

# Glubran 2: A New Acrylic Glue for Neuroradiological Endovascular Use

## Experimental Study on Animals

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### Summary

A new acrylic glue, Glubran 2, is now available on the European market. It bears the CE mark with several surgical indications and the specific indication for neuroradiological endovascular use. Despite this approval, to our knowledge its use is still limited to surgery and no injections have been made in human patients.

This study was designed to evaluate the behavior of Glubran 2 in endovascular injection in a simulation of brain AVM.

Six sheep were operated on opening a fistula between the right common carotid artery and the jugular vein. This fistula modifies blood flow in the skull base rete mirabilis, which then functions as an AVM. In two sheep, the rete mirabilis was occluded by injection of 1.5 ml of Histoacryl diluted 1:1 and 1:3 with Lipiodol. In two sheep, the rete was embolized by injection of 1.5 ml of Glubran 2 diluted 1:1 with Lipiodol. The last two sheep were embolized by injection of Glubran 2 diluted 1:3 with Lipiodol. The procedures were documented by DSA angiographic acquisitions and by fluoroscopic VHS. The sheep were killed immediately after the procedures and the rete mirabilis isolated for histologic examination.

Embolization was obtained with both kinds of glue. Glubran 2 diffuses in a very similar way to Histoacryl with an apparently more complete diffusion. Reflux in the ascending pharyngeal artery showed that Glubran 2 tended not to pro-

duce bubbles but diffused more homogeneously.

The subjective conclusion of this work is that Glubran 2 can be used in endovascular embolizations. Before approaching brain AVMs, a further study will investigate embolization of the external carotid territory.

### Introduction

Selective arterial embolization was first undertaken in the Sixties to treat brain arteriovenous malformations.

The technique was subsequently applied in the surgical and neurosurgical treatment of hypervascular tumours, haemorrhage following head injury, renal and gastrointestinal tumours and vascular head and neck lesions. In addition, embolization can be used as an adjunct procedure in orthopaedic oncology prior to surgery or as a palliative treatment for pain from metastases.

For more than twenty years, an acrylic glue, Histoacryl (Braun, Aesculap AG, Tuttingen, Germany), has been used for embolization of cerebral and extracerebral vascular diseases, especially brain arteriovenous malformations which are delicate conditions difficult to treat<sup>1,7</sup>.

In recent years, attention has focused on the fact that Histoacryl has no official recognition and its endovascular use is prohibited. This situation raises major legal problems deriving from its improper use.

A new acrylic glue, "Glubran 2" (GEM Srl, Viareggio, Italy), was first marketed a couple of years ago. This glue is authorised for surgical use and for "endovascular use in neuroradiology" with a CE mark for "internal use". Glubran 2 is not the same as other cyanoacrylate glues currently available (cf. Histoacryl) as it has a different chemical composition making it a comonomer rather than a simple monomer. The comonomer of Glubran 2 surgical glue is the result of mixing two monomers: N butyl 2 cyanoacrylate (NBCA) and MS (a monomer owned by GEM Srl). NBCA is a cyanoacrylate monomer common to many other glues like Histoacryl which are CE approved solely for topical use. The addition of MS to the basic monomer allows the NBCA to polymerize with an exothermic reaction at around 45° C, and a slightly higher polymerization time than Histoacryl thereby reducing the toxicity of the basic monomer. In fact, the longer the carboxylic side chain of the minor cyanoacrylate is, the less cytotoxic the resulting adhesive<sup>8</sup>. MS also gives surgical Glubran 2 a significant anti-inflammatory effect. Based on this premise, the addition of MS to NBCA yielded a glue complying with European regulations and hence receiving CE certification for internal and endovascular use.

Although Glubran 2 can be legally used for cerebral embolization procedures, practical clinical experience with the substance is lacking. For this reason, we performed an experimental animal study to test the endovascular behaviour of the glue. The study protocol conformed to Italian legislation (Decree Law n° 116 dated 27/01/1992) and was approved by the central veterinary service for the protection of animals used for experimental purposes.

### Material and Methods

We set out to simulate the conditions of brain AVM according to Qian et Al<sup>5</sup> by creating a fistula between the common carotid artery and the jugular vein thereby inverting arterial blood flow in the skull base rete mirabilis, which then simulates the conditions of an AVM: afferent arterial vessels from the internal maxillary artery, anastomotic arteries on the left side transformed into efferents on the right side.

