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Contrast agent comparison for three-dimensional micro-CT angiography: A cadaveric study

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Barium sulfate and lead oxide contrast media are frequently used for cadaver-based angiography studies. These contrast media have not previously been compared to determine which is optimal for the visualisation and measurement of blood vessels. In this study, the lower limb vessels of 16 embalmed Wistar rats, and four sets of cannulae of known diameter, were injected with one of three different contrast agents (barium sulfate and resin, barium sulfate and gelatin, and lead oxide combined with milk powder). All were then scanned using micro-computed tomography (CT) angiography and 3-D reconstructions generated. The number of branching generations of the rat lower limb vessels were counted and compared between the contrast agents using ANOVA. The diameter of the contrast-filled cannulae, were measured and used to calculate the accuracy of the measurements by comparing the bias and variance of the estimates. Intra- and inter-observer reliability were calculated using intra-class correlation coefficients. There was no significant difference (mean difference [MD] 0.05; MD 95% confidence interval [CI] -0.83 to 0.93) between the number of branching generations for barium sulfate-resin and lead oxide-milk powder. Barium sulfate-resin demonstrated less bias and less variance of the estimates (MD 0.03; standard deviation [SD] 1.96 mm) compared to lead oxide-milk powder (MD 0.11; SD 1.96 mm) for measurements of contrast-filled cannulae scanned at high resolution. Barium sulfate-resin proved to be more accurate than lead oxide-milk powder for high resolution micro-CT scans and is preferred due to its non-toxicity. This technique could be applied to any embalmed specimen model. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: micro-computed tomography; angiography; barium sulfate; lead oxide; contrast media

1. INTRODUCTION

A thorough and accurate knowledge of microvascular networks in the human body is necessitated by continued advances in reconstructive surgical procedures such as perforator and osteomyocutaneous flaps (1–3). This has led to renewed interest in advanced imaging techniques that enable comprehensive visualisation of microvascular networks in cadavers which can be used to inform surgical applications (2–5). Early anatomists used X-ray angiography to visualise and describe microvasculature morphology. This traditional technique has been considerably improved by recent advances in computerized tomography (CT) and micro-CT technology. However, there is a lack of clarity concerning which contrast medium is optimal to characterize vascular branching geometry and measure vessel dimensions accurately.

X-ray angiography overcame the disadvantage of tissue destruction associated with corrosion casting and dissection techniques to study blood vessels injected with latex, silicone or India ink (4). However, the two-dimensional (2D) x-ray images generated did not enable distinction between vessels in different tissue layers or the identification of vessels coursing through different tissue planes (3). The advent of CT angiography has created a non-destructive technique for 3D visualisation of microvascular networks (3,4,6). However, the accuracy of 3D reconstruction models generated from CT angiograms is limited due to the slice thickness being greater than the size of the microscopic vessels (7). To overcome these limitations, Ghanavati *et al*. (8) utilized micro-CT angiography to obtain high-resolution 3D images of the mouse cerebral microvasculature. The radiopaque contrast agents used for micro-CT angiography need to be low viscosity and contain atoms with a high atomic number to enable the contrast-filled microvasculature to be differentiated from surrounding soft tissue structures (6,8). Barium sulfate- and lead oxide-based contrast media are the most popular with lead oxide described as the 'gold standard' for visualization of the finest microvascular networks (9).

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Lead oxide mixed with gelatin is an inexpensive, simple and reliable method that results in very dense radiopacity (9). Milk powder has been substituted for gelatin in the x-ray angiography of embalmed cadavers with results equal to those achieved with the lead oxide and gelatin preparation injected into fresh cadaveric specimens (5,10). Lead oxide, however, is toxic (9,11). Stringent personal protective equipment (PPE) protocols are required for the use of lead oxide and any residues and contaminated equipment require specialist disposal (9). Barium sulfate, in contrast, is non-toxic. Typically mixed with gelatin or latex to achieve a thin consistency for optimal dispersal into the fine vessels, a new technique mixing barium sulfate with epoxy resin has recently been reported for CT angiography (4,9). There was agreement, to the smallest detectable arteries (0.1 mm diameter), between the CT angiographic images and corresponding dissection studies (4).

