A fundamental examination of the effect of N-butyl-2-cyanoacrylate (NBCA)-iodized oil mixtures on arterial embolization

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Purpose

N-Butyl-2-cyanoacrylate (NBCA; Histoacryl; B.Braun Medical Inc., Melsungen Germany) is a liquid permanent embolic material used for transcatheter arterial embolization (TAE) that polymerizes in the presence of anions. Clinically, it is used to embolize brain arteriovenous malformations\(^1\), dural arteriovenous fistulas\(^2\), wound-related arterial bleeding\(^4\), gastrointestinal bleeding\(^3\)\(^5\), postoperative pseudoaneurysms after liver excision and invasive treatments of the kidneys, such as percutaneous renal calculus enucleation, kidney partial excision, and biopsy\(^6\), in the bronchial artery to treat hemothysis\(^7\), and in polycystic kidneys\(^8\). Trauma patients and patients with coexisting disseminated intravascular coagulation (DIC) often have impaired clotting function, and embolism with coils or gelatin sponges tends to be ineffective. Because the embolization mechanism of NBCA does not depend on clotting function, it still has a marked embolic effect when clotting function is impaired\(^3\)\(^4\). For embolization, NBCA is mixed with iodized oil (Lipiodol; TERUMO, Tokyo, Japan) in a ratio between 1:1 and 1:9. This is done for two reasons: 1) to produce a radiopaque material; as NBCA is radiolucent and is not seen on fluoroscopy, it is mixed with radiopaque Lipiodol; and 2) to adjust the time from injection to polymerization (polymerization time) and the completion of embolization by changing the density of NBCA; this allows the material to arrive at target blood vessels.

For successful TAE with NBCA, the intravascular polymerization and the distance from the microcatheter tip to the target blood vessel should match. When they do not match, e.g., the embolic material polymerizes proximal to the target, recanalization may occur via the development of collateral vessels. The intravascular polymerization depends on the polymerization time and viscosity (intravascular polymerization factors), while the distance from the microcatheter tip to the target blood vessel may depend on the vessel anatomy and technical aspect of catheterization. The intravascular polymerization factors are presumably determined by the ratio of NBCA and Lipiodol (NBCA-Lip ratio).

Two conflicting opinions have been presented. One position proposes that the lower the NBCA density is, the more peripherally the embolic material reaches because the polymerization time is prolonged. Alternatively, the other position suggests that the lower the NBCA density is, the more proximally the embolic material ceases to flow because the viscosity of NBCA-Lip is increased due to high Lipiodol density. In clinical practice, the NBCA-Lip ratio is decided empirically by the physician performing TAE after considering the blood vessel diameter and blood velocity. However, the exact relationship between the NBCA-Lip ratio and intravascular polymerization factors is not known.

The purpose of this study was to find the optimal NBCA-Lip ratio for clinical use. We evaluated the viscosity, polymerization time, and diffusing capacity according to the NBCA-Lip ratio \textit{in vitro}. Furthermore, we evaluated the effect of the NBCA-Lip ratio...
on arterial embolization *in vivo*; various ratios of NBCA-Lip was injected into the renal arteries of adult beagles, after which the embolization effect after TAE was quantitatively investigated using computed tomography (CT) volumetry and histopathologically. Finally we discussed how the polymerization time and the viscosity of the NBCA-Lip affected arterial embolization.
Methods and Materials

-in vitro-

-the viscosity of the NBCA-Lip mixture

The viscosity of the NBCA-Lip mixture was determined using a tuning-fork-type viscometer (SV-10; A&D Company, Japan). We measured the viscosity of samples at nine different NBCA-Lip ratios #NBCA: Lipiodol = 1:1 (NBCA density 50%), 1:2 (33%), 1:3 (25%), 1:4 (20%), 1:5 (16.7%), 1:6 (14.3%), 1:7 (12.5%), 1:8 (11.1%), 1:9 (10%)# at a temperature of 37°C.