Six sheep underwent surgery according to

Italian legislation governing the protection of animals used for experimental purposes (Legislative Decree 116/92) and the "Guide for the Care and Use of Laboratory Animals" and Animal Welfare Assurance No. A5424-01 of the National Institute of Health (NIH-Rockville Maryland USA); the research protocol was authorized by the Bologna University Ethical Committee.

### Animals

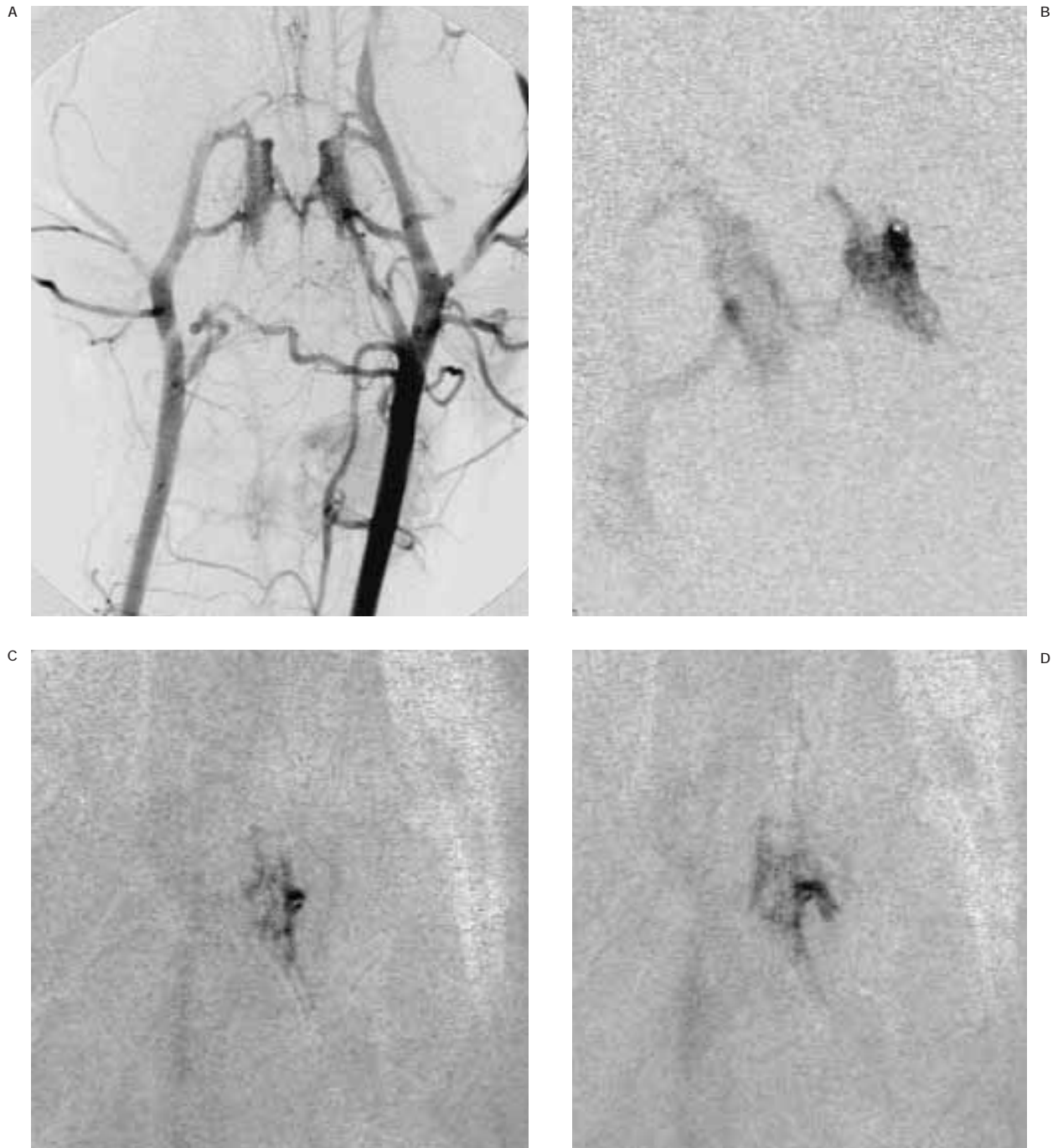
Six crossbred adult sheep (aged three years), weighing 75 ± 5 kg, were housed in individual cages with a grid floor at room temperature and controlled humidity: 20 ± 0.5° C and 50 ± 5%, with ventilation of ten air changes an hour. The animals were fed a standard maintenance diet (Mucedola, Settimo Milanese, Italy), clover and water ad libitum.