No studies to date have directly compared lead oxide and barium sulfate based preparations to characterize vascular branching geometry and accurately measure vessel dimensions using micro-CT in embalmed specimens. Although lead oxide is widely used, it is highly toxic and therefore sub-optimal in terms of safety. If barium sulfate preparations can produce equally detailed images then it is arguably a better contrast medium. We hypothesized that barium sulfate can produce micro-CT 3D angiographic images that are equivalent in detail and accuracy to those produced by a lead oxide preparation.

Thus, the aim of this study was to establish whether barium sulfate combined with resin or gelatin is an equally effective contrast medium compared to a lead oxide-milk preparation when used with micro-CT 3D angiographic images in embalmed rodents. The specific questions addressed include:

(1) Is barium sulfate resin (BS-R) and/or barium sulfate gelatin (BS-G) equivalent to lead oxide milk powder (LO-M) in terms of the extent to which it perfuses into the lower limb vessels for the characterization of vascular branching geometry in a rat model?
(2) Which of the contrast media most accurately represents a known diameter when measurements are made from 3-D reconstructed micro-CT images?

2. METHODS

2.1. Animals

Sixteen rats (male Wistar; 8 weeks of age) were perfused with a solution of 4% formaldehyde in phosphate buffered saline solution, following lethal barbitone injection, to enable fixation of the intact circulatory system whilst maintaining the patency of the blood vessels. These animals were obtained from another experiment and had undergone electrophysiological investigations of the sciatic nerve. Dissection of the anterior thorax and mediastinum of the rats was then undertaken to facilitate the insertion of a 20 G cannula (Introcan Safety, Braun, Melsungen, Germany) which was placed and secured into the thoracic aorta with 2-0 vicryl surgical ties (Ethicon Inc, Somerville, MA, USA), after the arch of the aorta was ligated proximal to the cannula insertion site. The study was approved by the Australian National University Animal Experimentation Ethics Committee (A2014/36) and was conducted in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the ACT Animal Welfare Act (1992).

The toes of the left foot of each rat were transected for visual inspection of when the contrast preparation had extended distally to the end of the limb. Ten millilitres (ml) of 6% hydrogen peroxide solution (Orion Laboratories Pty. Ltd., Balcatta, Australia) was injected into the cannula and left in the vessels for 1–2 hours. Oxygen bubbles from the hydrogen peroxide distend the vessels (8,12). The visible vessels were inspected for hydrogen peroxide leakage. Identified leaks were secured with 2–0 vicryl surgical ties and/or quick-drying super glue.

2.2. Contrast media preparation

The three contrast agents were prepared and injected as follows:

2.2.1. Barium sulfate resin (BS-R) preparation

Fifty grams of barium sulfate (E-Z-HD™, E-Z-EM, Westbury, NY, USA) was thoroughly combined with 80 ml of compound A (Kinetix® R118 Infusion Resin, ATL Composites, Molendinar, Australia) and 20 mL of compound B (Kinetix® H120 Infusion Hardener, ATL Composites, Molendinar, Australia) in a fumehood. The solution was then degassed in the fumehood prior to injection (4).

2.2.2. Barium sulfate gelatin (BS-G) preparation

One hundred mL of Milli-Q water was heated to 40 °C before 3 g of gelatin powder (Johnson & Johnson Pacific, Ultimo, Australia) and 5 mL of Listerine® (Johnson & Johnson Pacific, Ultimo, Sydney, Australia) was gradually added to the water and stirred continuously until the gelatin was dissolved. Fifty grams of barium sulfate (E-Z-HD™, E-Z-EM, Westbury, NY, USA) was then added while the mixture was continuously stirred until an emulsion was formed (13). This formulation was modified from the preparation described by Chan *et al*. (13) who used 40 g of barium sulphate in 100 mL of normal saline and 3 g of gelatin. The modification allowed for the concentration of barium sulfate to be equal in the BS-R and BS-G preparations.

