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EFFICACY AND LOW VASCULAR TOXICITY OF EMBOLIZATION WITH RADICAL VERSUS ANIONIC POLYMERIZATION OF N-BUTYL-2-CYANOACRYLATE (NBCA)

An experimental study in the swine

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SUMMARY

Background and purpose: To compare the efficacy and vascular toxicity of embolization with radical (NBCA+metacryloxysulpholane = CS) versus conventional anionic (NBCA alone = CA) polymerization of NBCA.

Materials and methods: Under continuous digital subtracted angiography (DSA) recordings, a 0.2 mL volume of identical glue mixtures were injected in a single-step procedure, concomitantly, in left and right (with CS and CA, respectively) renal arterial branches (RAB) and ascending pharyngeal arteries (APA) in 8 swines. Arterial histopathology and morphometry of inflammation were investigated at 2 weeks.

Results: Complete embolization was achieved with equivalent cast homogeneity on DSA with both NBCA mixtures in RAB and APA. Inflammatory crowns in APA and RAB were significantly lower in CS - than in CA-treated sites ($p<0.001$). CS plug was scarcely adhesive to the vascular wall, and pulled apart from the wall by a residual thrombotic lining; in contrast with CA casts that were strongly adhesive to walls with endothelium stripping.

Conclusions: Anionic and radical polymerization of NBCA embolization was identical with regards to occlusion rate; whereas radical pathway of polymerization with cyanoacrylates lowered histotoxicity with a less sustained adhesiveness of casts against vascular walls.

RÉSUMÉ

Efficacité et diminution de la toxicité de la polymérisation radicalaire par rapport à la polymerisation anionique lors de l'embolisation à l'aide du n-butyl-2-cyanoacrylate (NBCA). Une étude expérimentale chez le porc

Objectif : Le but de cette étude est de comparer l'efficacité et la toxicité vasculaire de deux différentes colles cyanoacryliques, dont le mécanisme de polymérisation est soit radicalaire (NBCA+métacryloxysulpholane = CS), soit anionique (NBCA seul = CA).

Matériel et méthodes : À l'aide d'un appareillage d'angiographie numérisée soustraite, des microcathéters ont été placés simultanément dans les artères pharyngiennes ascendantes droites et gauches puis dans des artères polaires des reins droits et gauches de huit porcs adultes. Un volume identique de 0,2 ml était injecté avec des concentrations différentes au niveau cervical et abdominal. Après deux semaines, les animaux ont été sacrifiés pour examens histologiques et analyse morphométrique de la réaction inflammatoires péri-vasculaire au niveau des sites d'embolisation.

Résultats : Angiographiquement, un niveau d'oblitération identique a été observé pour les différentes localisations anatomiques et les différentes concentrations utilisées. En revanche, du point de vue histologique, la réaction inflammatoire péri-artérielle a été significativement moins importante ($p < 0,001$) pour les artères oblitérées avec la solution CS que pour celle oblitérées avec la solution CA. Dans le premier cas l'embol respectait la morphologie de l'artère et parfois une fine couche de caillot le séparait de l'endothélium. Dans le second cas, on notait une destruction de l'endothélium associée a une importante infiltration inflammatoire pariétale artérielle.

Conclusion : Les modes de polymérisation radicalaire ou anionique apparaissent identiques quant à leurs propriétés d'oblitération endo-artériel. En revanche, du point de vue histologique, la polymérisation de type radicalaire (CS) diminue l'aspect d'adhésivité et de toxicité pariétale artérielle que l'on observe avec la polymérisation de type anionique (CA).

INTRODUCTION

Cyanoacrylates are the main liquid adhesives used to manage radiologic embolization of vascular abnormalities [10, 17-19, 22, 26, 27, 37, 38, 41-44]. The low physical viscosity of monomers enables injection through small diameter microcatheters to the target site of embolization. Chemical ability to mix with and entrap iodized oils enable radiologic procedures follow up and radiolocalization of deposits [5, 39]. Cyanoacrylates have also elevated polymerization rates in the presence of ionized anions in serum and ionized vessel proteins and strong reactivity and adhesives forces to vascular walls [1, 6, 7, 25-27, 43]. However, technical and therapeutic problems arise from both physical properties of glue and biological consequences of embolization. Poly-

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merization of glue produces heat with retropolymerization/repolymerization processes which release highly toxic components acrylacetate and formaldehyde [1, 2, 13, 28, 40].

This strong reactivity of the ionization-produced polymerization of cyanoacrylates causes major vascular cytotoxicity by damaging the vascular intima and by inducing inflammatory and immunological foreign-body responses, as well as accelerating the gradual resorption of occlusive polymers [6, 7, 16, 19, 23, 28, 36]. Vascular wall injury during polymerization is the prominent cause of subsequent vessel wall enlargement, neovascularization and finally recanalization of embolized vessels [14, 32, 37, 38]. Technically, this reactivity can also cause adhesion between the tip of the microcatheter and the artery and/or polymers [27].

Previous attempts to improve the practical use of cyanoacrylates were based upon changing the chemical nature of the hydrocarbon component which is prominent in defining the physical and biological properties of the glue. Optimization of the monomers by this way however is rendered difficult because decreasing the strong reactivity and adhesive forces is conversely associated with a lower polymerization rate and a lower hardening which could become too low to obliterate a vascular lesion with rapid blood flow, as the glue increases its tendency to fragment and to flow/run away from the point of application [6, 12, 25-27, 34, 35]. Instantaneous polymerization remains essential for embolization of vascular abnormalities, especially the high-flow AVM.

The present study investigates embolization of renal and brain arteries in a swine model comparing standard n-butyl-cyanoacrylate monomers alone (CA) which spontaneously undergo the conventional anionically catalyzed condensation polymerisation [1, 2, 6, 25, 27] to n-butyl-cyanoacrylate plus metacryloxysulpholane (CS) monomers which convert the polymerisation pathway into a radical mechanism [8, 11, 13], supposed to reduce heat and secondary toxic molecule production and so induce less acute biological reactions.

