"For one who has awakened in knowledge
there is no suffering"

Abstract
1. ABSTRACT

Coronary artery disease (CAD) is the major cause of morbidity and mortality worldwide. The introduction of metallic stents to treat coronary artery disease has been one of the most revolutionary breakthroughs in the history of cardiology. In the early stages of their development, these devices were mainly considered to represent the mechanical solution to abrupt vessel closure and elastic recoil following balloon angioplasty. For this reason, research and debate initially focused on issues surrounding stent design, including the assessment of different materials and surface treatments. More recently, following the introduction of drug eluting stents (DESs), the debate has shifted to the research of the best vector for local drug delivery and modification of coronary plaque pathophysiology. The rationale for the introduction of DESs was to reduce the formation of neointimal hyperplasia, a proliferative maladaptive healing response to bare metal stent implantation, potentially leading to restenosis and repeat revascularization. All preclinical and clinical studies received protocol approval from the animal and human ethics committees respectively.

We evaluated safety and efficacy of novel Supralimus-Core® sirolimus eluting coronary stent in preclinical and clinical testing methods. In preclinical, testing methods were like cytotoxicity, cytocompatibility, hemocompatibility, intradermal reactivity, local tolerance, pyrogen, Genotoxicity, histopathological, biofunctional and pharmacokinetics studies.

In vitro cytotoxicity tests using direct contact method test on extract and indirect contact method were performed with Supralimus-Core® stent as per ISO 10993-5. The Supralimus-Core® stent showed none to mild cytotoxic reaction to fibroblast cells.

The results of cytocompatibility study indicated that there is no endothelial cell proliferation or survival on bare / polymer coated / drug loaded stents. The results of cell staining and analysis indicated that there is no necrosis in 72 hours, whereas apoptosis progressed with time on both the drug loaded stents and lack of SMC proliferation on drug loaded stents is likely to be drug induced cell apoptosis.

Hemocompatibility studies using whole blood showed significant hemolysis with all types of stents. Though there is reduction in platelet count after exposure to whole
blood, platelet rich plasma (PRP) exposure and 1-125 PRP exposure results indicate that platelet adhesion is significant on bare metal and sirolimus eluting stents. Fibrinogen consumption and related prolongation in plasma clotting also observed in the case of all stents as compared to reference (whole blood). In studies with platelets, on platelet agreeability and secretion which is the least with polymer alone coated stents.

Local tolerance of Supralimus-Core® stent checked by closed patch test for delayed hypersensitivity and Intracutaneous (intradermal) reactivity tests conducted. In our study we observed Supralimus-Core® stent does not elicit any skin sensitization potential in Guinea pigs and zero irritation score in Intracutaneous (intradermal) reactivity test.

The testing for pyrogenic substances of either endotoxin or nonendotoxin origin was carried out in Albino rabbits. The result of the experiment indicated that, the rise in temperature is under acceptable level.

The Salmonella Reverse Mutation Assay (Genotoxicity) was conducted using Histidine auxotrophic strains of Salmonella typhimurium tester strains viz. TA97a, TA 98, TA 100, TA 1535 and TA 102. The sirolimus eluting stent was tested at the concentrations of 61.72, 185.18, 555.55, 1666.67 and 5000 μg/plate using Dimethyl sulphoxide as solvent. Based on these results it concluded that Extract of sirolimus eluting stents are not mutagenic in this Salmonella Reverse Mutation Assay.

The biofunctional study was carried out to in vivo stent occlusion, patency and other associated cardiac events in porcine coronary artery model in comparison to bare metal stent at 26 weeks and polymer coated stent was studied at 4 weeks. All the animals showed normal haematological and serum biochemical values at the end of the study. The surviving animals in this group showed no weight loss or adverse cardiac events during the study. There was a reduction of platelets from 2.65 to 2.15 x 10^5 (p = 0.02), bleeding time from 153 to 115 seconds (p = 0.007), clotting time from 416 to 353 seconds (p = 0.04) and BUN from 23.5 to 16.37 mg% (p = 0.04) were noted during explantation. Total protein and RBC count was increased from 6.95 to 76.68 g% (p = 0.03) and 4.5 to 5.25 (p = 0.03) respectively.

