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Modified Method to Increase the Volume and Stability of Bleomycin Foam: An Experimental Study

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BACKGROUND Bleomycin foam is an effective sclerotherapy method for venous malformations. The preparation method is rather complicated, and the volume and stability of the foam are limited.

OBJECTIVE To modify the currently used method for preparing bleomycin foam, to simplify the preparation procedure, and to produce foam with greater volume and increased stability.

MATERIALS AND METHODS Experiment 1: 6.0 IU of bleomycin powder was dissolved in different human serum albumin (HSA):saline solution (SS) ratios of 0.5:1.5, 0.75:1.25, 1:1, 1.25:0.75, 1.5:0.5, 1.75:0.25, and 2:0 in volume; then, an air:liquid ratio of 2:1 was used to create foam using the Tessari method. Experiment 2: 6.0 IU of bleomycin was dissolved directly in 2.0 mL of HSA; then, air:liquid ratios of 1:1, 2:1, 3:1, and 4:1 were used to create foam using the Tessari method. The optimum proportions of HSA:SS and air:liquid were screened by comparing the foam half-life (FHL).

RESULTS Experiment 1: the optimum proportion of HSA:SS was 2:0, and the FHL was 7.5 minutes. Experiment 2: the optimum proportion of air:liquid was 3:1, and the FHL was 9.0 minutes.

CONCLUSION The modified method is simpler and could produce more stable bleomycin foam with greater volume.

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Venous malformations (VMs) are common diseases in the head and neck and mostly occur in the buccal cavity, neck, eyelids, lips, tongue, or oral fundus. Extended VMs can cause deformities, dysfunction, infection, bleeding, and other complications. At present, the traditional treatment options mainly include surgical resection, sclerotherapy, laser, or a combination of these.[1] Percutaneous sclerotherapy is a well-established treatment option for VMs.[2–4] In recent years, bleomycin foam has been reported to be an effective sclerotherapy method for the treatment of VMs,[5,6] especially in areas where it is necessary to minimize swelling, and when extended, VMs require reduction in the dosage of bleomycin.

The currently used method for producing bleomycin foam is as follows[6–8]: first, a ratio of 6 IU of bleomycin is suspended in 1 mL normal saline solution (SS); second, 1 mL of human serum albumin (HSA) is added; and finally, an air:liquid ratio of 2:1 is used to create foam using the Tessari method. The foam half-

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life (FHL) and volume of foam produced by this method are about 3.5 minutes and 6 mL, respectively. However, according to our clinical experience, extended VMs in head and neck regions generally need to be injected with foam more than 6 mL in multiple points, so the treatment takes a longer time and a larger volume of foam. Therefore, the currently used method that is performed by 3 steps is rather complicated, and the volume and stability of the foam are limited. It is inconvenient when treatment for extended VMs is administered because precipitation of liquid easily occurs before the treatment ends, or secondary preparation of foam is required due to insufficient volume.

It is reported that the liquid–air proportion and sclerosant composition have influence on the stability of foam.[9,10] Human serum albumin added in this method is viscous colloidal solution, which mainly consists of small molecular compounds of short peptide chains, and the solution, with strong hydrophilic groups and hydrophobic groups, has higher surface activity to promote the development of the foam.[11] Here, a simpler modified method, by changing the proportion of HSA–SS and the proportion of air–liquid, is reported to produce larger quantities of bleomycin foam that is more stable.

Materials and Methods

Experiment 1. Preparation Method: Proportion of Human Serum Albumin and Saline Solution