2.2.3. Lead oxide milk (LO-M) powder preparation

One hundred grams of lead oxide (P_3O_4 red lead; Ajax Chemicals, Sydney, Australia), 18 g of baby milk powder (Johnson & Johnson Pacific, Ultimo, Sydney, Australia) and 20 mL of tap water were mixed using a pestle and mortar and ground into a smooth paste. Boiling water (40 mL) was added to the paste and thoroughly stirred and combined (10).

Each contrast agent was independently injected into the aorta of the 16 rats (six BS-R; five BS-G; five LO-M) using a 50 mL syringe in a pulsatile fashion to avoid supra-physiological pressures (10,12). The injections were conducted inside a fumehood. Appropriate personal protective equipment was used based on the relevant material safety data sheets and risk assessments. Perfusion was judged to be adequate when the contrast agent was observed to leak from the transected toes of the left foot. Polymerization of both the BS-R and BS-G preparations and solidification of the LO-M preparation was checked by assessing the residuals. In addition, four cannulae of known diameter (ranging from 18 G to 24 G in size [1.3, 1.1, 0.9, and 0.7 mm internal diameter]) were injected with each contrast agent. The rats and cannulae were labelled and placed in a coolroom at 4 °C and left for 48 hours.

2.3. Micro-CT scanning

The injected rats and cannulae were scanned using a Quantum FX Micro-CT scanner (PerkinElmer, Waltham, MA, USA). The rats were positioned prone head first in the scanner. Two sets of cannulae were scanned simultaneously to directly compare the contrast media. The cannulae were arranged so that the smallest cannula containing each contrast medium was positioned directly opposite each other. Two sequence parameters were used to produce: a low resolution/large scan area (field of view (FOV) = 70; voxel size = 295 μm; 90 kV; 200 μA; and a high resolution/small scan area (FOV = 30; voxel size = 59 μm; 90 kV; 200 μA).

2.3.1. Three-dimensional reconstructions

Three-dimensional reconstructions of the contrast-filled blood vessels and cannulae were created using the 3D volume rendering tool in Osirix MD (Pixmeo, Geneva, Switzerland). The 3D reconstructions of the blood vessels were then analysed. The number of branching generations of the vessels was counted in both the right and left lower limb of each rat.

2.3.2. Quantitative measures

The first branching generation was the bifurcation of the aorta and each continuous branching artery from this point was allocated a generation number. The greatest number of branching generations for each limb was recorded.

The diameter of the smallest visible vessel was then measured. Each 3D reconstruction of the rat lower limb vessels was divided into eight sections. The smallest vessel in each section was measured using the Osirix MD measurement tool. The diameter of the smallest vessel, out of the eight sections, was recorded for each rat.

Ten measurements were taken along the length of the 3D reconstruction of each cannula to quantify the internal diameter, using the Osirix MD measurement tool. The average of the ten measurements was calculated.

2.4. Data analysis

Question one was examined in two ways. First, the extent of perfusion was examined by counting the maximum number of branching generations as above. These were compared between contrast media using ANOVA adjusting for side (left/right). Predicted means, mean differences, standard errors of the means and 95% confidence intervals of the mean difference (MD 95% CI) were reported. Second, the smallest visible vessel diameter was measured and compared using the same analysis.

Question two was examined by comparing the bias and variance of the estimates relative to the known cannula diameter (14). Differences in the internal diameter between the known cannula diameter supplied by the manufacturer, and the measurements of the contrast-filled cannulae scanned at both low and high resolutions (bias), were graphed against the known diameter. In addition, differences in bias between the two contrast media were analysed using ANOVA controlling for resolution (low/high). The results of the ANOVA were reported as marginal means and standard errors for each contrast medium and the difference between the marginal means (MD) and 95% confidence intervals of the difference (MD 95% CI). Intra-observer reliability (measurements made two weeks apart by the same observer) and inter-observer reliability (measurements made by a second observer trained and blinded to the results of the first observer) were examined for all cannulae measurements using intra-class correlation coefficients (ICCs). All analyses were performed using SPSS version 20 for Windows (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