-the diffusing capacity of the NBCA-Lip

When mixed with blood, NBCA-Lip is polymerized to generate polymer. Therefore, diffusing capacity of the NBCA-Lip was evaluated by measuring the area of the generated polymer in blood after its mixing with blood. The NBCA-Lip (0.5 ml) was dropped on whole blood (1 ml) from an adult beagle on a petri dish and shaken with a shaker at 100 times per minute. Then, the area of the polymer was measured using Scion image (National Institute of Health, MD, USA).

-the polymerization time

To determine the polymerization time, NBCA-Lip was dropped on blood, and we recorded the process of polymerization by video (EXILIM EX-FH25 : CASIO, Japan) at a frame rate of 240 fps (frame/second) and observed the morphological changes in the polymer. The completion of polymerization was defined as the time when the morphological changes stopped.

-in vivo-

Twelve adult beagles were used for the in vivo experiment (mean body weight 14.9 kg: 13.8-15.8 kg). Selective embolization for abdominal or dorsal branch of renal artery with NBCA-Lip was performed by one radiologist(Fig.1). NBCA was mixed with Lipiodol in a ratio of 1:1, 1:3, or 1:9. Eight kidneys were embolized using each NBCA-Lip ratio.

-CT-volumetry(Fig.2)

Immediately after embolization, the kidneys were removed. CT of the specimen was obtained, and the intravascular distribution of Lipiodol was confirmed using 64-slice multi-detector computed tomography (Aquilion 64; TOSHIBA, Japan). Using CT volume rendering images of the kidney with a threshold CT value >500 HU, CT-volumetry of the
embolized vascular bed was performed on a workstation (Ziostation ver.1.3.0.2. AMIN, Inc., Japan). To evaluate how much the embolic material reached the peripheral vessels in the kidney, we measured the volume of embolized cortical vessels after removing the medullary vessels on source images. For the index of the peripheral embolization effect, we did not use the absolute value of the embolized cortical vessels but the ratio of embolized vessels of the cortex and those of the total kidney to exclude variation effect of different volume of renal vascular bed among different subjects.

#histopathological evaluation

A region with a high level of NBCA-Lip on CT was chosen for pathological preparation. This tissue was embedded in paraffin and stained with hematoxylin and eosin (H&E) and Elastica Masson (EM). Forty random fields in each kidney were observed at 400× magnification. A histopathological evaluation determined the following two points to evaluate how peripherally the NBCA-Lip reached in the artery.

(a) Item 1: The minimum minor diameter of the arteriole containing embolic material in the lumen was measured in each kidney.

(b) Item 2: The number of arterioles with a minor diameter #40 µm containing embolic material in the lumen was counted.

A sampling error due to specimen preparation may affect item 1. Therefore, item 2 was used as an index of the peripheral embolism effect. We evaluated the peripheral embolism effect quantitatively by item 2.

#Statistical analysis

The statistical analysis was performed using SPSS (ver. 15.0, IL, USA). The histopathological evaluations were compared using analysis of variance (ANOVA) with the Tukey-Kramer post-hoc test. P < 0.05 was considered to indicate statistical significance.
Fig. 0: Selective embolization of ventral branch of left renal artery by NBCA-Lip. (1:1) Yellow arrow head is ventral branch of left renal artery.

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Fig. 0: Volume Rendering images (CT) of the embolized kidney with a threshold CT value >500 HU. We evaluated the cortex/total kidney ratio of the vascular bed and considered this to be the index of the distal embolization effect.

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Results

*In vitro*

#Viscosity of NBCA-Lip (fig.1)

The viscosity of NBCA-Lip increased as the NBCA density decreased.

#Polymerization time of NBCA-Lip (fig.2)

The polymerization time prolonged as the NBCA density decreased. That were approximately similar time between 1:1 and 1:2 and between 1:3 and 1:5 and increased exponentially in 1:6~1:9.

#Diffusing capacity of NBCA-Lip (fig.3)

The area of the polymer increased as the NBCA density decreased.

