous assessment of the release profile. This is particularly true in the case of sirolimus, one of the widely used drugs in various applications. We studied the influence of various media on the release profile of sirolimus from DES. The data generated suggested that a release medium consisting of 9:1 (v/v) of normal saline and isopropanol is a most suitable one for assessing in vitro the release kinetics of sirolimus from DES.

1. Introduction

Drug eluting stents (DES) have increasingly been used in interventional cardiology largely considering the potential benefits of the procedure. DES can provide luminal scaffolding that virtually eliminates recoil and remodeling of the treated vessel. Currently available DES uses paclitaxel or sirolimus as the drug.

Sirolimus (SRL), also called rapamycin, was originally developed in 1975 as a macrolide antibiotic. It has potent antifungal, immunosuppressant, and antitumor properties [1,2]. It has possible applications in tuberous sclerosis treatment, as a potential therapeutic agent for the treatment of choroidal neovascularization and diabetic macular edema [3–5]. The ability of SRL to decrease keratinocyte proliferation may help patients with psoriasis [3]. When used as part of a DES system, SRL targets the very cause of in-stent restinosis, proliferating vascular smooth-muscle cells, arresting their proliferation. Since it does not kill the cells, it avoids the inflammation associated with massive cellular necrosis seen with cytotoxic approaches, such as brachytherapy. A recent literature review explains a new generation SRL eluting PTCA angioplasty balloon catheter for restinosis therapy [6]. In all the applications, stability and the release kinetics of the drug is an important factor to design the formulation and dosage of the drug. Drug release kinetics and applied dose are playing a major role in the duration and magnitude of arterial drug uptake [7].

In case of sirolimus DES, the range of in vitro release study from stents is limited compared to paclitaxel [8,9]. Generally phosphate buffered saline (PBS) with pH 7.4 at 37 °C is employed as a medium to monitor the in vitro release kinetics of drug eluting stents. In case of sirolimus eluting stent, this medium is found to be inappropriate since sirolimus hydrolyses to form newer compounds with opened lactam ring at alkaline pH and buffer salts. It is reported that the stability of sirolimus is very low if buffer with pH 7.4 as a medium for release studies is used [10]. The lower stability is reasoned due to hydrolytic degradation of sirolimus in buffer solutions though the solubility of sirolimus in aqueous solution is very low [11–13]. In the case of chromatographic analysis, the relatively more polar hydrolyzed product elutes at a lower retention time compared to sirolimus affecting the quantitative analysis of the drug [13]. The hydrolysis rate depends on the pH of the medium and the concentration of the buffer salts. In many studies, therefore, a residual release method by measuring the amount of residual drug in the polymer or stent was adopted [12] which require a large number stents for a single study. Hence it is imperative to optimize a suitable medium to examine the release kinetics of sirolimus. We studied the stability of the drug in various media and the data emerged is discussed in this communication.

2. Materials and methods

2.1. Materials

Sirolimus (rapamycin) was obtained from Biovision, USA. Normal saline (NS), 0.9% (w/v) was from Fresenius Kabi India Pvt.

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Limited, Pune, India. 2-Propanol, methanol, acetonitrile and other reagents of analytical or chromatographic grades were procured from E Merck, Mumbai, India. PBS with pH 7.4 was prepared according to Indian Pharmacopeia (IP). Stents (SUPRALIMUS-Stent with a balloon size of 3.0 mm × 17 mm and a stent size of 3.0 mm × 16 mm) used for this study were obtained from commercial sources.

2.2. Instrumental

pH of the release medium was measured using OAKTON Water proof pH Testr 20. Incubation of the samples was done using DAIHAN LABTECH Shaking incubator (Model No.: LSI-3016R, South Korea) at 37 °C and 120 rpm.