MATERIALS AND METHODS

Experimental protocol

All the experiments were conducted in accordance with the policies set by the local University Institutional Animal Care and Use Committee (UICUC), based upon the Guide for the Care and Use of Laboratory Animals (National Research Council, Natl Acad Press, Washington, DC, 1996) [29-31]. Eight male Pietrin swine (40.9±4.2 kg at 6 months of age) were kept in the facilities for 15 days prior to entering the protocol. Then, pre-procedural subtracted angiographies and glue-mediated occlusions were performed at the same time, and the animals were placed in post-op suite to recover for 24 hrs, prior to returning to the facilities [30]. Fourteen days after embolization, animals were submitted to angiographic investigations, euthanized (midazolam 15 mg and chlorpromazine 25 mg in KCl 15% (p/v), 20ml, i.v. bolus) and the occluded vessels (or vessel-containing organs) were surgically removed for pathological examinations [30].

Radiologic procedures, arterial embolizations

All procedures were performed on anesthetized pigs, with the lower limbs folded down onto the table, as referring to the radiological posture. A digitized subtraction angiographic Stenoscop system (General Electric Medical System, Minneapolis, USA) was used for radiological procedures. Percutaneous access was performed via the right and left superficial femoral arteries (SFA) under Doppler sonography guidance and using a 11 cm-long standard 5F vascular sheath (Cordis/J&J, Miami, Fl) with Seldinger technique under aseptic conditions [30]. A bolus of 5000 Units of Heparin was given intravenously after arterial femoral puncture.

Routine subtracted angiograms were obtained by injection of 10 mL of Visipaque (Nycomed Inc.) at 5 $ml.sec^{-1}$ after placement of 5F angiographic diagnostic catheters (5F, 100cm, Terumo, Japan) advanced to the left and right common carotid artery (CCA) upstream the corresponding ascending pharyngeal artery (APA) or renal artery, upstream the renal arterial branch (RAB) over a glidex-coated hydrophilic 0.035in guidewire (BSC-MediTech).

Right and left APA were selectively catheterized concomittantly with microcatheters (Rapid Transit 3F, 150 cm with 2.3F terminal part; Cordis, Miami, FLA or Excelsior microcatheters 2.6F, 150 cm with 2.0F terminal part, Boston Scientific/Target) over a 0.014in guidewire with hydrophilic coating (Transendex, BSC-MediTech). Identical microcatheters were used in one animal. A pre-procedural selective angiogram was obtained for both arterial sides, concomittantly using 1-3 mL of pure contrast agent through the microcatheters. Then, both microcatheters were connected with a 1-mL Luer-lock syringe (Medallion, Merit Medical, South Jordan, Utah), subsequently flushed with abundant isotonic 5% glucose solution. Pure NBCA (CA), (Histoacryl® 0.5mL capsule, Braun Gmbh, Melsungen, Germany) and NBCA-MS (CS), (Glubran2®, 1mL capsule, GEM, Viareggio, Italy) glues were identically mixed and deeply homogeneized with iodized oil (Lipiodol ultrafluid®, Guerbet, Aulnay, France) at 0.5:0.5 and 0.5:1.8 v/v proportions for occlusions in APA and RA, respectively. Under continuous subtracted radiological recordings, a 0.2 mL volume of the mixture was injected in a single-step procedure, concomitantly, with both syringes being handled in the same hand. Attempting to exclude operator-dependent influence on monomers delivery, the same experienced Neuroradiologist performed the paired embolization in APA and RAB, based on the clinical practice. Post-procedure angiograms were obtained as described above.

The same procedure was performed the same way thereafter in right and left RAB, with in-between removal of the first set of microcatheters and flush of the guiding catheters. The pure NBCA-oil (CA) mixture was always injected in the right side (right APA and right RAB) and the NBCA-MS-oil (CS) mixture on the left side (left APA and left RAB). All things considered, four arteries were embolized

for each animal. After compression (8 min.) of puncture site and wound, the pig was transferred to the post-operative suite for recovery, and then returned to the facilities. Fourteen days after arterial occlusions, control angiograms were obtained from a single introduced sheath inserted into the right SFA.

Histopathology

Immediately, after radiologic controls at 2 weeks, and a submentooccipital projection performed for optimal delineation of the rete, the animals were euthanized and APA with surroundings tissues in a single excision block, as well as right and left kidneys with complete renal arteries was surgically obtained, immersed in Bouin's liquid fixation medium for 2 days. From gross examination under radiologic control, occluded vessels were precisely localized and sampled, prior to further re-fixation in 10% formalin for another day. Serial pathologic slides, orthogonally sectioned (5 µm) were obtained from paraffin blocks, prior deparaffined slides were routinely stained with eosine-phloxine-saffran for microscopic observations [29-31].

Anatomic analysis of inflammatory/immunologic reaction (IIR) in the periarteriel space was performed using an automatic videoanalyzer as previously described for evaluating angioplasty-induced restenosis response in the swine [31]. Morphometric analysis of the arterial section geometry consisted of (1) delineating on the computer screen, the boundaries of the lumen, intima-media and periarterial IIR, (2) centering 24 radial pairs of 2 orthogonal diameters on the arterial section, and (3) measurement of 96 triplets of radial lumen, intima-media and IIR thickness. In each sample at the lesional site, mor-