### General anaesthesia

After five days' quarantine, the animals underwent surgery in general anaesthesia. As pre-operative preparation, the sheep were fasted for 48 hours without water for the 24 hours prior to the operation. The animals were premedicated by subcutaneous administration of atropine sulphate 0.125 mg/kg and intramuscular injection of ketamine 10mg/kg (Ketavet Farmaceutici Gellini Spa, Italy) and xylazine 0.3 mg/kg (Rompun, Bayer, Italy). General anaesthesia was induced by bolus infusion of thiopentone sodium (Pentothal, Hoechst AG, Germany) in 2.5% solution at a dose of 6 mg/kg. On loss of the swallowing reflex, an endotracheal tube and a ruminant probe were inserted to avoid meteorism. Anaesthesia was maintained with assisted ventilation (Servo Ventilator 900D Siemens, Germany) with administration of an O<sub>2</sub>/N<sub>2</sub>O (50/50%) mixture with fluothane at 2.3% (Halothan, Hoechst AG, Germany).

### Intra-operative monitoring

The animals were monitored throughout surgery using a modular system for three-way ECG (M1001 A ECG Module Hewlett Packard, Germany), end-tidal CO<sub>2</sub> (M1016A CO<sub>2</sub> Module Hewlett Packard, Germany), average arterial pressure (AAP) using a 20 G catheter inserted into the cephalic artery (Component Monitoring System Hewlett Packard,



*Figure 1* A) Left Carotid angiography with clear visualisation of the rete mirabilis. Flow inversion in the right carotid artery, due to the carotid-giugular fistula. B) Supraselective injection of the rete mirabilis through a left anastomotic artery. C,D) Injection of the glue, Glubran 2: Lipiodol 1:1.

Germany) and body temperature measured by a rectal probe (M1029° Temperature Module, Hewlett Packard, Germany). Assisted ventilation was administered at a frequency of 12 breaths/min maintaining an end-tidal CO<sub>2</sub> of

30-35 mmHg: these parameters were maintained throughout the operation. The temperature of the operating theatre was kept stable at 23 ± 2° C throughout the procedure and the animals' vital parameters kept constant.



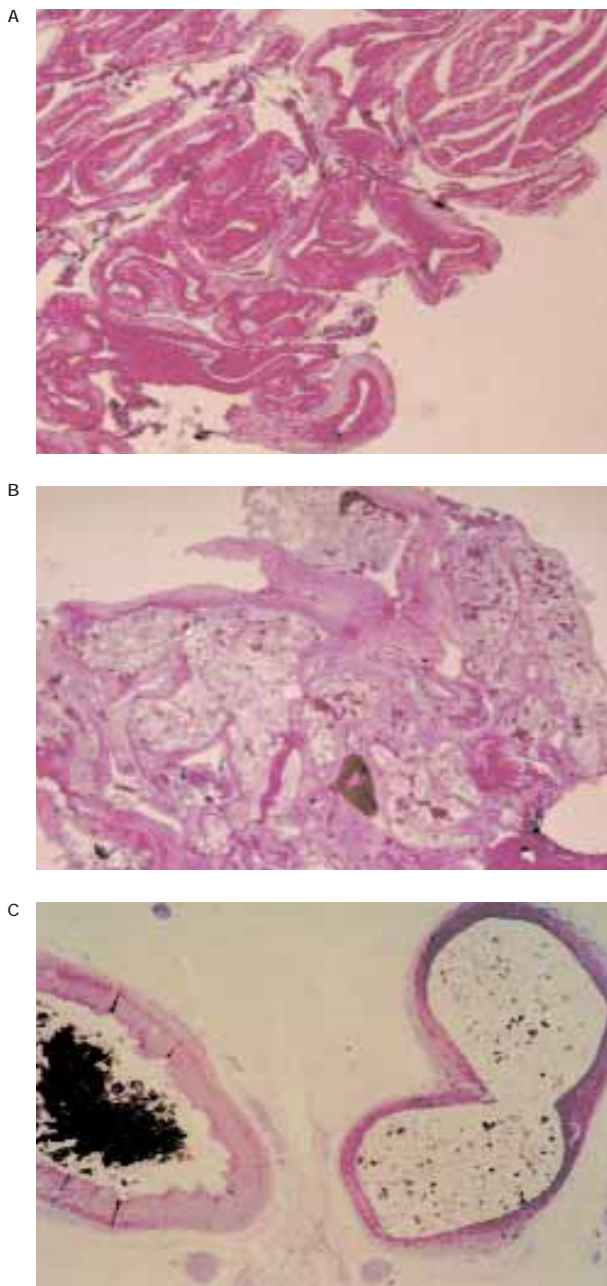


Figure 2 Histological findings: A) rete mirabilis. B) rete mirabilis filled with glue: Glubran 2: Lipiodol 1:1. C) at higher magnification, on the left side a normal rete mirabilis capillary vessel; on the right side a capillary filled with glue.