*In vivo*

TAE of the renal artery succeeded in all cases. On average, 0.43 ml (0.3-0.6 ml) of NBCA-Lip was required to achieve embolization in all kidneys. There was no significant difference in the quantity injected among the three groups.

CT of the isolated kidney confirmed the distribution of the embolic material from the hilum to the cortex in each kidney. The vascular bed of the embolized blood vessel calculated using CT volumetry tended to increase as the NBCA density decreased (fig.4). In addition, the embolized vascular bed was significantly bigger with NBCA-Lip ratios of 1:3 and 1:9 than with 1:1, no significant difference between 1:3 and 1:9 was observed.

(a) Item 1: (fig.5)

Regarding evaluation item 1, the minimum minor diameter of an arteriole that contained embolic material in the lumen was 3.4, 2.2, and 2.3 µm with NBCA-Lip ratios of 1:1, 1:3, and 1:9, respectively. With ratios of 1:3 and 1:9, the embolic material was found in significantly narrower arterioles than at a 1:1 ratio.

(b) Item 2: (fig.6)

Regarding evaluation item 2, the average number of embolized arterioles in 40 fields was 1.1, 5.6, and 6.3 with ratios of 1:1, 1:3, and 1:9, respectively. More arterioles were embolized at 1:3 and 1:9 ratios than at 1:1, whereas no significant difference was found between 1:3 and 1:9.
At a low NBCA density, narrow arterioles were embolized, and the peripheral distribution of the embolic material tended to be broad.
**Fig. 0:** Viscosity of NBCA-Lip. As NBCA density decreased, viscosity of NBCA-Lip increased.
Fig. 0: Polymerization Time of NBCA-Lip. As NBCA density decreased, polymerization time was prolonged.

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**Fig. 0:** Diffusing Capacity of NBCA-Lip. As NBCA density decreased, diffusing capacity increased.

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**Fig. 0:** Embolized Vascular Bed. The embolized vascular bed calculated using CT volumetry tended to increase as the NBCA density decreased. The embolized vascular bed was significantly bigger with NBCA-Lip ratios of 1:3 and 1:9 than with 1:1, no significant difference between 1:3 and 1:9 was observed.

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Fig. 0: The minimum minor diameter of the arteriole containing embolic material. With ratios of 1:3 and 1:9, the embolic material was found in significantly narrower arterioles than at a 1:1 ratio.

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Fig. 0: Number of embolized artery (/40 fields). More arterioles were embolized at 1:3 and 1:9 ratios than at 1:1, whereas no significant difference was found between 1:3 and 1:9.
Conclusion

Discussion

Clinically, NBCA-Lip is used in mixture ratios of 1:1 to 1:9. The ratio is decided by the interventional radiologist empirically depending on the blood vessel diameter, blood velocity, and distance between the microcatheter tip and target. High-density NBCA (NBCA:Lip=1:1~1:3) tends to be used when the distance from the microcatheter tip to the target is short, whereas low-density NBCA (NBCA:Lip=1:6~1:9) is used when the microcatheter tip cannot be inserted close to the target. Successful embolization has been performed at the following ratios for different target arteries: bronchial artery 1:7\textsuperscript{9}, gastrointestinal organs 1:1-1:7, splenic artery 1:3-1:4, the lower dorsal branch of the renal artery 1:4\textsuperscript{4}, lumbar artery 1:1, inferior epigastric artery 1:7, and iliac artery 1:5.

However, little is known of intravascular polymerization factors of NBCA-Lip, and there is a risk of excessive or incomplete embolization or adhesion of the catheter tip to the vascular wall. There is no conclusive evidence of the optimal NBCA-Lip ratio. For safer, more effective embolization, fundamental research is needed to determine the optimal NBCA-Lip ratio. Nevertheless, no study has quantified the relationship between the NBCA-Lip ratio and the embolic effect or the level in the artery attained. Therefore, we evaluated the embolic effect of various NBCA-Lip ratios quantitatively. In other words, we evaluated both the difference in its properties \textit{in vitro} and the difference in embolic effect \textit{in vivo} and considered the correlation between them.