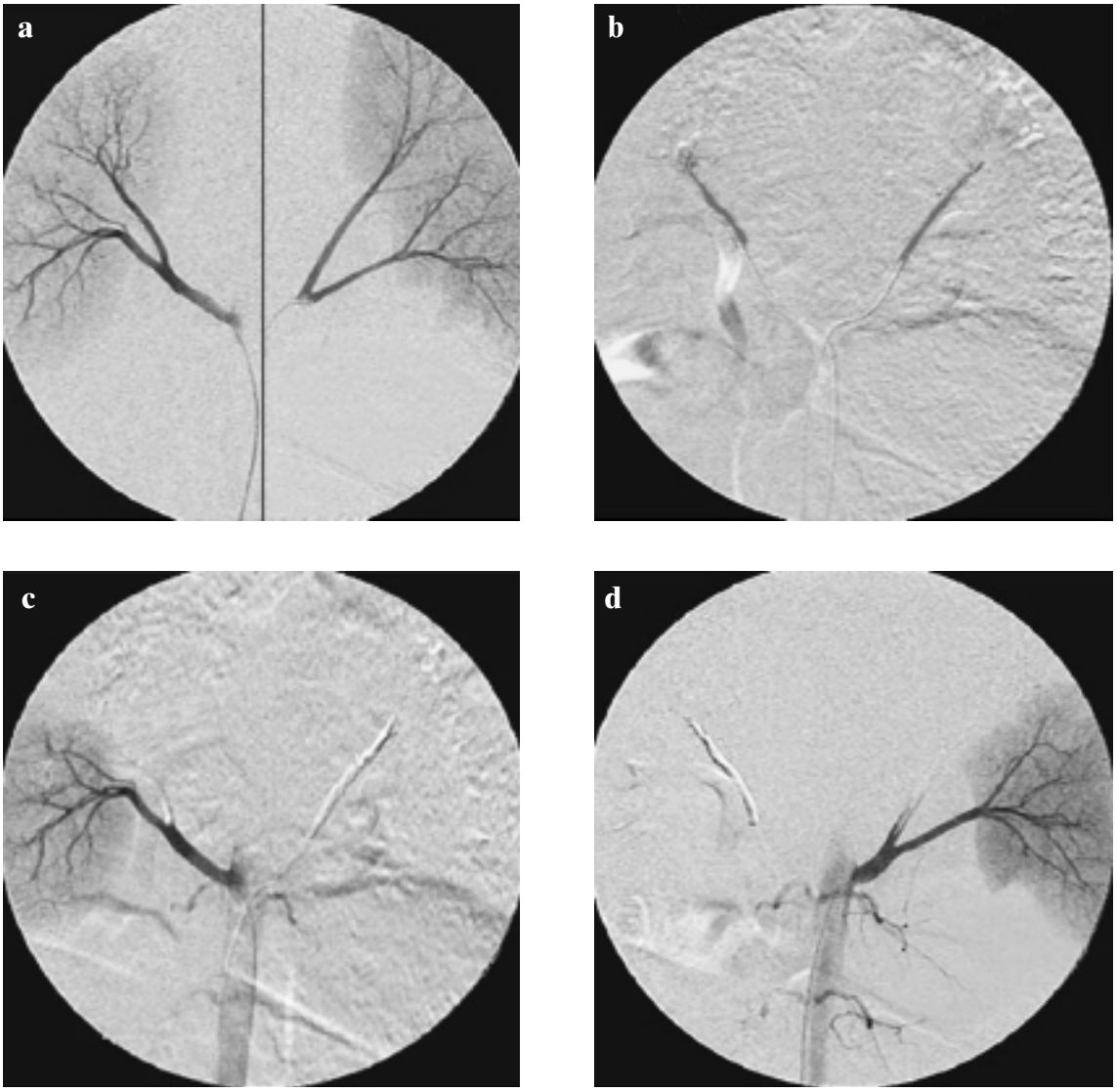

FIG. 1. – Angiograms show the control appearance of the renal arterial branches (RAB) (upper panel, left and middle), the subtracted angiogram of the polymer casts (CA et CS in left and right RAB, respectively) (upper panel, right), and post-embolization angiograms of RAB (lower panel).

FIG. 1. – *Injections sélectives simultanées de produit de contraste dans les artères rénales droite et gauche (en haut à gauche de la figure). Injections hypersélectives simultanées de mélanges de produit de contraste huileux et de colle acrylique respectivement « CA » dans les artères polaires supérieures droites et « CS » dans les artères polaires supérieures gauches (en haut à droite de la figure). Contrôle angiographique permettant de visualiser l'oblitération des artères polaires rénales supérieures droite et gauche (en bas à droite et à gauche de la figure).*

phometric data were further averaged per individual arterial site and per animal.

Statistical analysis

Data treatment and statistical analyses were performed by using Systat 9.0 software (SPSS Inc, Chicago, IL) (29-31). Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed by using the Kruskal-Wallis test, (i.e. the non-parametric analogue of a one-way analysis of variance), which reduced to the Mann-Whitney test (i.e. the nonparametric analogue of the two-sample t test) when there are only two groups.

RESULTS

Animals

The animals remained healthy throughout the program period, except for a transient episode of neurological disorders in one animal for 3 days, prior to recovering a normal ambulatory behaviour. Specifically, a one-side foot stepping syndrome was observed in the right posterior limb of the animal having received pure NBCA glue in the left APA without detectable procedural problems.

Angiography, arterial occlusions

Based upon experience and clinical practice, infusion microcatheter device was placed in all cases and no acute complication occurred during arterial insertion, tracking, glue-iodized solution delivery or retrieval of the device along the guidewire. Typical pre- and post-embolization angiograms, and glue casts in APA and RAB are shown in *figure 1 and figure 2*, respectively. Whatever the nature of the glue, and by using the volume and respective percentage of the mixture composition used in APA and RAB, complete embolization was achieved in each case, both at the time of post-embolization and 14 days later, no recanalization was observed, and angiographic results illustrated arterial occlusions with equivalent efficacy at both sites.

