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VANTRIS®, A BIOCOMPATIBLE, SYNTHETIC, NON-BIODEGRADABLE, EASY-TO-INJECT BULKING SUBSTANCE. EVALUATION OF LOCAL TISSULAR REACTION, LOCALIZED MIGRATION AND LONG-DISTANCE MIGRATION.

María Ormaechea[4], Mario Paladini[1], Roberto Pisano[1], Miguel Scagliotti[2], Rubén Sambuelli[1], Santiago Lopez[1], Andrés Guidi[1], Juan Muñoz[1], Victor Rossetti[3], Manuel Carnerero[1], Dante Beltramo[5], Roxana Alasino[5], Ismael Bianco[5], Osvaldo Griguol, Daniela Valladares[6] and Francisco De Badiola[4].

[1]*Universidad Católica de Córdoba. School of Medicine. Córdoba. Argentina.*
[2]*Veterinaria San Isidro. Córdoba. Argentina.*
[3]*Veterinaria Medicina Animal Córdoba. Córdoba. Argentina.*
[4]*Hospital Italiano. Buenos Aires. Argentina.*
[5]*Agencia Córdoba Ciencia. Unidad CEPROCOR. Córdoba. Argentina.*
[6]*PROMEDON. Córdoba. Argentina.*

Summary.- *Biodegradable injectable bulking agents of animal origin present a fast rate of bio-reabsorption and may cause an allergic reaction. Biodegradable elements of synthetic origin have a high rate of reabsorption after a year. Non-biodegradable agents of synthetic origin lead to the formation of a fibrotic capsule, giving stability and long-term permanence. VANTRIS® is categorized into this last group; it belongs to the family of Acrylics, particles of polyacrylate polyalcohol copolymer immersed in a glycerol and physiological solution carrier. Molecular mass is very high. When injected in soft tissues, this material causes a bulkiness that remains stable through time.*

The carrier is a 40% glycerol solution with a pH of 6. Once injected, the carrier is eliminated by the reticular system through the kidneys, without metabolizing. Particles of this polyacrylate polyalcohol with glycerol are highly deformable by compression, and may be injected using a 23-gauge needle. The average of particles size is 320 mm. Once implanted, particles are covered by a fibrotic capsule of up to 70 microns.

Particles of this new material are anionic with high superficial electronegativity, thus promoting a low cellular interaction and low fibrotic growth.

The new polyacrylate polyalcohol copolymer with glycerol was tested for biocompatibility according to ISO 10993-1:2003 in vitro, showing that they are not mutagenic for the Salmonella T. strains analyzed. The extract turned out to be non-cytotoxic for cell lines in culture and non-genotoxic for mice. In in vivo studies, acrylate did not cause sensitization in mice. The macroscopic reaction of tissue irritation was not significant in subcutaneous implants and in urethras of rabbits. Seven female dogs were injected transurethrally with VANTRIS® to evaluate short and long-term migration (13 weeks and 12 months respectively).

No particles or signs of inflammation or necrosis are observed in any of the organs examined 13 weeks and 12 months after implantation. To conclude, this new material meets the conditions of ideal tissue bulking material.

Keywords: *Endoscopic bulking-treatment substance. Vesicoureteral reflux. Urinary incontinence. Bulk.*

Correspondence
Francisco I.P. de Badiola
Ruta 6, Km. 37,5.
Open Door. Lujan.
Pcia de Buenos Aires. 6708 (Argentina).

francisco.debadiola@hospitalitaliano.org.ar
maria.ormaechea@hospitalitaliano.org.ar

Resumen.- *Los agentes inyectables biodegradables de origen animal presentan una tasa rápida de bioreabsorción y pueden provocar reacciones alérgicas. Los elementos biodegradables de origen sintético tienen una alta tasa de reabsorción después de un año. Los agentes no-biodegradables*