**Surgical procedure**

All surgical procedures were carried out under sterile conditions with the animals in supine decubitus.

The surgical approach involved incision in the right laterocervical neck region along the

anterior margin of the sternocleidomastoideus muscle, revealing and isolating a 5 cm long segment of the right external jugular vein and common carotid artery. After careful removal of perivascular tissue, each part of the isolated vessels was clamped with vascular miniclamps to block blood flow for the duration of shunt preparation. A 2 cm incision was made in both vessels, washing away residual blood within the vessels with saline solution. A laterolateral shunt was created using prolene suture thread 7-0. When completed, the clamps were removed and the common carotid artery isolated 1 cm below and the jugular vein 1 cm above the shunt. After the procedure the incision was sutured with separate stitches along the anatomical planes.

**Postoperative course**

After surgery the animals underwent postoperative treatment for five days with intramuscular administration of cephalosporin at a dose of 1 gr/die (Totacef, Bristol Myers Squibb SPA, Italy) and 500 mg/die ketoprofen (Orudis, Rhone-Poulenc-Rorer SPA, Italy); the surgical wound was medicated daily with betadine. Clinical veterinary examination was performed daily and the animals presented no lateral signs after surgery: they were able to stand two hours after the procedure and organic functions resumed after twelve hours.

**Angiography and embolization**

Angiography and embolization were subsequently performed under the following anaesthesia protocol: pre-operative fasting from solid food for 36 h, fluids for 12 h; Premedication: t.- 30 min atropine sulphate (0.04 mg/Kg i.m.); t.-15 min acetylpromazine maleate (0.25 mg/Kg i.m.) + xylazine ( 0.5 mg/Kg i.m.) in a single syringe; Induction: t. 0 min sodium thiopental dilution 1g/ 20 ml to action; Orotracheale intubation + ruminant probe; Deep anaesthesia: isoflurane 2.5% in O<sub>2</sub> (0.10 l / Kg/ min); Maintenance: isoflurane 1.25% average in O<sub>2</sub> (0.10 l / Kg/ min) administered in an open circular system under assisted spontaneous breathing.

Arteriography was subsequently performed under general anaesthesia (G.E. OEC 9800 System, Courtesy of General Electric Medical Systems) and microcatheterization with access

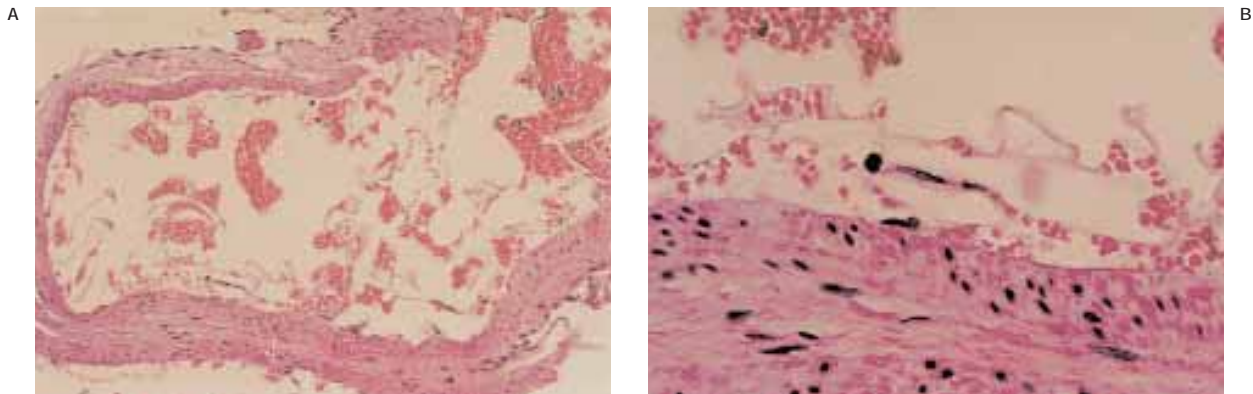


Figure 3 Histological findings: endothelial damage after injection of Histoacryl: Lipiodol 1:1. A) Panoramic view, B) magnification: modest detachment of the endothelium probably due to the thermic reaction to polymerization.

through the left femoral artery (Microcatereteri Elite, courtesy of Boston Scientific). The rete mirabilis was embolized by injection of: 1.5 ml of Histoacryl diluted 1:1 and 1:3 with Lipiodol: one sheep each.