Histopathological Observations

Delivery of CA in APA *(figure 3)* returns the basic pathologic characteristics previously reported for CA histotoxicity, as:

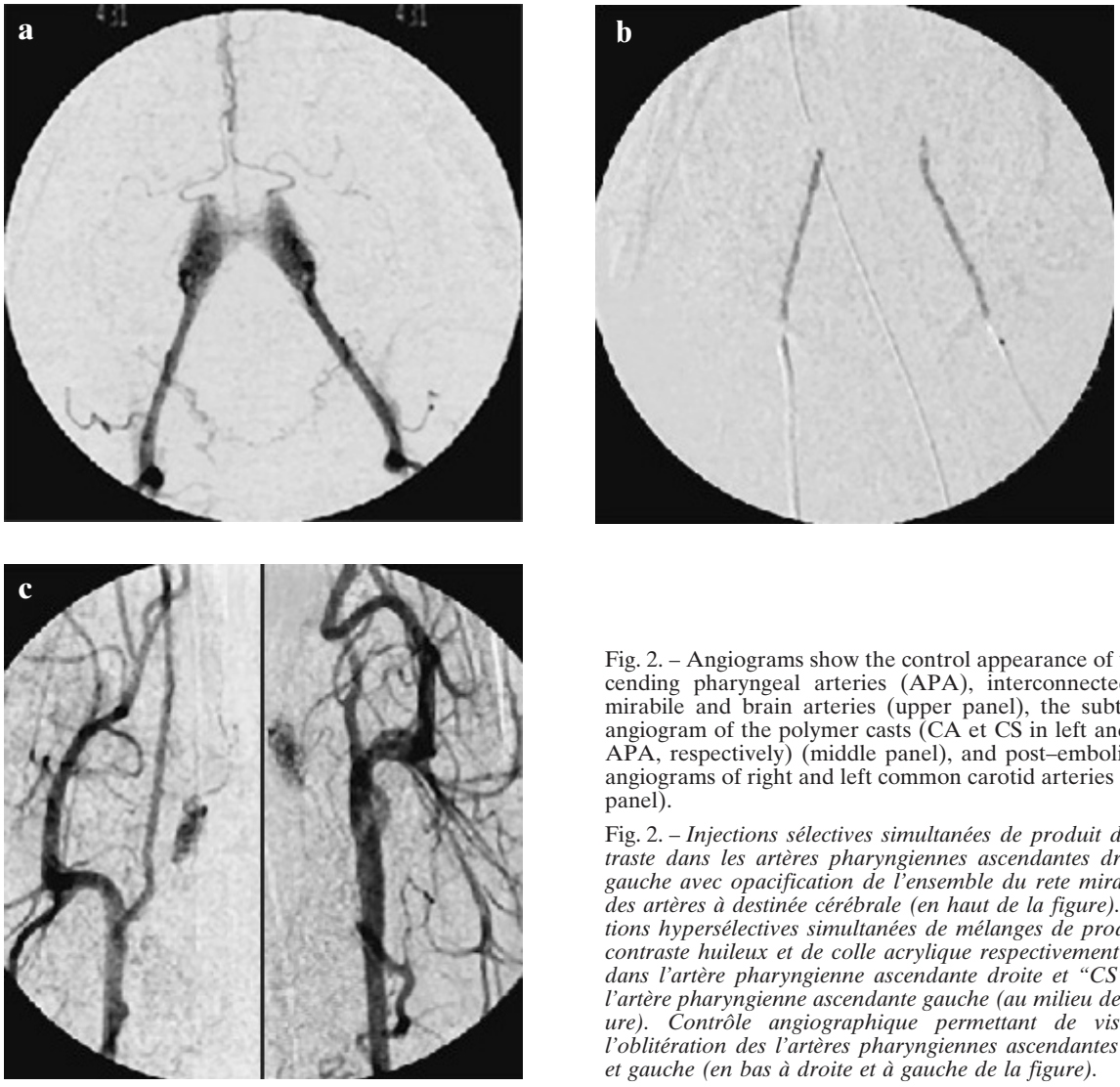

Fig. 2. – Angiograms show the control appearance of the ascending pharyngeal arteries (APA), interconnected rete mirabile and brain arteries (upper panel), the subtracted angiogram of the polymer casts (CA et CS in left and right APA, respectively) (middle panel), and post–embolization angiograms of right and left common carotid arteries (lower panel).

Fig. 2. – *Injections sélectives simultanées de produit de contraste dans les artères pharyngiennes ascendantes droite et gauche avec opacification de l'ensemble du rete mirabile et des artères à destinée cérébrale (en haut de la figure). Injections hypersélectives simultanées de mélanges de produit de contraste huileux et de colle acrylique respectivement "CA" dans l'artère pharyngienne ascendante droite et "CS" dans l'artère pharyngienne ascendante gauche (au milieu de la figure). Contrôle angiographique permettant de visualiser l'oblitération des l'artères pharyngiennes ascendantes droite et gauche (en bas à droite et à gauche de la figure).*