de origen sintético dan lugar a la formación de una cápsula fibrótica, dando estabilidad y permanencia a largo plazo. VANTRIS® se clasifica en este último grupo; pertenece a la familia de los acrílicos, partículas de copolímero poliacrida polialcohol inmersas en una solución vehiculante de glicerol y fisiológico. Su masa molecular es muy alta. Cuando se inyecta en tejidos blandos, este material produce un abultamiento que permanece estable a lo largo del tiempo. El vehículo contiene un 40% de solución de glicerol con un pH de 6. Una vez inyectada, el vehículo es eliminado por el sistema reticular a través de los riñones, sin metabólizar. Las partículas de este poliacrilato polialcohol con glicerol son altamente deformables por compresión, y pueden inyectarse utilizando una aguja del 23 Gauge. El tamaño medio de las partículas es de 320 mm. Una vez implantadas, las partículas se recubren de una cápsula fibrótica de hasta 70 micrones. Las partículas de este nuevo material son aniónicas y tienen una gran electronegatividad en superficie, promoviendo así una baja interacción celular y un bajo crecimiento fibrótico. El nuevo copolímero de poliacrilato polialcohol con glicerol fue sometido a pruebas de biocompatibilidad in vitro de acuerdo con la normal ISO 10993-1:2003, mostrando que no es mutagénico para las cepas de salmonela T. analizadas. El extracto no fue citotóxico en cultivos de líneas celulares ni en ratones. En los estudios in vivo, el acrilato no produjo sensibilización en ratones. Los implantes subcutáneos y en uretra de conejos no produjeron reacción de irritación tisular macroscópica significativa. Para evaluar la migración a corto y largo plazo se inyectó Vantris® por vía transuretral en siete hembras de perro (13 semanas y 12 meses respectivamente).
No se observaron partículas o signos de inflamación con necrosis en ninguno de los órganos examinados ni a las 13 semanas ni a las 12 meses del implante. En conclusión, este nuevo material cumple con las condiciones del material inyectable tisular ideal.

Palabras clave: *Tratamiento endoscópico con sustancias inyectables. Reflujo vesicoureteral. Incontinencia urinaria. Inyectable.*

INTRODUCTION

Endoscopic injection of bulking agents has been gaining attention as a therapy for urinary incontinence and vesicoureteral reflux, because this therapy is simpler, less time-consuming, and less painful than traditional surgeries. The ideal bulking agent for the injection therapies must be easily injectable, biocompatible, volume-stable, non-antigenic and non-migratory (1, 5).

VANTRIS® is composed of anionic microparticles of polyacrylate polyalcohol copolymers, immersed in a glycerol and physiological solution carrier. Polyacrylates are super-absorbent polymers due to their structure. In the case of the polyacrylate polyalcohol copolymer, the sodium carboxylate groups hang off the main chain. When in contact with water, sodium ions come off leaving negative groups free. These repel one another, since they are negatively charged, so the polymer unfolds and absorbs water. The polyacrylate polyalcohol copolymer has a very high molecular weight (~10 million Daltons) and it comes in the form of deformable particles.

VANTRIS®, just like other bulking agents, can be used not only for stress urinary incontinence and vesicoureteral reflux treatment, but also for the treatment of gastroesophageal reflux disease (GERD), fecal incontinence and aesthetic surgery.

We evaluated polyacrylate polyalcohol particles (VANTRIS®) as an injectable bulking agent for urologic injection therapies. To determine whether VANTRIS meets the requirements of an ideal bulking agent, all properties were evaluated, according to ISO 10993-1:2003 standards.

A protocol was made to evaluate the local reaction, the localized migration and long-distance migration of the new substance in crossbred female dogs to:

1. Evaluate bulk permanence in the urethras of female dogs in the short-term (13 weeks) and long-term (12 months) after implantation.

2. Evaluate if there was any migration of the implanted substance to organs in the short term (13 weeks) and long term (12 months).

3. Histopathologic evaluation of the tissues surrounding the urethra.

METHODS

Polyacrylate polyalcohol was from Emerging Technologies inc (Greensboro, NC, USA). The material consists of particles of polyacrylate polyalcohol that were purified by supercritical carbon dioxide treatment to remove impurities. The purified particles of polyacrylate polyalcohol were dissolved in a dilution 1/25, in a biocompatible glycerol and physiological solution (40 % of glycerol).

Determination of Particles Size: The size of the particles was measured after being extruded through 23-gauge needles, using a laser diffractometer with measurement capacity between 0.02 to 2000 microns.