1.5 ml of Glubran 2 diluted 1:1 with Lipiodol: two sheep. 1.5 ml of Glubran 2 diluted 1:3 with Lipiodol: two sheep.

### Histology

The sheep were killed immediately by i.v. injection of a solution of Embutramide (200 mg/ml) mebenzoniim iodide (50 mg/ml) and tetracaine chloride (50 mg/ml) (Tanax®) at a dosage of 0.15 ml/Kg at the end of the procedures and the rete mirabilis isolated for histological examination. Specimens were fixed in 10% buffered formalin with paraffin embedding and staining with haematoxylin-eosin. Histological features were evaluated blindly. The pathologist (FR) did evaluate the distribution of the material within the rete mirabilis, endothelial damage or necrosis. Acute and chronic inflammation, giant cell reactions and fibrosis were beyond the scope of the study because of too short a time between the injections animal sacrifice.

### Results

The main angiographic findings showed that:

- embolization was obtained with both Histoacryl and Glubran 2;
- Glubran 2 diffuses in a way very similar to Histoacryl closely corresponding to the angio-

graphic appearance of superselective injection of contrast media (figure 1);

- reflux in the ascending pharyngeal artery showed that Glubran 2 tended not to produce bubbles but diffused homogeneously;
- the microcatheter did not stick to Glubran 2;
- we did not observe changes in sheep vital parameters during injection of Glubran, which could be linked to the absence of an exothermic reaction (and pain) due to glue polymerization.

The pathological findings demonstrated that distribution of both glues in the rete mirabilis was complete with vascular stretching and dilation (figure 2).

Focal necrosis was detected with both glues<sup>4</sup>, as described for Histoacryl<sup>2,3,6,9</sup>. Focal endothelial damage was disclosed in one sheep (figure 3), subsequently identified as Histoacryl injection: Histoacryl/Lipiodol 1/1, and interpreted as probably due to the high thermic reaction of Histoacryl polymerization<sup>8-9</sup>. The pathologist failed to find significant differences between the two glues

### Conclusions

Glubran 2 behaves in a very similar way to Histoacryl and is probably less harmful because of the lower thermic level of polymerization. It is suitable for use in endovascular embolisation procedures.

Before tackling human brain AVMs a further study will investigate embolization of the external carotid territory and spinal orthopaedic tumors and metastases.

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## EDITORIAL COMMENT

*M. Leonardi and coworkers report an interesting paper on a promising new embolic agent (Glubran 2) that could be considered as a substitution agent for the "classical" cyanoacrylate glue. Histoacryl\* has indeed till now no CE trademark and is described by the manufacturer as contraindicated for endovascular purposes, despite the fact that it has been used worldwide by most teams for safe AVM or tumor embolization for many years.*

*The authors' report tackles most of the immediate questions that arise when one deals with such a new material, but certain points remain debatable or subject to discussion and further evaluation: will Glubran be usable in each circumstance where Histoacryl\* has proven its efficacy (such as rapid closure of high-flow fistulas where pure glue is needed)? Will it be stable at long-term follow-up as the currently used glue is, and as is the experience by teams who have used this material for more than 25 years? Will Glubran be innocuous for the surrounding tissues at long-term follow-up? Can it be used in every territory where Histoacryl\* is used with safety (brain, spinal cord, maxillo-facial area)? These questions overstep largely the immediate debate on more "manipulable" embolic agents that can be used by a larger medical population. Indeed, the fear of many interventionalists of using Histoacryl because of its reported (well known and avoidable) hazards (escape of glue into the venous system and risks of haemorrhage, glueing the catheter, etc ...) has led recently to attempts to develop new agents (as Onyx) that would not have the same potential dangers. It is not our aim to claim that medical research on that topic should be stopped and that we should be happy with the emboli we have and manipulate nowadays. Improvement of materials must be regularly looked for. We should, however, be cautious that the message that a new embolic agent replaces an old one because of its apparent safety is a dangerous thought that tends to say that knowledge and medical behaviour can be universalized without proper training or understanding of the disease, of which the radiological picture ("the target") is only an expression. The authors should be encouraged to pursue their investigations with Glubran so that our questions can be adequately answered.*

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