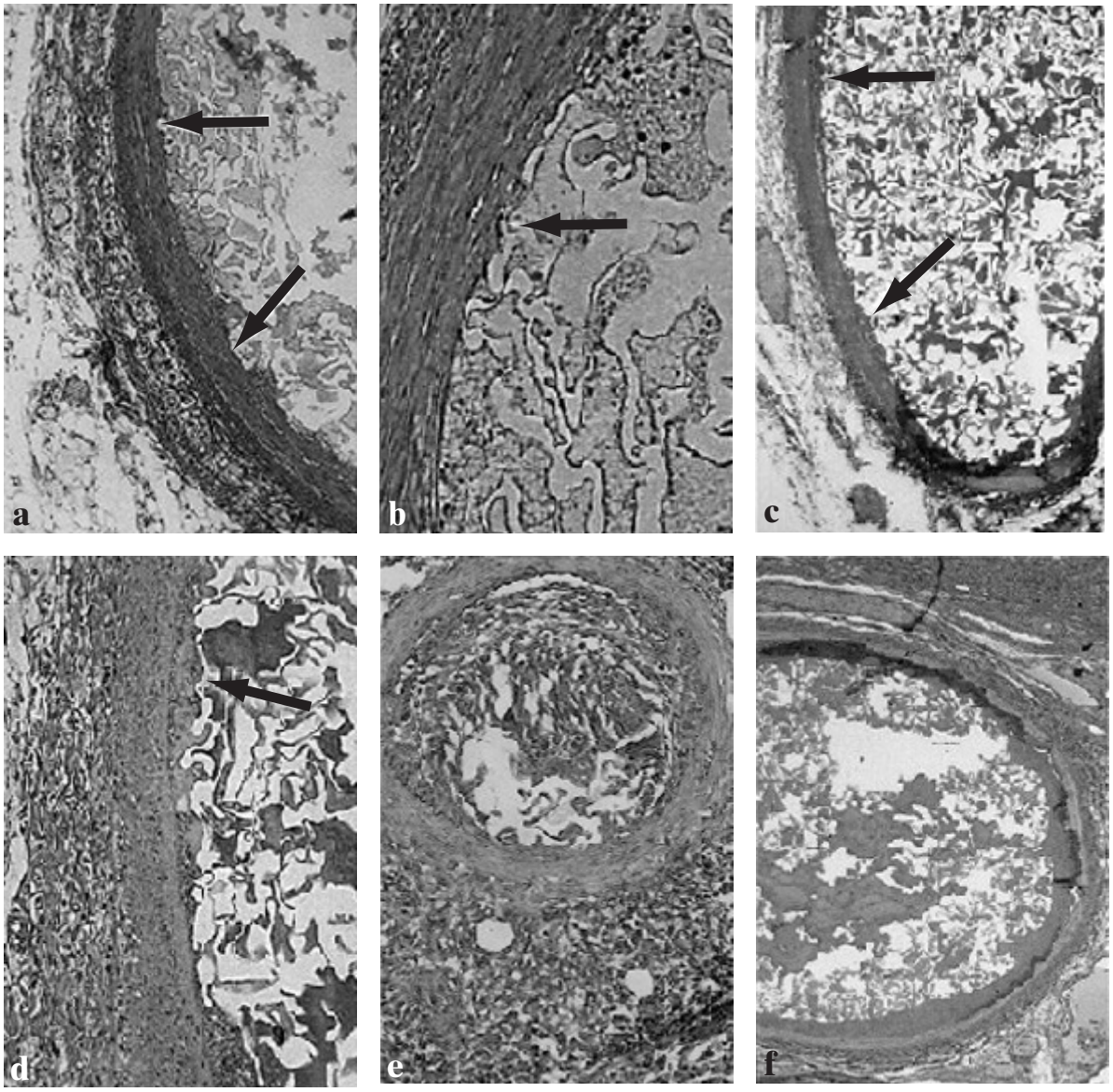

FIG. 3. – Microphotographs show in APA the close adherence of CA to the intima (arrows) with stripping of the endothelium lining, and the intense periadventicial inflammatory reaction crown (upper panel, × 75, × 125 magnification); the low inflammatory reaction around APA after embolization with CS, as well as the permanent thin presence of thrombotic residual lining (white arrows) covering preserved endothelium (medium panel, × 50, × 150 magnification); and similar findings in RAB for CA (left) and CS (right), (lower panel, × 75, × 125, magnification).

FIG. 3. – *Analyses histologiques : Mise en évidence au niveau de l'artère pharyngienne ascendante de l'adhérence de l'embol de "CA" à l'intima (flèche) avec destruction de l'endothélium et de la présence d'une importante réaction inflammatoire péri-adventicielle (grossissement × 75 et × 125 ; en haut de la figure). On note la faible réaction inflammatoire autour de l'artère pharyngienne ascendante avec l'embol "CS" ainsi que la persistance d'une fine couche de matériel thrombotique (flèche blanche) recouvrant l'endothélium resté intact (au milieu de la figure, grossissement × 50 et × 75). Des observations similaires sont effectuées lors de l'examen des artères rénales en présence de "CA" (à droite) et de "CS" (à gauche) (en bas de la figure, grossissement × 75, × 125).*

1. marked inflammatory reaction in the periadventitial space with marked fibroblastic contribution to the process, i.e. an arteritis-like process and

2. strong, direct adhesiveness of the glue to the vascular wall. Adhesion of the glue directly onto the luminal vascular wall was associated with stripping, disruption and disappearance of the endothelium lining, in a major portion of the circumferential lumen. At two weeks, the former inflammatory burst was associated with some disorganization in the stacking-up of the elastic laminae in the vascular media. The rupture of internal elastic laminae was not commonly observed. Histological responses to CA in the renal arteries were found closely resembling the situation in APA.

Histological observations in CS embolized vessels also were of the same type in APA and RAB, but markedly contrasted the CA cytotoxicity. Specifically, scarce adhesion of the glue plug was observed on the vascular wall, being most of the space along pulled apart from the wall by a residual thrombotic lining. In addition, the inflammatory-fibroblastic reactions in the periadventitial space were low to moderate, and in some places could even not be detected as differing from the usual presence of inflammatory cells in the perivascular areas. Morphometric analy-

sis revealed that, by measuring the inflammatory crowns as percentage of the media thickness to eliminate changes in the vessel size, arteritis-like processes were 105.4 ± 18.2% and 18.3 ± 9.3%; and 221.1 ± 62.4% and 35.8 ± 12.6% in APA and RAB embolized with CA and CS, respectively ($p<0.001$). These results demonstrate that CS had less local toxicity than CA alone.