The chosen hosts were 7 crossbred female dogs that weighed between 20 and 28 kg, and they were injected with acrylate transurethrally.

This kind of animal has been successfully used in other studies where the migration of particles, like Teflon and silicon particles injected in the urethra, was evaluated (2, 3).

For the implantation of the substance in the female dogs, the material used was an endoscope with a 7 French (Fr) working channel, 30º optics, sleeve with double irrigation system and Albarran lever. The source of light was a

Xenon 300, by the company Storz, with endo-camera of the same origin. The injection line used was a 5 Fr catheter, 33 cm length, and 23-gauge needle and 1,5 cm long.

The animals were placed in the supine position with the distal portion (sacrum coccyx) free to allow movement and facilitate the insertion of the endoscope.

Once all the animals were anesthetized with atropine/acedan/diazepam in an antebrachial vein, the animals' vaginas were examined, previous antisepsis with providone iodine solution. To facilitate the entrance to the urethra and prevent injuries in the mucous membrane, a gel of Xylocaine 3% was used and the faucet of washing liquid was opened (sodium chloride solution). The bladder was entered and a complete examination was made, especially of the bladder neck and urethral meatuses. The injection line had to be drained with the substance and distally oriented 1 cm from the edge of the bladder neck. The urethral mucosa was punctured positioning the bevel of the needle.

Punctures were made at hours 3, 6 and 9 with enough volume to achieve complete closing of the urethral gap. The total average volume of injection needed was 3.2 ml (1.2-5.6 ml). The integrity of the bladder was monitored by cystoscopy, as well as the proper injection of the material with the consequential bulk formation.

To prevent material from coming out, the position of the needle was maintained in each puncture for 30 seconds.

The endoscope was not reintroduced in the bladder to prevent "flattening" of the bulks.

It wasn't necessary to probe any animals for retention.

The periodic control examinations were made, avoiding entering the bladder without the due observation and documentation of proximal urethra.

• Three of the female dogs were evaluated 13 weeks later (short-term) and the other 4, 12 months later (long-term). The evaluation consisted in:

1) Observation with cystoscopy: Preimplant on weeks 0 and 13 in the short-term evaluation, and preimplant on months 0, 3, 6, 9 and 12 in the long-term evaluation with video recording.

2) Ultrasound observation of the bulk formed: Post-implant on week 13 in the short-term evaluation, and post-implant on months 3, 6, 9 and 12 in the long-term evaluation with video recording.

3) Migration study: Once the established terms had been completed (13 weeks and 12 months), the animals were slaughtered, and the following organs were extracted: lung, bronchus, brain, heart, kidney, spleen, periureteral lymph nodes and liver, they were placed in separated flasks in a 10% formalin solution for subsequent histopathologic analysis, which consisted in microscopic observations of extracted organs.

4) Histopathology of the urethra: together with the organs mentioned above, the urethra was extracted and preserved in 10% formalin. The following were determined: Thickness of the capsule and the presence of: Polymorphonuclear inflammation, lymphocytes, plasmatic cells, macrophages, giant cells, necrosis, fibroplasias, fibrosis, fatty infiltration and foreign object.

RESULTS

Particles of polyacrylate polyalcohol copolymers are anionic. This means that they have a high superficial electronegativity that confers a very low cellular interaction and low fibrotic growth around them to the medium where they are implanted. This material, when injected in soft tissues, produces a bulkiness that remains stable through time.