3. RESULTS

3.1. General aspects

Each micro-CT dataset was composed of 512 images. The arterial network from the aortic bifurcation to the digital arteries of the toes was visible on four BS-R and four LO-M 3D reconstructions (Fig. 1). Two injections failed (one BS-R and one LO-M). In both

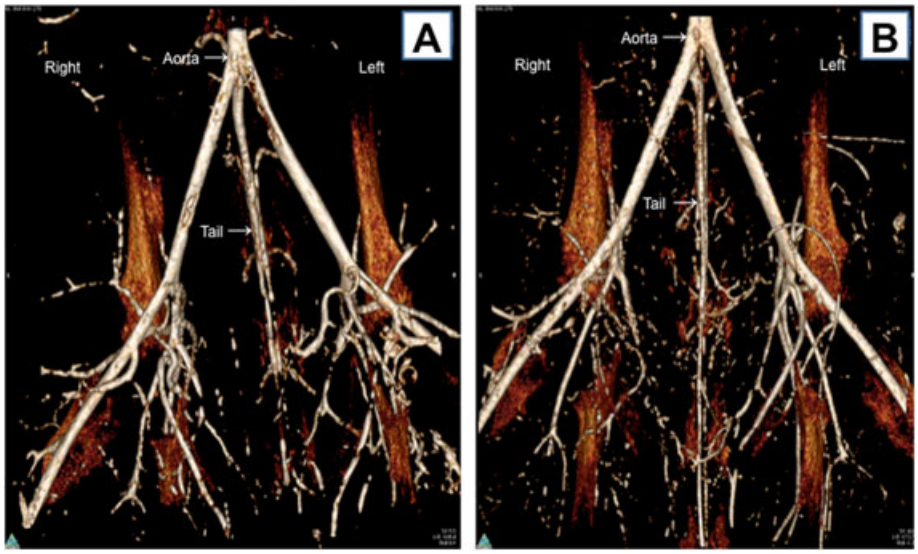

Figure 1. Anterior view of three-dimensional reconstruction images of the embalmed rat lower limb vessels injected with barium sulfate resin (A) and lead oxide milk powder (B) contrast media.

failed injections there was no evidence of vessel filling. Four out of five BS-G injections failed to fill the vessels and therefore no further analysis of the BS-G injected rats and cannulae was undertaken.

3.2. Vascular branching geometry

The number of continuous branching generations ranged from four to six (BS-R) and three to seven (LO-M). There was no significant difference (MD 0.05; MD 95% CI -0.83 to 0.93) between the number of branching generations of the rat lower limb vessels counted for the BS-R (mean 4.80; SE 0.30) and LO-M (mean 4.75; SE 0.34) (Table 1). The smallest vessel diameter visible on the rat arterial network 3D reconstructions was 0.07 mm for both the BS-R and LO-M preparations.

3.3. Measurement accuracy

The bias (mean difference) between the contrast medium and true cannula diameter was greater for LO-M (0.11 mm) compared to BS-R (0.03 mm) when the cannulae were scanned at high resolution (Table 2). At low resolution, the mean difference was the same for BS-R and LO-M. The variance of the estimates (1.96*standard deviation of the differences) was wider for LO-M compared to BS-R at both low and high resolutions (Fig. 2). There was no significant difference (MD -.03; MD 95% CI -0.12 to 0.06) between the bias (known minus measured) for the BS-R (mean 0.04; SE 0.03) and LO-M (mean 0.07; SE 0.03). The ICC intra-observer ($ICC_{3,1}$) and inter-observer ($ICC_{2,1}$) reliability was excellent (Table 2).

4. DISCUSSION

Barium sulfate resin (BS-R) was superior to lead oxide milk powder (LO-M) in terms of accuracy at high resolution and also in terms of reduced variance at both high and low resolution. Both media were equivalent in terms of extent of perfusion.

