EXPERIMENTAL INFUSION PHLEBITIS: TOLERANCE pH OF PERIPHERAL VEIN

Takashi KUWAHARA, Shougo ASANAMI, Yoshiyuki KAWAUCHI and Shuji KUBO

*Naruto Research Institute, Otsuka Pharmaceutical Factory, Inc.,
115 Tateiwa, Muya-chō, Naruto, Tokushima 772-8601, Japan*

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ABSTRACT — This study aims to determine the pH that peripheral veins can tolerate. Intravenous nutrient solutions with different pHs (from 4.52 to 6.71) were infused into rabbit ear veins, and the veins were examined histopathologically. After 6-hr infusion at 10 mL/kg/hr, a commercial 2.72% amino acid/7.5% glucose solution with electrolytes (AG) caused obvious phlebitic changes, such as loss of venous endothelial cells, inflammatory cell infiltration, and perivascular edema, in all 6 rabbits because of its low pH (4.52) and high titratable acidity (22 mEq/L). The phlebitis was reduced when the solution was neutralized with NaOH to pH 5.93, and was almost eliminated when the pH was neutralized to 6.49. After 8-hr infusion at 15 mL/kg/hr, AG-adjusted pH to 6.30 caused slight phlebitic changes, but AG-adjusted pH to 6.71 scarcely caused any change. Furthermore, 24-hr infusion of the pH 6.49 solution caused no histopathological changes in 3 rabbits. These results suggest that the tolerance pH for the peripheral vein is about 6.5, and that an infusion solution does not cause phlebitis due to acidity if the pH is not lower than the tolerance pH.

KEY WORDS: Infusion phlebitis, Tolerance pH, Peripheral vein, Peripheral intravenous infusion, Rabbit ear vein

INTRODUCTION

Because infusion phlebitis is a major complication of peripheral intravenous infusion, the phlebitic potential of infusion solutions should be reduced to a minimum. Factors involved in the development of infusion phlebitis include acidity and hyperosmolality of the infusion solution (Lewis and Hecker, 1985; Hesson, 1985; Nordenstrom, 1992). Clinical (Elfving and Saikku, 1966; Fonkalsrud *et al.*, 1968a; Eremin and Marshall, 1977) and animal studies (Kauste and Rantakallio, 1962; Fonkalsrud *et al.*, 1968b) show that infusion phlebitis is reduced by neutralizing the pH of the infusion solution. Fonkalsrud *et al.* (1968a) indicated that neutralizing the pH of the infusion solution to 7.4 significantly reduced the incidence of infusion phlebitis, and they concluded that neutralization of the infusion solution was the most significant factor for reducing infusion phlebitis. Eremin *et al.* (1977) also demonstrated that neutralizing to pH 7.0 significantly

reduced the incidence of infusion phlebitis, and they concluded that neutralization enhanced the functional life span of the infusion. Although neutralizing to pH 7.4 is effective to prevent phlebitis, it may cause problems in the stability of the infusion solutions, such as calcium phosphate precipitation (Driscoll *et al.*, 1994; Hill *et al.*, 1996). It has not been studied yet whether the pH of the infusion solution should be neutralized to 7.4, or whether a pH value of around 6.0 would be adequate to prevent phlebitis. We therefore determined the tolerance pH of peripheral vein by histopathological evaluation of the infused vein. In the present study, different pH solutions were infused into rabbit ear veins for 6 or 8 hr, then a solution at the tolerance pH was infused for 24 hr to see if the tolerance pH was affected by infusion duration.

MATERIALS AND METHODS

Animals

Male rabbits (Kbl;JW, Kitayama Labes Inc., Nagano, Japan) weighing from 2.5 to 3.5 kg were housed in individual cages in an animal room maintained at $23 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ relative humidity with ventilation 13-16 times/hr and a 12-hr light-dark cycle. The animals were allowed free access to diet and water except during the infusion period. Six rabbits were used for each 6- or 8-hr treatment, and three were used for the 24-hr treatment.

Test solutions

A commercial 2.72% amino acid/7.5% glucose solution with electrolytes (PLASAMINO, Otsuka

Pharmaceutical Factory, Inc., Tokushima, Japan; AG) was used as the base solution. The composition of AG is shown in Table 1. The test-solution codes denote the base solution and the approximate pH value; i.e., AG65 was a solution with a pH of about 6.5. AG45 was AG as is. AG59, AG65, AG63 and AG67 were prepared by adding NaOH to AG. For the 24-hr infusion, AG65 was diluted with water (DAG65) to prevent hyperosmolality effects, because the tolerance osmolality of peripheral venous endothelial cells for 24-hr infusions is about 550 mOsm/kg (Kuwahara *et al.*, 1998a). All test solutions were prepared within 1 hr before the start of infusion. The pH of the test solutions and the titratable acidity to pH 7.4 were measured with a pH meter (PH82, Yokogawa Electric Co., Tokyo, Japan). The osmolality was measured with an osmometer (OM-6040, Kyoto Daiichi Kagaku Co., Kyoto, Japan). The composition, pH, titratable acidity, and osmolality of the test solutions are shown in Table 2.