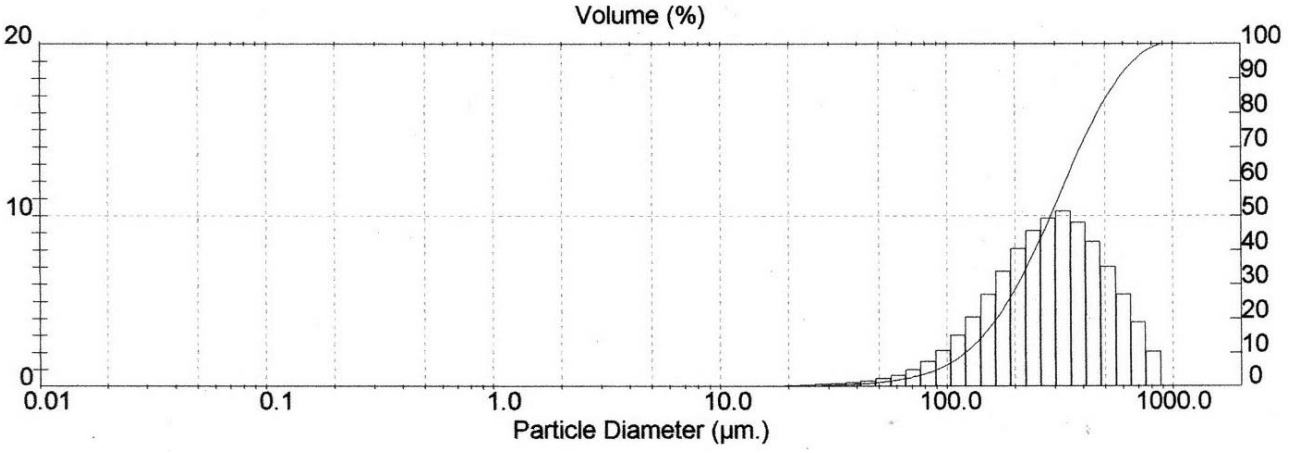

FIGURE 1. Analysis of particle size of VANTRIS®, extruded through transurethral injection using a 5-Fr catheter and a 23- gauge needle. Method for measurement: Laser diffractometer - Malvern Mastersizer.

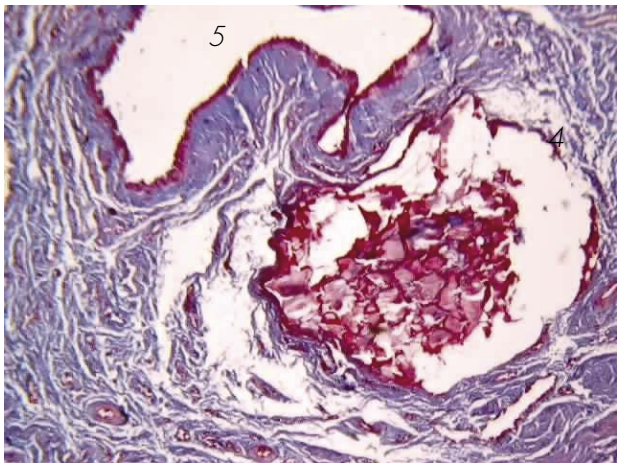

FIGURE 2. Stain: Masson's Trichrome. Material implanted within the submucosa with hypersensitive capsule of connective tissue (< 70 microns) (4). The urethra is marked as (5) post-implant Dog Nº 3 – 13 weeks post implant.

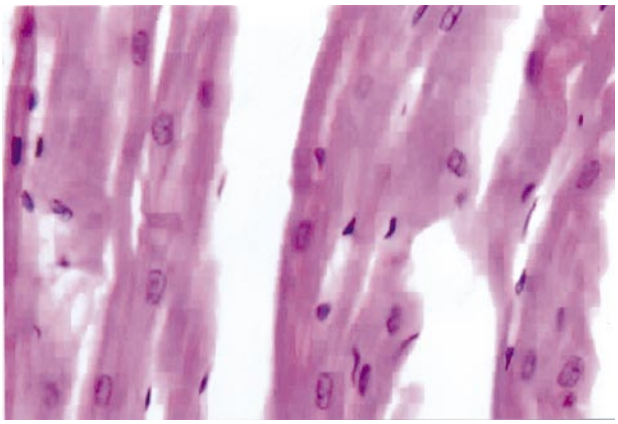

FIGURE 3. Heart 40 x HE No peculiarities. Post-implant Dog Nº 7 -12 months post implant.

The carrier is a solution of 40% glycerol with a pH of 6. Once implanted, the glycerol carrier is eliminated by the reticulo-endothelial system, without metabolizing, and excreted through the kidneys, while the particles remain for permanent bulking.

Particles of polyacrylate polyalcohol copolymer are highly deformable by compression, and may be easily extruded manually through 23-gauge needles. After the extrusion process, the analysis by laser diffractometer shows an average of particles size of 320 mm (Figure 1).