The histopathological evaluation of sirolimus eluting stents carried out in porcine carotid artery model. Each parameter was assessed in the intima, media and adventitia. The
histopathological evaluation of sirolimus eluting stents, lumen patency was observed at 8 weeks. An average injury score of 0.52 was noted. The neointimal thickness (NIT) was moderate with an average of 356 μm at the strut sites and 122 μm at mid strut region. The average total neointimal area was 2.77 mm² with a 23% average in-stent stenosis of lumen and an average luminal area of 9 mm². Partial endothelization was seen in all. Mild inflammation predominantly mononuclear cells were present in all cases with minimal angiogenesis, necrosis, haemorrhage, and thrombosis / fibrin / fibrinoid deposits.

The pharmacokinetic study was carried out in male New Zealand white rabbit. The animal pharmacokinetic study, implantation of 1 (n=15) sirolimus eluting stents, show that $C_{\text{max}}$ were closely dose-proportional. The sirolimus levels in blood immediately after 1 day of stent implantation was 2.4 ng/ml while necropsy at 3rd day showed 0.8 ng/ml but on 7th day it was below LLOQ (0.5 ng/ml). Tissue levels varied from 0.6 to 4.0 ng/mg tissue wet weight over the first 7 days with a peak value of 4 ng/mg on 1st day of implantation. At 15 days after implantation, tissue Sirolimus levels ranged between 0.7 to 2.1 ng/mg tissue wet weight. This range was reduced to 70%, at 28th day after implantation (0.2 to 0.6 ng/mg tissue wet weight). The peak Sirolimus tissue level occurs within the first week after stent placement while systemic detection of the drug was negligible.

The sirolimus eluting stent human pharmacokinetic study, the average peak blood concentration for patients receiving two stents was about twice the concentration as was observed in patients receiving one stent (one stent, 1.92±0.9 ng/mL vs. two stents, 4.05±0.12 ng/mL, p < 0.05). Generally, all AUC parameters, which describe systemic exposure to sirolimus, were about 2-fold greater in patients receiving two stents than in patients receiving one stent. In contrast, the parameters that describe the release of sirolimus from the stent and its clearance from blood were similar in the two groups. For example, $T_{\text{max}}$ was 4.82±3.15 vs. 4.00±0.00hr, t½ was 94.14±41.20 and 114.46±8.84 hr, and clearance was 0.83±0.23 and 0.76±0.03 L/hr in patients treated with one or two stents, respectively (p = not significant for all comparisons). The sirolimus drug released from stent is in a controlled and predictable fashion.

In vitro stent dilation and recoil study, the dilation behaviours of four Supralimus-Core® coronary stents at the diameter of 3.5 mm and after recoil were examined. In vitro
study demonstrated that the acute stent recoil was 3.78%, while, the recoil study in human, acute absolute mean recoil of the Supralimus-Core® stent was 0.08±0.19 mm (2.42%) while in 24 hours angiographic follow-up mean stent recoil was 0.05±0.21mm (1.12%). In vivo acute recoil of the vessel wall immediately after sirolimus eluting coronary stent system implantation was 0.08 mm (2.42%) this is less than in vitro recoil (3.78%). The Supralimus-Core® stent has greatest radial strength.

First-in-man (MAXIMUS study) was a single-centre, prospective and non-randomized. The study included 105 patients with de novo native coronary artery lesions including multi-vessel disease patients. The average age of enrolled patients was 58.8 years and 79% were males. Diabetes, hypertension and smoking were present in 37%, 48% and 30% respectively. At quantitative coronary angiography 8 month luminal late loss was 0.39 ± 0.33 mm in-stent and 0.33 ± 0.35 mm in-segment. The binary angiographic restenosis rate in-stent and in-segment was 3.3% and 4.6% respectively. The incidence of any major adverse cardiac event at 30 days, 8 months and 12 months was 1%, 6% and 7% respectively. The Supralimus-Core® stent is effective in reducing neointimal hyperplasia.