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STENTING OF THE ABDOMINAL AORTA IN RABBITS

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Abstract. Before starting clinical trials of stents, the stage of testing them on animals is mandatory. It is rabbits that are best suited for these purposes, since their arteries are similar to the human reaction of the vascular wall to the stent. Installation of arterial implants in the abdominal aorta of a rabbit with access to vessels through the carotid artery is an inexpensive and convenient experimental model in vivo for preclinical testing of endovascular implants, in particular coronary stents, to consider the impact of new models. The first experiments on rabbits in the field of X-ray surgery of blood vessels and the heart were already at the beginning of the 20th century. Surgical intervention techniques do not require specialized instruments: standard medical devices for endovascular interventions (introducers, conductors, inflators, diagnostic catheters) are used.

Key words: stenting; iliac arteries; abdominal aorta; restenosis.

СТЕНТИРОВАНИЕ БРЮШНОЙ АОРТЫ У КРОЛИКОВ

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Резюме. Перед началом клинических испытаний стентов обязательным является этап испытания их на животных. Именно кролики лучше всего подходят для этих целей, так как их артерии схожи с человеческими по реакции сосудистой стенки на стент. Установка артериальных имплантатов в брюшную аорту кролика с доступом к сосудам через сонную артерию представляет собой недорогую и удобную экспериментальную модель *in vivo* для проведения доклинических испытаний эндоваскулярных имплантатов (в частности, коронарных стентов), рассмотрения влияния новых моделей. Первые опыты на кроликах в области рентгенохирургии сосудов и сердца проводились уже в начале XX века. Техники оперативного вмешательства не требуют специализированного инструментария: используются стандартные медицинские изделия для проведения эндоваскулярных вмешательств (интродьюсеры, проводники, индефляторы, диагностические катетеры).

Ключевые слова: стентирование; подвздошные артерии; брюшная аорта; рестеноз.

INTRODUCTION

Preclinical animal studies are mandatory steps before starting clinical trials of high-risk medical devices and implantable medical devices. The most widespread device of this class is arterial implants, in particular, coronary stents (risk class III due to rules for medical devices classification, depending on potential risk of use). Rats are the best objects for studies on an occurrence of atherosclerosis and the effect of stents on their arteries. Since metabolic, morphological and functional changes in vascular wall during the development of atherogenesis have been studied in detail over many years, as well as vessels size, it is possible to fully visualise and evaluate intima damage. Installation of arterial implants in the abdominal aorta of a rabbit with access to vessels through the carotid artery is an inexpensive and convenient experimental model *in vivo* for preclinical testing of endovascular implants, in particular coronary stents, to consider the impact of new models.

In 1933, the first experimental coronary angiography was performed on rabbits. Rousthoi inserted a catheter through a common carotid artery into ascending aorta of a rabbit and contrasted the coronary arteries with thorotrast (25 % thorium dioxide solution). In 1936, in the USSR, the first coronary angiography was performed by P.N. Mazaev using such method on animals. In 1949, the doctoral thesis was published, in which conditions for the use of radiocontrast agents were described [9].

Intravascular stent is an intravascular implant, placed in a lumen of artery, vein or other hollow organ, used to eliminate narrowing of the lumen, ducts, respiratory tract, esophagus or intestinal tube by creating mechanical support (support) to ensure and maintain the lumen of the vessel or other hollow anatomical structures [1–5]. The stent, mounted on a delivery system, is delivered into blood vessel in a folded state, positioned and deployed, after which the delivery system is removed. The use of a polymer coating on a metal stent as a matrix for encapsulating a drug is due to the need for targeted delivery of the active substance in order to reduce the intensity of vascular intimal cell proliferation. Gradual resorption of the coating ensures local release of a sufficient amount of drug necessary to limit hyperplasia in response to implantation [6].

Despite the fact that rabbit's iliac artery has significant anatomical differences from human coronary arteries, both of them have a similar vascular wall reaction to stent implantation. Unlike the vascular wall of pig's iliac arteries, there is no pronounced granulomatous reaction, an endothelialisation process is similar to that in humans. In addition, stents can be implanted in both rabbit's iliac arteries. This allows to compare the stent under study with control, excluding the influence of individual characteristics on healing process. In addition, the model is relatively inexpensive, easy to use, does not require difficult preparation, and can be used with known instruments and accessories available on the market [8].