-Viscosity and diffusing capacity-

The viscosity of NBCA-Lip increased as the density of NBCA decreased, although the diffusing capacity of the polymer tended to be larger. The fact that the polymer spread widely on shaking suggests that the embolic material would reach peripherally in blood vessels. This suggests that clinically, NBCA-Lip tends to flow more peripherally when the NBCA density is low, refuting the opinion that NBCA-Lip does not reach the peripheral artery when the Lipiodol density is high, at least for ratios in the range of 1:1~1:9.

-Polymerization time-

Several experimental studies have measured the polymerization time. Brothers\textsuperscript{10} dropped glue onto human plasma and measured the time from contact of the glue with the plasma to polymerization with a stopwatch. Kailasnath\textsuperscript{11} recorded the tension on the NBCA injection catheter using a blood vessel model and used the precipitous increase in the tension curve to indicate the completion of polymerization. David\textsuperscript{12} dropped NBCA-Lip onto blood and judged the polymerization time by direct vision under a dissecting
microscope and recorded it with a stopwatch. These methods lack accuracy because there is no clear definition of the completion of polymerization, and the time was recorded manually. Therefore, we recorded the polymerization reaction with a high-speed camera at 240 fps. We defined the completion of polymerization as when the morphological changes stopped and judged the polymerization time from the video record when NBCA-Lip was dropped onto blood. We thought that an objective evaluation was possible by this method.

The mixture of high density NBCA polymerized comparatively quickly (#40 sec). By contrast, the mixture of low density NBCA polymerized in around 60 sec, and only where the mixture contacted blood. Elsewhere, the NBCA-Lip remained fluid. We thought Lipiodol inhibited the contact and polymerization between NBCA and blood. Therefore, when the density of NBCA is low, its polymerization in an artery occurs slowly. The polymerization occurred more quickly as the density of NBCA increase. With our method, the mixture polymerized incompletely when the density of NBCA was very low. Additionally, our study was conducted on a flat petri dish, without the influence of pulsatile flow or infusion pressure. It is likely that the actual polymerization time during TAE would be shorter. However, objectivity is higher in this method for measurement than the conventional method. We believe that we have enough results to evaluate the relative difference in the polymerization time according to the mixture ratio.

Our results in vitro suggest that it is easier for NBCA-Lip to flow more peripherally when the density of NBCA is lower. Furthermore, this tendency was thought to be influenced by prolongation of the polymerization time rather than the increase in viscosity.

-Histopathological evaluation-

In previous reports, NBCA was not identified using a standard staining process. Instead, the embolic material was recognized by the presence of Lipiodol, which is an oily contrast medium subject to oil-red staining. To evaluate NBCA itself, one report used the rare metal europium as a fluorescent chelate composite\textsuperscript{13}. No other method has been reported. Therefore, we performed renal artery embolization with NBCA alone, NBCA-Lip, and Lipiodol alone in a preliminary experiment. We prepared cross-sections of the embolized renal artery and kidney with H&E, EM, and oil-red staining and evaluated the pathological characteristics of the embolic material. In both tissues, we recognized an amorphous material in the artery that stained with H&E, EM, and oil red. This was not seen in the normal kidney and was interpreted as the embolic material. Even in arteries embolized with NBCA alone, an amorphous structure that stained with oil red was recognized. Therefore, the amorphous structure in the vessel embolized with NBCA-Lip was regarded as the mixture of NBCA and Lipiodol.

In this study, as the density of Lipiodol increased, embolic material was recognized in smaller-diameter arteries, and the embolized vascular bed was larger. During TAE,
NBCA-Lip mixture with a low density of NBCA-Lip would reach more peripherally from the catheter tip and embolize more peripheral and smaller-diameter arteries.

Conclusion

For NBCA-Lip ratios of 1:1~1:9, embolization occurred more peripherally and in wider vascular bed, as NBCA density decreased. We consider that polymerization time had greater influence to this tendency than viscosity.
References


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