Waters HPLC system equipped with Waters C_{18} column (3.5 μm, 4.6 mm × 150 mm), 717 plus Auto sampler and Waters 2487 dual λ absorbance detector was used for the quantitative estimation of sirolimus. HPLC analysis was done using a mixture of acetonitrile, methanol and water in the ratio of 45:40:15 as mobile phase with a flow rate of 1.2 mL/min. Column temperature was 60 °C and the injection volume was 20 μL. Waters dual λ absorbance detector with a wave length of 278 nm was used as the detector. A calibration plot was constructed for sirolimus quantification with an r^2 value of 0.9999. The amount of drug released from the DES was calculated after considering the extraction efficiency.

2.3. Methods

2.3.1. Development and optimization of release medium

Different media were tried to study the release profiles. These studies were conducted by spiking 10 μg sirolimus into the medium selected and subjected to aging at 37 °C at an rpm of 120 for 1 day and subsequently analyzed after extracting with dichloromethane. High performance liquid chromatography was used for the quantitative determination of sirolimus after aging. From these trials of different release mediums, a mixture of normal saline (NS) and 2-propanol (IPA) in the ratio of 9:1 (v/v) was selected to assess the release profile of sirolimus from DES.

2.3.2. Estimation of extraction efficiency

Extraction efficiency was assessed for two ranges of concentrations of sirolimus. To get a view on the efficiency in the lower range, 1, 3 and 5 μg of the drugs were spiked into 2 mL of NS and IPA in the ratio of 9:1 (v/v). The drug was extracted after an hour from the medium using 2 mL of dichloromethane. The extraction was repeated thrice and the combined extract was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1 mL methanol and 20 μL was injected onto the chromatographic column. The amount of drug was estimated from a calibration plot constructed using standard solutions of the drug versus peak area. All the studies were carried out in triplicate and the average value was taken. The efficiency in the higher range was estimated by spiking 5, 15 and 20 μg of the drug as stated above. The extraction (%) was calculated using the relationship

$$\frac{C}{C0} \times 100,$$

where $C0$ is the amount of drug spiked and C is the amount of drug extracted. The extraction efficiency in the higher range was 96% while it was 80% in the lower range.

2.3.3. Estimation of the in vitro release kinetics of sirolimus from DES using the developed release medium

To the deployed stent, added 2 mL of release medium, incubated at 37 °C with a speed of 120 rpm. After 24 h, the medium was replaced with a new solution of NS–IPA mixture. The drug released medium was extracted with HPLC grade dichloromethane.

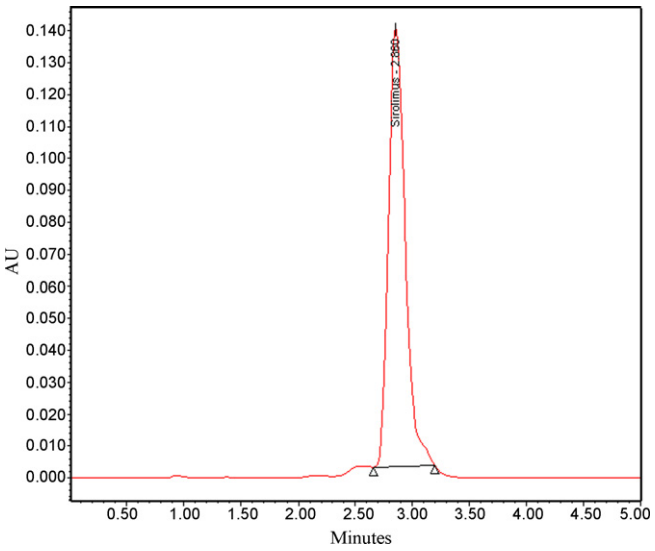

Fig. 1. Chromatogram of sirolimus dissolved in methanol and injected onto the column.

Extraction was done thrice. The combined extracts were collected, evaporated the solvent under a blanket of nitrogen and the drug residue was dissolved in 1 mL HPLC grade methanol. 20 μL of this solution was injected into HPLC and the amount of drug released was calculated by comparing the peak area obtained with that of standard sample. A triplicate analysis was done to examine the release profile of the drug from DES.