DISCUSSION

The results in this swine model were especially effective with CA and CS. Radiological images did not reveal differences in the architecture of NBCA: Iodized oil casts, in particular, there was no difference in homogeneity and/or fragmentation of the polymerized deposits. There were significant histological differences for both glues. At two weeks, the degree of blood vessel injury and the foreign body tissue reaction to the respective glues were markedly different. Specifically, pure NBCA after embolization displayed, as already known, strong adhesiveness to the vascular wall with associated injury to the endothelium with stripping and intima losses, and shrinking of the media; the process was also associated with an intense inflammatory/immunological reaction in the immediate periadventitial spaces [6, 7, 14, 16, 19, 23, 28, 36, 37].

By contrast, CS was poorly gripping and attached to the intima, and subsequently intima was not injured and vascular wall presented with a thin, if any, inflammatory crown surrounding the adventitia. The thin space lying in between the latter glue plug and vascular intima was filled and occupied by a film of residual thrombus. Very significantly, the polymerization pathway was not associated with intramural haemorrhage and extravasations of glue after embolization.

Anionic NBCA polymerization pathway

Attempting to evaluate the immediate and early (2 weeks) embolization properties and consequences of the ring-opening polymerization pathway for NBCA, we used CA as the gold standard reference for the embolization procedure. The results show that, as expected from the clinical practice of an experienced neuroradiologist, efficient embolizations in two different vascular beds were achieved with CA, and, with complementary investigations at two weeks, we found tissue reactions now acknowledged to occur after embolization. Data from the litterature demonstrate that the physico-chemical characteristics of the anionic polymerization pathway provide the rationale for the results. The anionic polymerization is initiated with bonding of the ethylene units the monomeric form of the cyanoacrylates consists of [1, 2, 6, 25, 27]. By investigating the hardening rate and gelation time of common NBCA in an in vitro fluid circuit model as compared with the in-vivo matched situation, the lone electronic pairs on the NH2 groups within proteins of serum and vascular wall were found prominent in the anionic polymerization processes thereby leading to a fast hardening rate and strong adhesiveness to the vascular wall [20, 21]. The mechanism of anionic polymerization of butyl cyanoacrylate in the aqueous dispersions, slowed down by dispersion in acidified water, has been shown to proceed by generation of discrete populations of oligomers. Polymerization then is a step-wise cycle of initiation, termination, re-initiation and re-termination until diffusion is limited by internal viscosity within the particles [1, 2]. At physiologic pH values, reaction is ultra-rapid to yield the final casts, and heat production in the exothermic polymerization and release of formaldehyde during termination processes have been described as causative factors for the embolization-related tissue lesions encountered [1, 2, 6, 25, 27]. Owing to the qualitative difficulties in distinguishing between tissue, iodized oil and residual blood/blood products, a quantitative method for determining NBCA concentration within embolized tissue using europium fluorescence as specific dying for NBCA [7] demonstrated that NBCA distribution throughout the lumen concentrates in a band adjacent to the wall with the form of irregular, interwoven folds within the lumen which adhered to the vessel wall with stripping of the endothelium [7].

Radically NBCA polymerization pathway

The major result from the study is that the radically formed cyanoacrylate casts were identical in embolization efficacy and topographic organization to the anionically formed casts, but they were not as adhesive as CA to and subsequently not injuring the vascular wall. Again, the present results are understandable by considering the physical-chemical characteristics of the polymerization pathway. Radical polymerisations for copolymers having controlled microstructures are alternative pathways to anionic polymerizations which basic principle is to drive the polymerization radically to develop CC-scission due to the activation of C-C bonds by electronic "push-pull" mechanism [8]. The radical polymerization there for was promoted by introducing of proper substances in the monomers so that the monomer did not polymerize under anionic conditions because of the stability of α-carbon radical formed at the initiating reaction [8]. This concept was extended to the monomers by introduction of two-electron-withdrawing groups (as the sulfones) for the stabilization of growing radical species, thus forming push-pull system to activate the C-C bond between substitution sites. The final non-crystalline copolymers are obtained at body temperature with a polymerization mechanism there for independent of the reactive proteins in the vascular wall which obviously accounts for the absence of adhesiveness of the casts to the vessel walls.

Are the radically formed NBCA casts a permanent embolotherapy?

The alternative pathway for recanalization originates from glue-mediated marked injury of intima and vascular walls and subsequent acute inflammatory reactions in the wall and surrounding tissues progressing to a chronic and granulomatous process with foreign body giant cells and fibrosis [5, 6, 27, 38,

39] which on the long-term was detected in 88% of cases within the cast areas with a recanalization of embolized vessels in 50% of cases [14]. The dilatation of pre-existent collateral vessels and/or vascular ectasia, reconstituting the blood flow, is important mechanisms for recalanization of embolized malformations [15]. Although it is not clearly established whether or not recanalization only develops in cases where total and solid casting of the nidus was not achieved [14, 15, 22, 40-44], it is likely that angiogenic or arteriogenic (i.e. vascular enlargement) reaction of embolized vessel is triggered by the enhanced release of cytokines in the presence of vascular endothelium injury and/or inflammation, as vasoactive growth-related factors, such prominently vascular endothelial growth factor, were shown to be produced by activated vascular cells, macrophages and T lymphocytes [3, 4, 9, 14]. Of major interest to the present study is to determine the therapeutic relevance of the findings that radically polymerized NBCA did not markedly injure the vascular wall and was poorly pro-inflammatory, and, consequently, it remains to establish to which extent the vascular harmlessness will decrease the arterial recanalizations.

CONCLUSION

We report here that efficient embolizations in RAB and APA were equally obtained with NBCA by using either the anionic or ring-opening polymerization pathways (especially there was no difference in homogeneity and/or fragmentation of the polymerized deposits) and that the radically polymerized NBCA was poorly promoting blood vessel injury and inflammatory reactions to the casts. It is not definitely established that the new polymerization pathway results in improved procedural techniques, since in the hands of an experienced Neuroradiologist no procedural complications were observed by using either of the glues. Finally, it remains to establish whether, and to which extent, or not the low vascular wall adhesiveness (by favouring the presence of a potentially angiogenic residual thrombotic lining between the glue plug and the vascular wall), and/or subsequent low vascular wall injury (by lowering the release of angiogenic factors by both the injured vascular cells and activated inflammatory cells), prevent recanalization of the embolized vessels.