Once implanted, particles encourage adhesion to host tissues, generating a fibrotic capsule of up to 70 microns around them. Results are shown in Table I.

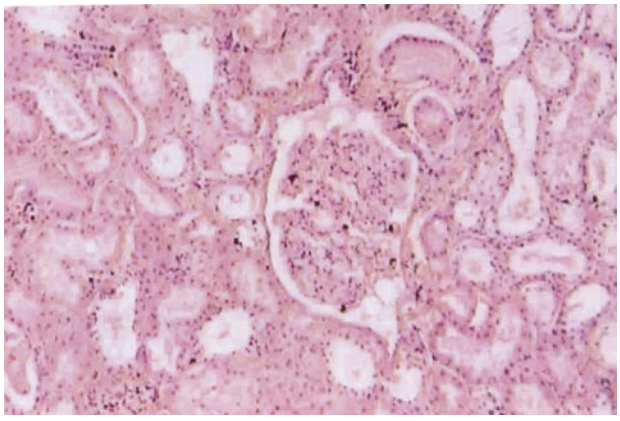

FIGURE 4. Kidney10 x HE Glomerules and tubules with no special peculiarities. Post-implant Dog Nº7 -12 months-post implant.

Short and long term controls with cystoscopy, at the different times of observation, according to the suggested protocol, prove that "bulks" can be appreciated in all the animals with varying levels of closing of the proximal urethra. Preimplant cystoscopy verified the good condition of the bladder, bladder neck and urethra.

During implantation, enough material was placed (between 1.2 and 5.6 ml, containing 48 mg and 224 mg of Polyacrylate polyalcohol particles) until urethral closing was verified. After implant, original bulks undergo a slight readaptation but preserve intraluminal urethra closing observed by urethroscopy in the female dogs.

Bibliography quotes that success of endoscopic therapy depends more on the positioning of the implants and on the ability of the surgeon as well as the selection of patients, than in the choice of material (6).

Injections were administered in the urethral submucosa; nonetheless, in some punctures material was implanted past the submucosa. This was demonstrated by the histopathologic analysis of the 7 urethras analyzed, where in 3 of the 7 female dogs the material was found in the subserous and muscular layers. In those cases, instead of appearing inside the urethral lumen, the bulk was found in deeper layers.

Images obtained with anal and/or vaginal ultrasound in the post-implant period, in the short-term evaluation as well as in the long-term evaluation evidenced a homogeneous anechoic image, with defined borders located in the periurethral area, with approximate measures of 0.3 cm (anteroposterior) x 0.5 cm (longitudinal) x 0.2 cm (transverse).

On week 13, no particles, signs of inflammation or necrosis were observed in any of the organs analyzed in the 3 female dogs with 70 cuts of the lung, heart, bronchus,

TABLE I. IN VITRO AND IN VIVO TEST ACCORDING TO ISO 10993-1:2003 (BIOLOGICAL EVALUATION OF MEDICAL DEVICES).