STENTING OF ILIAC ARTERIES. PREPARATION FOR SURGICAL OPERATION. COURSE OF AN OPERATION. POSTOPERATIVE PERIOD

Before a surgical operation animals were limited in food for 8 h. Cefazolin (25 mg/kg) was administered intramuscularly before the surgical operation to prevent infectious complications. The operation was conducted using general anaesthesia [15–17]. Ketamine 75 mg/kg was injected intramuscularly [14, 20]. Depth and course of anaesthesia were monitored by the reaction of the animal's pupils to light, heart rate and respiratory rate [12]. When deep anaesthesia (level III) was reached, sanitary shaving of fur in the area of carotid artery was performed (field size 10 × 10 cm). Then an animal was transferred to surgical unit, placed on operating table and the operation began.

Operation field was disinfected using antiseptic solution. Then under aseptic conditions, a longitudinal skin incision 3–5 cm long was made 0.5–1 cm lateral to neck midline, along the anteromedial surface of the neck. After skin incision, internal jugular vein was discovered 1–1.5 cm lateral to trachea. It was necessary not to damage internal jugular vein and not to cause massive haemorrhage [14, 20].

Between the internal jugular vein and trachea, medial edge of sternocleidomastoid muscle was isolated, and right common carotid artery was exposed and mobilised. It is important to carefully free the artery in the area of planned puncture from perivascular tissues so that tissues do not interfere with the insertion of introducer into the artery. In this regard, holders were formed in distal and proximal sections of the common carotid artery using a ligature (silk thread 3/0) (in 12 animals) [14]. This method allowed the surgeon's assistant to fix the isolated artery, which later made it easier to puncture it. In 28 animals, a combination of holder on the distal segment of artery and application of a bulldog clamp (length 46 mm, working part 8 mm) to the proximal section of the common carotid artery were used to further open surgical field. Immediately before the puncture of the common carotid artery, the distal holder was slightly pulled in order to fill the artery, increase its diameter and facilitate the puncture. Puncture of the common carotid artery was performed with a 22G needle. Then, a 0.014" diameter coronary guidewire was inserted into the needle and advanced into the ascending or descending aorta. The Balance guide wire (Abbot, USA) was used in 15 animals, Balance Elite (Abbot, USA) was used in 8 animals, and Runthrough NSTM (Terumo, Japan) was used in 17 animals. When passing the guide wire, no force, sudden jerks or pushes of the guide wire are allowed to avoid the development of aortic dissections. If the advancement of the guide wire is difficult, its rotation, changing the radius of a tip of the guide wire, and an angle of the needle facilitate its safe passage. Next, a Check-Flo Performer 4F introducer for radial access (Cook, USA) was inserted into the artery through the guide wire in 34 animals, and a Radiofocus II 4F introducer (Terumo, Japan) was used in 6 animals [10, 29]. The introducer was fixed to skin with threads to prevent its dislocation from the artery [18, 19].

To control a position of the instrument and assess anatomy of the aortic arch, angiography was performed on a mobile X-ray diagnostic surgical installation RTS-612 (ZAO NIPK Electron, Russia) in angiography mode with voltage parameters of 73 kV/2.5 mA. For angiographic examination, Omnipaque 350 contrast agent (General Electric) diluted with saline in 1:1 ratio was used. Then, the installation was switched to fluoroscopic mode, and a 65 cm long BERENSTEIN 4 F diagnostic catheter (Merit Medical) was inserted into the aortic arch under X-ray control. The tip of the catheter was oriented in direction of the descending aorta. The tip of the guide wire was given a slight bend at an angle of 45° and length of 2–3 mm. A guide wire was inserted through the catheter into the aortic arch and then into the descending aorta. Then the entire "catheter-guide wire" system was brought to the terminal section of the aorta. The guide wire was removed and aortography of the terminal aorta was performed with contrast of the common iliac arteries and arteries of the hind limbs [7]. Position of the stents, presence of blood flow in the distal arteries, and arterial spasm in the area of stent implantation were assessed. Then, the introducer was removed and puncture hole was sutured [8].

The operation time from incision to wound suturing averaged 34.3 ± 12.4 min (20 to 55 min). The X-ray tube operation time averaged 8.3 ± 3.2 min (5 to 14 min). The operation was successfully completed in 39 animals. As it was noted before, 1 rabbit died from intraoperative bleeding. The operation was relatively easy, quickly to perform, and accompanied by a low radiation exposure [8].