3. Results

Fig. 1 shows the chromatographic trace of drug dissolved in methanol and injected onto the column. To get an understanding on the effect of PBS on the drug, known amount of sirolimus in methanol was spiked into 0.01 M PBS and kept overnight at 37 °C under 120 rpm. The chromatogram of the extracted drug is shown in Fig. 2. The formation of additional peaks reflects the deleterious effect of the medium on the drug. Sirolimus upon contact with aqueous medium gets hydrolyzed at the lactide ring forming further polar components [11–13]. The additional peaks are assumed to be associated with the species originated from hydrolysis.

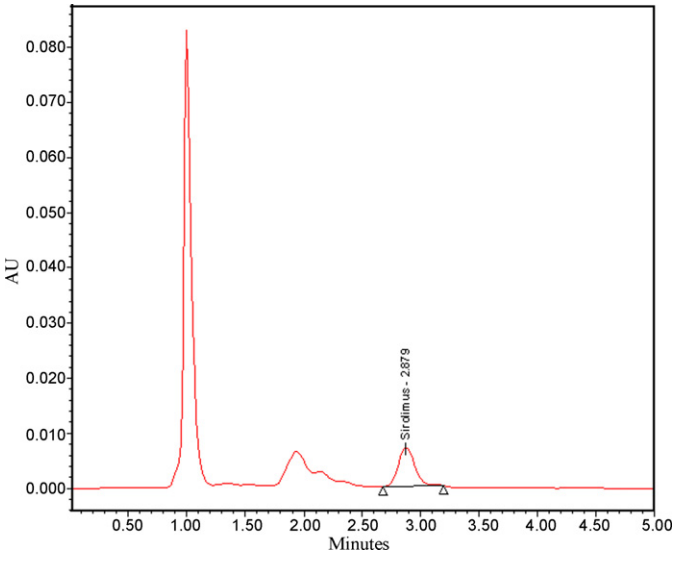

Fig. 2. Chromatogram showing the degradation of sirolimus in PBS with pH 7.4.

Table 1
The effect of the nature of medium on the stability of sirolimus. Significant degradation of the drug can be seen in media such as PBS, Tris–HCl, etc.

Medium	RT, min[a]	Peak area[a]	Amount obtained after extraction, μg[a]
Methanol, 20 μg/mL	2.880	1,111,230	19.985
PBS, 0.01 M	2.882	68,387	0.737
PBS, 0.001 M	2.899	21,228	0.229
PBS/IPA, 9:1	2.883	36,661	0.395
PBS/IPA, 8:2	2.882	71,395	0.769
PBS/IPA, 7:3	2.885	153,741	1.657
PBS/β-CD	2.882	69,884	0.753
Tris–HCl-pH 7.4	2.903	49,905	0.538
Tris–HCl-pH 6.0	2.903	26,919	0.290
NS/IPA, 9:1	2.890	557,229	9.675
NS/IPA, 8:2	2.891	581,135	9.732
NS/IPA, 7:3	2.893	586,523	9.775

Volume of injection, 20 μL.
Amount of drug spiked to each media, 10 μg.
[a] All the data are within 95% confidence limit from triplicate analysis.

This observation warranted the need for a stable medium for determining the release profile of the drug. The chromatographic profile of the spiked drug in various media like PBS with 10, 20 and 30% IPA, PBS containing Beta Cyclodextrin, 0.01 M Tris–HCl buffer with pH 7.4 and 6 and 0.001 M of PBS were assessed. All the chromatograms showed additional peaks indicating the transformation of sirolimus in the media. We monitored the stability of the drug in NS–IPA mixture (90/10, 80/20, 70/30). The amount of drug in each media was quantified and the data is shown in Table 1. Apparently the data suggest that the stability of the drug is severely affected by the nature of the media.

The extent of drug transformation is negligible in media constituted of NS and IPA. The formation of additional entities is significantly reduced with the volume of IPA. A typical chromatogram is shown in Fig. 3. NS/IPA (70/30, v/v) is found to be more ideal since the degree of degradation of the drug is minimal in this medium. However, more IPA in the medium favoring the solubility of the drug showed faster release kinetics. To get a comparative view of the in vitro data with the in vivo release profile of the drug, a medium which provide a slower release rate would be more appropriate. In that sense, it seems NS:IPA (90/10, v/v) is more suitable to study the release profile of the drug.