The experiment was designed and conducted in 7 groups. In Group 1, 6.0 IU of bleomycin hydrochloride for injection (Hanhui Pharmaceutical co., Ltd., Zhejiang City, China) was dissolved in 1.5 mL of SS (0.9% sodium chloride; Otsuka Pharmaceutical Co., Ltd., Tianjin City, China), which was then dissolved in 0.5 mL of HSA (20%, the ingredients include HSA, water, and sodium chloride; 50 mL; CSL Behring AG, Bern, Switzerland) in 1 10-mL syringe (WEGO, Weihai City, China) so that the ratio of HSA:SS was 0.5:1.5. In Group 2, 6.0 IU of bleomycin was dissolved in 1.25 mL of sodium chloride, which was then dissolved in 0.75 mL of HSA in 1 syringe so that the ratio of HSA:SS was 0.75:1.25. In Group 3, 6.0 IU of bleomycin was dissolved in 1.0 mL of sodium chloride and then dissolved in 1.0 mL of HSA in 1 syringe so that the ratio of HSA:SS was 1:1. In Group 4, 6.0 IU of bleomycin was dissolved in 0.75 mL of sodium chloride and then dissolved in 1.25 mL of HSA in 1 syringe so that the ratio of HAS:SS was 1.25:0.75. In Group 5, 6.0 IU of bleomycin was dissolved in 0.5 mL of sodium chloride and then dissolved in 1.5 mL of HSA in 1 syringe so that the ratio of HAS:SS was1.5:0.5. In Group 6, 6.0 IU of bleomycin was dissolved in 0.25 mL of sodium chloride and then dissolved in 1.75 mL of HSA in 1 syringe so that the ratio of HSA to SS was1.75:0.25. In Group 7, 6.0 IU of bleomycin was directly dissolved in 2.0 mL of HSA in 1 syringe, and no additional SS was added. Seven additional syringes were used to extract 4 mL of air at room temperature. A medical 3-way tap (WEGO) was used to connect 2 syringes at 90° for 7 groups. The plungers of 2 syringes were subsequently moved back and forth together 20 times to produce bleomycin foam.

To test the foam stability, once the foam formed, it was then immediately pushed into a standard 10-mL syringe that was vertically placed under a stopcock. The appearance of the foam was observed under optical microscopy, and the change of its volume in the syringe was recorded. The foam in the syringe gradually resolved into liquid and air, with some liquid sclerosing agent gathered at the bottom. The time it took for foam to lose half of its initial volume was recorded as the FHL.

The tests aforementioned were performed 10 times by the same operators. Each foam was prepared by using a new sclerosant, and all the experiments were conducted at room temperature. Experimental values were analyzed using SPSS 19.0 software (Chicago, IL). The Kruskal–Wallis test was used for the difference detection of multiple-sample, and the *t*-test was used for the difference detection between 2 independent samples. A p-value $\leq .05$ was considered to be significant.

Experiment 2. Preparation Method: Proportion of Air–Liquid

Four group experiments were designed and conducted as follow. First, 6.0 IU of bleomycin was directly

dissolved in 2.0 mL of HSA in each group. Then, 2-mL liquid and 2-mL air (the air–liquid ratio was 1:1) was mixed in Group 1, 2-mL liquid and 4-mL air (the air–liquid proportion was 2:1) was mixed in Group 2, 2-mL liquid and 6-mL air (the air–liquid proportion was 3:1) was mixed in Group 3, and 2-mL liquid and 8-mL air (the air–liquid proportion was 4:1) was mixed in Group 4. According to the classic Tessari method, 2 syringes were connected and pushed back and forth in 4 groups for a total of 20 times. The test method and statistical analysis were same as Study 1.

Results

The Proportion of Human Serum Albumin and Saline Solution

The results indicated that as the proportion of HSA increased and that of SS decreased, the FHL was gradually prolonged and the smooth and creamy degree of the foam was gradually increased. When HSA, without additional saline, was used to dissolve the bleomycin powder, the FHL became the longest, and the bubbles in the foam exhibited the smallest and smoothest feature, indicating that the foam in that case was also the most stable. The FHL values were shown in Table 1, and the foam surface profiles were more intuitively seen in Figure 1.

The Proportion of Air–Liquid

The findings showed that when the air–liquid proportion was 3:1, the FHL was the longest. The FHL values were shown in Table 2.

Therefore, based on the previous experiments, a modified bleomycin foam-producing method was established as following: 6.0 IU of bleomycin was directly dissolved in 2.0 mL of HSA in 1 syringe, and 6.0 mL of air was extracted in the other syringe. A medical 3-way tap was used to connect the 2 syringes at 90°, and then, the plungers of the 2 syringes were moved back and forth 20 times to produce the sclerosing foam.

Discussion

Foam stability has been reported to be influenced by different gases, methods of preparation, the liquid–air ratio, temperature, viscosity, and sclerosant composition.[9,10,12,13] Therefore, the modified preparation method for bleomycin foam consists of directly dissolving bleomycin in HSA, and then, a 3:1 air–liquid ratio is used to create foam. This not only simplifies the preparation process, increasing the amount of foam, but also greatly increases the stability of the foam.