IN VITRO STUDIES		
TEST	**TEST DESCRIPTION**	**RESULTS**
Genotoxicity test through reverse mutation in Salmonella Typhimurium strains (AMES Test) in saline and organic extract (DMSO)	Bacterial strain used: Salmonella Typhimurium mutants (5) Positive controls (4): 2-nitrofluorene, 2-aminofluorene, sodium azide and 9-aminoacridine Negative control: DMSO and PBS	The two product extracts tested (organic and saline) produce no mutations in the bacterial strains analyzed
Cytotoxicity test	Cells used: Vero Positive control: NaCl in MEM, 0.5 and 2% Negative control: MEM	The extract tested produces no cellular toxicity
Genotoxicity test: Study of chromosomal aberrations in mammal cells	Cells used: Chinese hamster ovary cells Positive control: Mitomycin C with no metabolic activation and Cyclophosphamide with metabolic activation Negative control: McCoy's medium	The product produces no chromosomal aberrations in mammal cells with or without metabolic activation
	IN VIVO STUDIES	
Sensitivity test in mice	Host: CBA/J mouse strain Contact time: 6 days Positive control: 1 Chlorine, 2.4 DNB Negative control: DMSO and acetone	The product causes no sensitivity
Test of subcutaneous implant and in urethras of rabbits on weeks 1, 4, and 13	Host: male rabbits (4) Negative control: Solid silicone Regions analyzed: 3 subcutaneous regions and 1 region in urethra	• Non-significant macroscopic reaction of tissue. • Slight irritation in microscopic reaction of tissue • No active inflammatory processes are observed
Test of bulk migration and permanence on week 13 and on month 12 (implant in the urethras of female dogs)	Host: female dogs Organs analyzed: lung, heart, brain, bronchus, kidney, spleen, periurethral lymph nodes and liver Amount of sections from each organ: 70	No particles are observed at the level of the lung, heart, brain, bronchus, kidney, spleen, periurethral lymph nodes and liver. Cystoscopy and ultrasound showed that the implants remained stable
Carcinogenicity and chronic toxicity	According to OSHA Hazard Communication Standard (US Department of Labor)	The product causes no carcinogenicity or chronic toxicity.
Genotoxicity test: Micronucleus test in the bone marrow of mice	Host: 30 Mus musculus mice Positive control: cyclophosphamide Negative control: 0.9% ClNa in USP Contact time: 48 hours	The product is not genotoxic for mice

kidney, spleen, peribronchial, paratracheal and abdominal lymph nodes, pancreas, ovaries, oviduct uterus, intestine and liver. 70 serial cuts were made to the urethra, and the histopathologic report demonstrated the presence of implanted material distributed in the submucosa in 2 female dogs, and in the others, an annular distribution of implanted material was observed in the muscular area of the urethra (Figure 2).

A hypersensitive capsule of connective tissue of up to 70 microns developed around the implant, which protrudes from the rest of the tissue, in the three female dogs studied (Figure 2).

The cellular infiltrate adjacent to the implant includes lymphocytes with few plasmatic cells, with no eosinophils or neutrophils in the three female dogs studied. No necrosis was observed either, or contact with the urethral epithelium.

On month 12 no particles, signs of inflammation or necrosis were observed in no one of the organs analyzed in the 4 female dogs with 70 sections of the lung, heart (Figure 3), brain, kidney (Figure 4), spleen, peribronchial, paratracheal and abdominal lymph nodes, including retroperitoneal and suprarenal lymph nodes, pancreas, and liver.

70 serial cuts were made to the urethra, and the histopathologic report showed the presence of implanted material distributed in the subserous membrane in 2 of the 4 female dogs studied, while in the other two no material was observed, only fibrosis in the submucosa and among the muscular layers.

Inflammatory infiltrate with lymphocyte prevalence was observed in the four female dogs studied, with giant cells and plasmatic cells, making evident a fibrotic capsule of up to 70 microns.

DISCUSSION

The physiochemical properties of VANTRIS® make it suitable for treatment, not only of vesicoureteral reflux but also of urinary incontinence on effort due to intrinsic sphincter deficiency. When these particles are implanted in the ureterovesical junction, the material acts enlarging the volume of the area and correcting the anatomy of the meatus and the distal ureter, preventing urine to return to the ureter after being stored in the bladder. In the case of the treatment of intrinsic sphincter deficiency particles are implanted between the middle urethra and the bladder neck, and the material acts enlarging the volume of the area and assisting to return to urinary continence. Other possible applications of the substance are: treatment of gastroesophageal reflux disease (GERD), fecal incontinence and aesthetic surgery.

This study demonstrated that periurethral injection of VANTRIS® particles does not cause their migration to the tissues examined and that it is easily injected manually, meeting two of the conditions for and ideal injectable substance.

In the short-term as well as long-term, the presence of implanted material was observed in the urethra. During the whole study, the presence of a thin fibrotic capsule around the implant was shown, meeting one more requirement for an ideal implant (1,5).

After the implant, the original bulks undergo a slight readaptation, but the bulk effect is preserved, closing the lumen of the urethra checked by urethroscopy in all 7 female dogs studied.

The long and short-term histologic evidence demonstrated the presence of material in the urethra, located not only in the urethral submucosa, but also in deeper urethral layers, which supports the reduction of intraluminal volume observed through time (Figure 2).

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