Table 1. Mean bias (95% CI) from low and high resolution micro-CT scan three-dimensional reconstructions, for cannulae of known ('true') internal diameter filled with barium sulfate resin or lead oxide milk powder

Micro-CT Scan Resolution	Bias from known diameter, MD (95% CI)	
	Barium Sulfate (n = 4)	Lead Oxide (n = 4)
Low	0.03 (0.01 to 0.06)	0.03 (−.03 to 0.1)
High	0.05 (−0.04 to 0.14)	0.11 (−0.05 to 0.26)

Table 2. Reliability of the internal diameter measurements of the contrast-filled cannulae three-dimensional reconstructions

Contrast	Intra-observer ($ICC_{3,1}$)	Inter-observer ($ICC_{2,1}$)
Barium	0.97	0.98
Lead	0.98	0.89

Barium sulfate is a non-toxic alternative contrast medium for micro-CT angiography. It has been used in previous studies but has not previously been assessed for accuracy compared to traditional standard preparations using lead oxide. Lead oxide supplanted barium sulphate as a contrast agent in the 1980s because it was believed to provide optimal visualization of small vessels (9,11). It was commonly combined with gelatin to prevent leakage. In this study we show for the first time that barium sulfate combined with resin represents both a safer and more accurate option.

Lead oxide demonstrated less accuracy at high resolution compared to barium sulfate. In this study we included both high and low scan resolutions in order to examine whether resolution altered the accuracy of our measurements. The relative decrease in accuracy at higher resolution in the lead oxide preparations was due to 'scatter' or 'sun-burst' artefact. Artefact of this type has been previously reported with lead preparations which are very high density (3). The degree of artefact can be minimized by reducing the concentration of lead oxide. Tregaskiss *et al.* (3) reduced the concentration of lead oxide by 66% compared to that previously used by Rees and Taylor (5) for x-ray studies, in order to minimize the artifact. They used a gelatin preparation with a lower concentration of lead (65 g PbO per 100 mL H_2O) compared to that used in this study (100 g Pb_3O_4 per 60 mL H_2O). Tregaskis *et al.* (3) reported that this concentration was optimal for preventing artefact however, in this study we employed a technique established for use in embalmed specimens which was developed for x-ray angiography. It is possible that a lower concentration of lead oxide would have yielded better results. However, by not using lead oxide the problem is avoided altogether.

Lead oxide is not an ideal material to be used in the anatomical research and teaching environment for a number of reasons. The most important consideration is toxicity. Lead oxide is highly toxic and therefore requires personal protective equipment and specialist disposal (9). So, although lead oxide is cheaper to buy, this advantage is offset by the expense of disposal. Further, if lead oxide is used in a cadaveric specimen for imaging purposes it is rendered useless for further research of teaching. It is therefore preferable to use a preparation like barium sulphate and resin which, although more expensive, is safer and actually enhances the specimen for visualization of the vessels for teaching.

In this study the barium sulfate and gelatin injections failed due to incomplete filling of the vessels but the reason is not clear. Solidification and blockage of fine gauge needles has been previously reported (12). Therefore it is possible that the fine vessels targeted in this study were too small for the gelatin preparation to penetrate. Barium sulfate and gelatin injections have previously been used with success in fresh cadavers (9). It is possible that the vessels in our embalmed specimens lacked sufficient elasticity to allow adequate ingress. Gelatin is used to prevent leakage (3) but resin also performs this function. It is therefore suggested that gelatin preparations are potentially less suitable for use in embalmed specimens.

The extent to which a contrast agent perfuses into the vessel tree is indicative of its utility for micro-CT angiography. No previous studies have compared contrast preparations for use in micro-CT angiography. In fresh human cadavers injected and scanned with conventional CT scanners (3,4), the smallest vessel measured was 0.4 mm (lead oxide gelatin), and 0.3 mm (barium sulfate resin) (3,4). In this study the smallest vessel diameter measured was 0.07 mm for both barium sulfate resin and lead