Normal saline is used as medication intravenously and can be considered for in vitro release studies as an alternative to PBS. To assess the suitability of the proposed medium consisting of NS and IPA in the ratio of 90:10 (v/v), we carried out in vitro release of sirolimus from stent procured from the market. Fig. 4 shows the cumulative release profile of sirolimus from DES in a medium of 2 mL 9:1 NS/IPA.

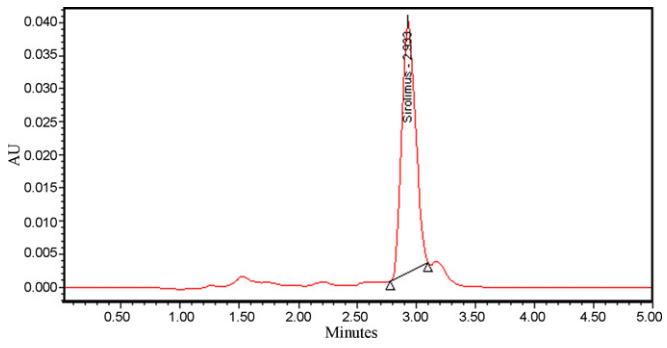

Fig. 3. Chromatogram of sirolimus extracted from a medium of NS with 30% IPA.

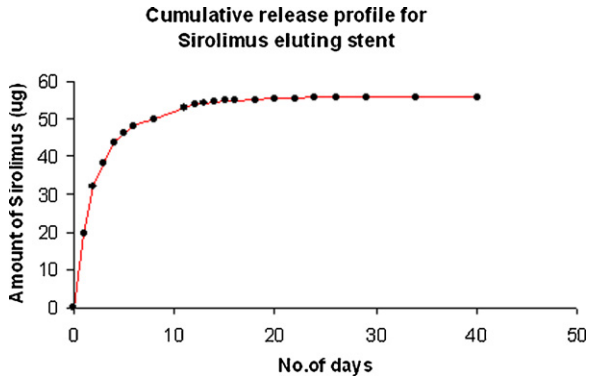

Fig. 4. The in vitro release profile of sirolimus from DES into NS:IPA (90:10, v/v) at 37 °C and 120 rpm.

4. Discussion

The instability of sirolimus in PBS is widely documented [11–13]. The actual amount released will be lower even if the analysis is conducted as quickly as possible. The poor stability of the drug in the 0.01 M phosphate buffered saline with pH 7.4 affect the quantification of the drug released during definite intervals which are required for constructing the release profile.

The drug content of the stents employed in the study was 60 ± 1.2 μg and the quantity of drug released was 58 ± 0.9 μg. The residual drug content on these stents after the stipulated period shown in the trace was found to be 0.016 μg. These parameters suggest that the amount of drug transformed in the medium is negligibly small (3.3% of the total drug content) and strongly substantiate that the proposed methodology is a more suitable one for assessing the release kinetics of sirolimus. The medium used in this study based on our trial and error approach is less hostile for sirolimus. This conclusion was drawn based on the chromatograms of samples spiked into various media. Currently, for assessing the release profile of sirolimus from devices such as DES, the residual drug remaining in the stents after a specific time period is used. An exercise of this kind is highly expensive since it requires a large number of stents. The proposed methodology can also be adopted for sirolimus release studies as a regular quality check in production and also in the development of new sirolimus embedded matrices.

5. Conclusion

Sirolimus is an important component in designing novel devices to tame the life threatening diseases. To optimize the formulation, however, a thorough understanding on the in vitro release profile of the drug entrenched in various matrices is mandatory. Several studies point out that sirolimus is unstable in aqueous media such as PBS which is universally used in determining the release kinetics of the drug. We attempted here to develop a medium in which drug undergoes minimum changes. The data generated using a wide variety of media suggested that normal saline and IPA in the ratio of 9:1 (v/v) is an appropriate medium to assess the time bound release of sirolimus. The efficacy of our method is demonstrated by estimating the release kinetics of sirolimus from a DES.

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