REFERENCES

[1] Behan N, Birkinshaw C, Clarke N. Poly n-butyl cyanoacrylate nanoparticles: a mechanistic study of polymerisation and particle formation. *Biomaterials* 2001 ; *22* : 1335-1344.

[2] Behan N, Birkinshaw C. The mechanism of polymerisation of butyl cyanoacrylate in aqueous dispersions. *Colloid and polymer science* 2000 ; *21* : 884-886.

[3] Brogi E, Winkles JA, Underwood R, Clinton SK, Alberts GF, Libby P. Distinct patterns of expression of fibroblast growth factors and their receptors in human atheroma and nonatherosclerotic arteries. Association of acidic FGF with plaque microvessels and macrophages. *J Clin Invest* 1993 ; *92* : 2408-2418.

[4] Brogi E, Wu T, Namiki A, Isner JM. Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only. *Circulation* 1994 ; *90* : 649-652.

[5] Brothers MF, Holgate RC. Intracranial angioplasty for treatment of vasospasm after subarachnoid hemorrhage: technique and modifications to improve branch access. *AJNR Am J Neuroradiol* 1990 ; *11* : 239-247.

[6] Brothers MF, Kaufmann JC, Fox AJ, Deveikis JP. n-Butyl 2-cyanoacrylate--substitute for IBCA in interventional neuroradiology: histopathologic and polymerization time studies. *AJNR Am J Neuroradiol* 1989 ; *10* : 777-786.

[7] Calvo WJ, Lieber BB, Hopkins N, Wakhloo AK. Europium fluorescence to visualize N-butyl 2-cyanoacrylate in embolized vessels of an arteriovenous malformation swine model. *Am J Neuroradiol* 2001 ; *22* : 691-697.

[8] Cho I. New ring-opening polymerizations for copolymers having controlled microstructures. *Progress in Polymer Science* 2000 ; *25* : 1043-1087.

[9] De Marchis F, Ribatti D, Giampietri C, Lentini A, Faraone D, Scoccianti M, Capogrossi MC, Facchiano A. Platelet-derived growth factor inhibits basic fibroblast growth factor angiogenic properties in vitro and in vivo through its alpha receptor. *Blood* 2002 ; *99* : 2045-2053.

[10] Debrun GM, Aletich V, Ausman JI, Charbel F, Dujovny M. Embolization of the nidus of brain arteriovenous malformations with n-butyl cyanoacrylate. *Neurosurgery* 1997 ; *40* : 112-120.

[11] Diaz CR, Riande E. Comparative study of mechanical and dielectric properties of glassy acrylic polymers containing 1,3-dioxane rings in their structures. *Macromolecular Symposia* 1999 ; *147* : 191-199.

[12] Duffner F, Ritz R, Bornemann A, Freudenstein D, Wiendl H, Siekmann R. Combined therapy of cerebral arteriovenous malformations: histological differences between a non-adhesive liquid embolic agent and n-butyl 2-cyanoacrylate (NBCA). *Clinical Neuropathology* 2002 ; *21* : 13-17.

[13] Garcia F, Garcia BA, Compan V, Diaz CR, Guzman J, Riande E. Relaxation behavior of acrylate and methacrylate polymers containing dioxacyclopentane rings in the side chains. *Journal of Polymer Science Part B Polymer Physics* 2001 ; *39* : 286-299.

[14] Gruber A, Mazal PR, Bavinzski G, Killer M, Budka H, Richling B. Repermeation of partially embolized cerebral arteriovenous malformations: a clinical, radiologic, and histologic study. *AJNR Am J Neuroradiol* 1996 ; *17* : 1323-1331.

[15] Guo WY, Wikholm G, Karlsson B, Lindquist C, Svendsen P, Ericson K. Combined embolization and gamma knife radiosurgery for cerebral arteriovenous malformations. *Acta Radiol* 1993 ; *34* : 600-606.

[16] Hood TW, Mastri AR, Chou SN. Neural and vascular tissue reaction of cyanoacrylate adhesives: a further report. *Neurosurgery* 1982 ; *11* : 363-366.

[17] Kerber CW, Wong W. Liquid acrylic adhesive agents in interventional neuroradiology. *Neurosurgery Clinics of North America* 2000 ; *11* : 85-99, VIII, IX.

[18] Kerber CW, Cromwell LD, Zanetti PH. Experimental carotid aneurysms: Part 2. Endovascular treatment with cyanoacrylate. *Neurosurgery* 1985 ; *16* : 13-17.

[19] Klara PM, George ED, McDonnell DE, Pevsner PH. Morphological studies of human arteriovenous malformations. Effects of isobutyl 2-cyanoacrylate embolization. *J Neurosurg* 1985 ; *63* : 421-425.

[20] Lin JC, Lin CW, Lin XZ. In vitro and in vivo studies for modified ethyl cyanoacrylate regimens for sclerotherapy. *Journal of Biomedical Materials Research* 2000 ; *53* : 799-805.

[21] Lin SY, Chen KS, Liang RC. Organic esters of plasticizers affecting the water absorption, adhesive property, glass transition temperature and plasticizer permanence of Eudragit acrylic films. *Journal of Controlled Release* 2000 ; *68* : 343-350.

[22] Lundqvist C, Wikholm G, Svendsen P. Embolization of cerebral arteriovenous malformations: Part II--Aspects of complications and late outcome. *Neurosurgery* 1996 ; *39* : 460-467.

[23] Massoud TF, Vinters HV, Chao KH, Vinuela F, Jahan R. Histopathologic characteristics of a chronic arteriovenous malformation in a swine model: preliminary study. *Am J Neuroradiol* 2000 ; *21* : 1268-1276.