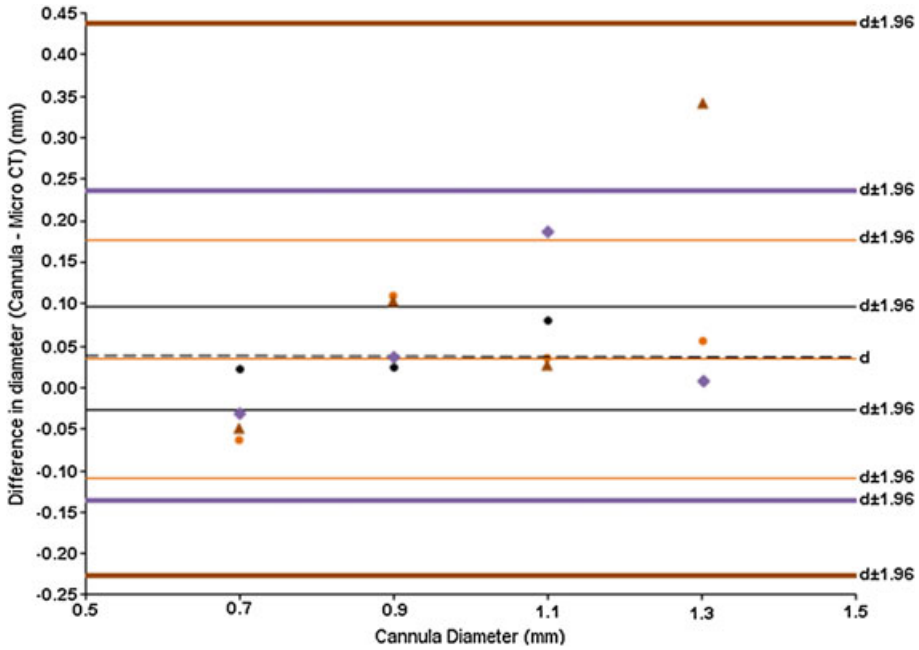

Figure 2. The mean difference (MD) and the variance of the estimates (1.96SD = 1.96*standard deviation of the differences) between the lead oxide milk powder (grey) and barium sulfate resin (black) and known internal cannulae diameter when micro-CT scanned at high (thick line) and low (thin line) resolution – lead low resolution scan; - barium low resolution scan; - lead high resolution scan; - barium high resolution scan

oxide milk powder preparations. This increase in ability to visualize smaller vessels is unsurprising given the better resolution offered by micro-CT compared to conventional CT. However, the ability to visualize the small vessels depends on both the contrast agent and the substance in which it is suspended. We used milk powder and water to suspend the lead oxide because it has been successfully used previously in embalmed specimens (12). Similarly barium sulfate mixed with resin has also been used previously but it is not clear whether the specimens were embalmed (4). We have demonstrated that both contrast preparations were able to perfuse far enough into the vascular tree to take advantage of this new technology.

The technique used in this study demonstrated less bias than has previously been reported. Tregaskiss *et al*. (3) reported a mean difference in known glass tube length of 0.2 mm with variability of 2.28 mm. In this study barium sulfate resin produced the most accurate results with a mean difference of 0.03 mm and variability of 0.27 mm. Therefore anatomical examination of microvasculature is significantly enhanced by the use of micro-CT together with a barium sulfate resin contrast medium.

There are a number of limitations to this study. We used a small number of rats in each arm of the study, however, this is consistent with similar previous studies (3,4,12). A limitation of using micro-CT is that the resolution is inversely proportional to the size of the specimen i.e. the higher the resolution required the smaller the field of view therefore a smaller sample size is accommodated. Although the technology is improving rapidly and larger sample sizes are being accommodated, at this time longer scanning times are required.

5. CONCLUSION

In this study barium sulphate-resin and lead oxide milk powder preparations were compared for efficacy when visualizing microvasculature using micro-CT in embalmed specimens. We found that barium sulfate combined with a low viscosity resin provided superior results at high resolution and was equivalent to lead oxide and milk powder in terms of extent of perfusion. We therefore recommend barium sulfate resin as an effective contrast preparation when assessing embalmed cadaveric microvascular anatomy using micro-CT due to its non-toxicity and minimal artefact at higher resolutions.

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