Suturing a wound of the common carotid artery ensures reliable hemostasis and stroke prevention, increases the survival rate after surgery, and shortens the time of manipulation and anaesthesia in laboratory animals [8].

Foreign studies also compare the polymer stents Resolute and Xience. Bilateral iliac artery stents were installed in rabbits with induced atheroma: a stent with everolimus coating (Xience V EES; Abbott Vascular), stent with zotarolimus coating (Resolute ZES; Medtronic Cardio Vascular) or a bare metal stent (BMS; Multi Link Vision; Abbott Vascular). The XienceV EES stent showed the best results, it more intensively suppressed neointimal thickening with normal endothelial growth and reduced inflammation in the vascular wall [24].

RESTHENOSIS OF THE ABDOMINAL AORTA IN RABBITS

Percutaneous coronary intervention (PCI) is the mainstay of treatment for diseases of coronary artery, but complications such as in-stent restenosis and thrombosis remain problematic. Radiofrequency balloon angioplasty (RBA) can widen the lumen, fuse intimal tears, and dissection, but is associated with a higher rate of restenosis.

Thus, in the study, after creating a model of atherosclerosis based on endothelial abrasion and a high-cholesterol diet, 45 rabbits were randomly divided into three groups: radiofrequency balloon angioplasty (RBA) ($n = 20$), percutaneous transluminal angioplasty (PTA) ($n = 20$), and controls ($n = 5$). Then, aortic segments were isolated for haematoxylin and eosin staining, Masson trichrome staining, immunohistochemistry, and Western blotting for TLR-4, NFκB, MCP-1, and VCAM-1 expression. After 28 days, the intimal area was significantly lower in the RBA group compared with the PTA and controls. At the same time, the luminal and medial areas were comparable in the RBA and PTA groups but higher and lower than those in the controls, respectively. The expression of TLR-4, NFκB, MCP-1, and VCAM-1 showed no significant difference between the RBA and PTA groups [21].

The conclusion of the study was that RBA could suppress intimal hyperplasia and promote arterial dilation to greater extent than PTA after 28 days. However, this did not involve the TLR-4 signalling pathway, which likely plays a minor role in mediating restenosis. The reduction in intimal hyperplasia may be due to damage to the tunica media ablation and inhibition of vascular smooth muscle cell proliferation and migration [11].

One study also showed that endovascular stenting resulted in significant reduction in arterial wall compliance in the abdominal aorta pf rabbit [25].

A peak in smooth muscle cell and macrophage DNA synthesis was noted 3–5 days after angioplasty, typically in dissection but also in normal arterial sites. Adventitial injury and subsequent adventitial cell proliferation and collagen production were observed. After 3 days, a rapid decrease in the radiographic minimum of lumen diameter was noted as the result of vascular retraction or thrombus filling the dissection. After 7–14 days, only 24 to 33 % of the lumen loss was due to intimal enlargement, with neointima accounting for 22 to 28 % of the intima [27].

STENTS IN THE ABDOMINAL AORTA IN RABBITS. RESULT

In 2017, one research described using a pure zinc stent implanted in the abdominal aorta of a rabbit for 12 months. The pure zinc stent maintained mechanical integrity for 6 months and degraded by 41.75 ± 29.72% of the stent volume after 12 months of implantation. There was no severe inflammation, platelet aggregation, thrombus formation, or overt intimal hyperplasia at any time point after implantation. The destruction of the zinc stent played a positive role in the process of arterial remodelling and healing [13]. Favourable behaviour when there is a physiological degradation makes zinc a promising candidate for future stent applications [22, 23].

In 2020, there was a study which also proposed a bioresorbable Firesorb-C stent coated with a biodegradable Poly-L-lactic acid film. It was also tested on the abdominal aorta of rabbits and showed excellent efficacy, biosafety and biodegradability. It provided complete patency in the iliac arteries immediately after the procedure and after 6 months on control angiography and CT [26].

CONCLUSION

Comparative study on the effect of drug-eluting stents (everolimus and zotarolimus) and uneluting stents showed that stents coated with everolimus have a better effect on the vascular wall than others and can be used in further clinical practice without any particular concerns. In addition, a study on rabbits showed that radiofrequency balloon angioplasty causes fewer restenoses compared to percutaneous transluminal angioplasty [28].

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Experiments with animals were carried out in accordance with international rules (Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes).

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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