It is known that the protein solution, with higher surface activity, forms a liquid film with a double electron layer on the surface and then surrounds the air and forms bubbles.[11] The drainage of the liquid membrane is an important factor to affect the stability of foam.[14,15] The relationship between the half-time of drainage and viscosity of the liquid is as following[16]: $T_{1/2} = \frac{580\eta h}{\rho g d^2 V}$, where $T_{1/2}$ is the half-time of drainage, η is the viscosity of the liquid, h is the initial height of foam column, ρ is the density of the

TABLE 1. Foam Half-Life (FHL) of Different Proportions of Human Serum Albumin (HSA) and Saline Solution (SS)

Group	*Bleomycin Dose (IU)*	*HSA (mL)*	*SS (mL)*	*Air (mL)*	*Total Bleomycin Foam Volume (mL)*	*The FHL (min)*
1	6.00	0.50	1.50	4.00	6.00	2.25 ± 0.17
2	6.00	0.75	1.25	4.00	6.00	2.67 ± 0.19
3	6.00	1.00	1.00	4.00	6.00	3.51 ± 0.10
4	6.00	1.25	0.75	4.00	6.00	4.34 ± 0.30
5	6.00	1.50	0.50	4.00	6.00	4.78 ± 0.32
6	6.00	1.75	0.25	4.00	6.00	6.25 ± 0.52
7	6.00	2.00	0.00	4.00	6.00	7.48 ± 0.47

The average FHL is expressed as the mean ± SD.

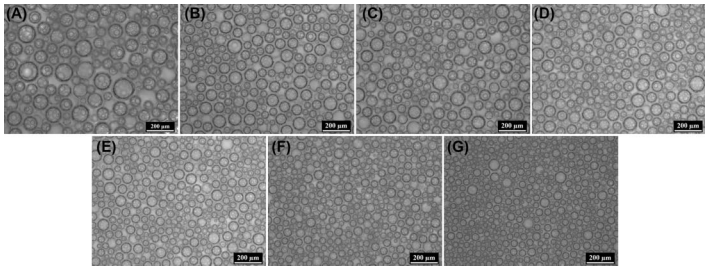

Figure 1. Foam surface profiles in different proportion of human serum albumin (HSA) and saline solution (SS). (A) Group 1 (HAS:SS = 0.5 mL:1.5 mL). (B) Group 2 (HAS:SS = 0.75 mL:1.25 mL). (C) Group 3 (HAS:SS = 1.0 mL:1.0 mL). (D) Group 4 (HAS:SS = 1.25 mL:0.75 mL). (E) Group 5 (HA:SS = 1.5 mL:0.5 mL). (F) Group 6 (HAS:SS = 1.75 mL:0.25 mL). (G) Group 7 (HAS:SS = 2.0 mL:0 mL).

TABLE 2. The Foam Half-Life (FHL) of Different Proportions of Air and Liquid

Group	*Bleomycin Dose (IU)*	*HSA (mL)*	*Air (mL)*	*Total Bleomycin Foam Volume (mL)*	*The FHL (min)*
1	6.00	2.00	2.00	4.00	3.52 ± 0.26
2	6.00	2.00	4.00	6.00	7.22 ± 0.33
3	6.00	2.00	6.00	8.00	9.03 ± 0.59
4	6.00	2.00	8.00	10.00	7.87 ± 0.51

The average FHL is expressed as the mean ± SD.
FHL, foam half-life; HSA, human serum albumin.

liquid, g is the gravitational constant, d is the diameter of bubble, and V is the initial liquid fraction of bubble. The equation shows that with the increase of viscosity of the liquid, the half-time of drainage is longer and the foam is more stable.

The components of the commercial HSA solution used in these experiments mainly include HSA, water, and sodium chloride. We think that the water in the solution makes it possible to dissolve the bleomycin powder directly. Besides, the stability of produced foam is positively related to the viscosity of albumin solution, and the higher the ratio of HSA, the higher the viscosity of the colloidal solution formed. Therefore, bleomycin powder is wholly dissolved by commercial HSA in our modified preparation method, instead of dissolving SS first and then adding commercial HSA,[6] which increases the concentration of albumin; as a result, the foam is more stable compared with the currently used method.

We also found that the FHL produced by the modified method is 9.0 minutes, which is nearly 3 times that of currently used method6 (3.5 minutes). This is statistically significant which suggests that the stability of the foam is appreciably increased. In addition, the change in the ratio of air:liquid from 2:1 to 3:1 led to a 33% increase in the volume of foam and a decrease in the therapeutic dose per unit volume.

However, our modified method has only been tested in vitro and not in the clinic. We speculate that the potential risk of foam distant side-effects such as pulmonary embolism will increase due to the prolonged FHL. Therefore, it is necessary to study the clinical safety and efficacy further.

To conclude, the modified method is simpler and could produce a greater volume of more stable bleomycin foam than the currently used one.

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