[24] MURAYAMA Y, VINUELA F, ULHOA A, AKIBA Y, DUCKWILER GR, GOBIN YP, VINTERS HV, GREFF RJ. Nonadhesive liquid embolic agent for cerebral arteriovenous malformations: preliminary histopathological studies in swine rete mirabile. *Neurosurgery* 1998 ; *43* : 1164-1172.

[25] OOWAKI H, MATSUDA S, SAKAI N, OHTA T, IWATA H, SADATO A, TAKI W, HASHIMOTO N, IKADA Y. Non-adhesive cyanoacrylate as an embolic material for endovascular neurosurgery. *Biomaterials* 2000 ; *21* : 1039-1046.

[26] PEVSNER PH, GEORGE ED, DOPPMAN JL. Interventional radiology polymer update: acrylic. *Neurosurgery* 1982 ; *10* : 314-316.

[27] POLLAK JS, WHITE RI. The use of cyanoacrylate adhesives in peripheral embolization. *Journal of Vascular and Interventional Radiology* 2001 ; *12* : 907-913.

[28] RAO VR, MANDALAM KR, GUPTA AK, KUMAR S, JOSEPH S. Dissolution of isobutyl 2-cyanoacrylate on long-term follow-up. *AJNR Am J Neuroradiol* 1989 ; *10* : 135-141.

[29] ROLLAND PH, FRIGGI A, BARLATIER A, PIQUET P, LATRILLE V, FAYE MM, GUILLOU J, CHARPIOT P, BODARD H, GHIRINGHELLI O. Hyperhomocysteinemia-induced vascular damage in the minipig. Captopril-hydrochlorothiazide combination prevents elastic alterations. *Circulation* 1995 ; *91* : 1161-117.

[30] ROLLAND PH, CHARIFI AB, VERRIER C, BODARD H, FRIGGI A, PIQUET P, MOULIN G, BARTOLI JM. Hemodynamics and wall mechanics after stent placement in swine iliac arteries: comparative results from six stent designs. *Radiology* 1999 ; *213* : 229-246.

[31] ROLLAND PH, BARTOLI JM, PIQUET P, MEKKAOUI C, NOTT SH, MOULIN G, AMABILE P, MESANA T. Local Delivery of NO-Donor Molsidomine Post-PTA Improves Haemodynamics, Wall Mechanics and Histomorphometry in Atherosclerotic Porcine SFA. *Eur J Vasc Endovasc Surg* 2002 ; *23* : 226-233.

[32] SADATO A, WAKHLOO AK, HOPKINS LN. Effects of a mixture of a low concentration of n-butylcyanoacrylate and ethiodol on tissue reactions and the permanence of arterial occlusion after embolization. *Neurosurgery* 2000 ; *47* : 1197-1203.

[33] SIEKMANN R, WAKHLOO AK, LIEBER BB, GOUNIS MJ, DIVANI AA, HOPKINS LN. Modification of a previously described arteriovenous malformation model in the swine: Endovascular and combined surgical/endovascular construction and hemodynamics. *Am J Neuroradiol* 2000 ; *21* : 1722-1725.

[34] STOESSLEIN F, DITSCHERLEIN G, ROMANIUK PA. Experimental studies on new liquid embolization mixtures (histoacryl-lipiodol, histoacryl-panthopaque). *Cardiovasc Intervent Radiol* 1982 ; *5* : 264-267.

[35] TSENG YC, HYON SH, IKADA Y, SHIMIZU Y, TAMURA K, HITOMI S. In vivo evaluation of 2-cyanoacrylates as surgical adhesives. *J Appl Biomater* 1990 ; *1* : 111-119.

[36] VINTERS HV, GALIL KA, LUNDIE MJ, KAUFMANN JC. The histotoxicity of cyanoacrylates. A selective review. *Neuroradiology* 1985 ; *27* : 279-291.

[37] VINTERS HV, LUNDIE MJ, KAUFMANN JC. Long-term pathological follow-up of cerebral arteriovenous malformations treated by embolization with bucrylate. *N Engl J Med* 1986 ; *20* : 477-483.

[38] WHITE RI, POLLAK J, PERSING J, HENDERSON KJ, THOMSON JG, BURDGE CM. Long-term outcome of embolotherapy and surgery for high-flow extremity arteriovenous malformations. *Journal of Vascular and Interventional Radiology* 2000 ; *11* : 1285-1295.

[39] WIDLUS DM, LAMMERT GK, BRANT A, TSUE T, SAMPHILLIPO MA, JR., MAGEE C, STARR FL, ANDERSON JH, WHITE RI, JR. In vivo evaluation of iophendylate-cyanoacrylate mixtures. *Radiology* 1992 ; *185* : 269-273.

[40] WIKHOLM G, LUNDQVIST C, SVENDSEN P. Embolization of cerebral arteriovenous malformations: Part I--Technique, morphology, and complications. *Neurosurgery* 1996 ; *39* : 448-457.

[41] WIKHOLM G, LUNDQVIST C, SVENDSEN P. Transarterial embolization of cerebral arteriovenous malformations: improvement of results with experience. *AJNR Am J Neuroradiol* 1995 ; *16* : 1811-1817.

[42] WIKHOLM G, LUNDQVIST C, SVENDSEN P. The Goteborg cohort of embolized cerebral arteriovenous malformations: A 6-year follow-up. *Neurosurgery* 2001 ; *49* : 799-805.

[43] WIKHOLM G. Occlusion of cerebral arteriovenous malformations with N-butyl cyano-acrylate is permanent. *AJNR Am J Neuroradiol* 1995 ; *16* : 479-482.

[44] WIKHOLM G. Role of transarterial embolization in the management of cerebral arteriovenous malformations. *Acta Radiol Suppl* 1996 ; *404* : 1-25.