Table 1. Composition of the commercial solution AG used as the base solution.

Component	
L-Leucine	4.1
L-Isoleucine	1.8
L-Valine	2.0
L-Lysine · HCl	6.2
(as L-Lysine	5.0)
L-Threonine	1.8
g/L N-Acetyl-L-Tryptophan	0.7
(as L-Tryptophan	0.6)
L-Methionine	2.4
L-Phenylalanine	2.9
L-Arginine	2.2
L-Histidine	1.0
Glycine	3.4
Total amino acid (as free amino acid)	27.2
Glucose	75.0
mEq/L Na ⁺	34
Cl ⁻	34

Experimental methods

We undertook two experiments with the different infusion conditions to confirm the generality of the results. In addition, a solution at the tolerance pH was infused for 24 hr to see if the tolerance pH was affected by infusion duration.

Infusion, tissue sampling, and histopathological examination were performed as described in our previous studies (Kuwahara *et al.*, 1996 and 1998b). Test solutions were infused through a polytetrafluoroethylene catheter (24G $\times$ 3/4"; 0.67 mm in external diameter and 19 mm in length, Terumo Co., Tokyo, Japan) into an ear vein (Fig. 1). AG45, AG59, and AG65 were infused for 6 hr at 10 mL/kg/hr; AG63 and AG67 for 8 hr at 15 mL/kg/hr; and DAG65 for 24 hr at 5 mL/kg/hr. The rabbits were sacrificed by injection of excess sodium pentobarbital (Nembutal, Abbott Laboratories, IL, USA) 20 - 24 hr after the end of infusion. Two samples

Table 2. Composition, pH, titratable acidity, and osmolality of test solutions.

Test solution	Base solution AG (mL)	1/2 N NaOH (mL)	Distilled water (mL)	pH	Titratable acidity (mEq/L)	Osmolality (mOsm/kg)
AG45	as is	-	-	4.52	22.0	796
AG59	500	12.6	-	5.93	11.0	789
AG65	500	15.8	-	6.49	6.4	787
AG63	500	14.5	-	6.30	7.4	785
AG67	500	17.5	-	6.71	5.4	782
DAG65	500	15.8	240	6.49	4.4	531

AG, a commercial 2.72% amino acid/7.5% glucose solution with electrolytes.

of the ear, one from a region 3 - 10 mm downstream from the catheter tip (proximal region) and the other from a region 20 - 30 mm downstream from the catheter tip (distal region), were obtained (Fig. 1) and fixed with phosphate-buffered 10% formalin. As the untreated control, a sample was obtained from the contralateral ear. Cross sections of the vein were stained with hematoxylin and eosin (HE). The histopathological examination was performed blindly, in which the findings were graded according to the criteria shown in Table 3, although there is no standard criteria to evaluate infusion phlebitis.

Statistical analysis

The grades of histopathological findings following 6-hr infusion of AG45, AG59, and AG65 were analyzed statistically by the Steel-Dwass test (Imamizo *et al.*, 1992a). The grades following 8-hr infusion of AG63 and AG67 were also analyzed by the Wilcoxon rank sum test (Imamizo *et al.*, 1992b).

RESULTS

6-hr infusion at 10 mL/kg/hr

The results of the histopathological examination are shown in Table 4, and the cumulative grades of the findings and the results of the statistical analysis are shown in Fig. 2.

AG45 caused loss of venous endothelial cells (grade 2 or 3), inflammatory cell infiltration (grade 1, 2, or 3), and perivascular edema (grade 2 or 3) in both the proximal and distal regions in all 6 rabbits and thrombi in 4 rabbits (Photo 1). AG59 also caused some phlebotic changes in all 6 rabbits, but of lower grade (1 or 2) and without thrombi (Photo 2). AG65 caused slight loss of venous endothelial cells or slight inflammatory cell infiltration, each in the proximal region of 1 rabbit, and caused no changes in the proximal region in the other rabbits or in the distal region in all 6 rabbits (Photo 3). The differences between AG45 and AG59 were significant in loss of venous endothelial cells in the proximal and distal regions ($p < 0.05$, 0.01), inflammatory cell infiltration in the proximal and distal regions ($p < 0.05$), and edema in the distal region ($p < 0.05$). The differences between AG45 and AG65 were significant in loss of venous endothelial cells, inflammatory cell infiltration, and edema in both the proximal and distal regions ($p < 0.01$).

The differences between AG59 and AG65 were significant in loss of venous endothelial cells in the proximal and distal regions ($p < 0.05$), and edema in the

proximal region ($p < 0.05$).

8-hr infusion at 15 mL/kg/hr

The results of the 8-hr infusion are shown in Table 5. AG63 caused slight venous endothelial cell loss in the proximal region of 5 of 6 rabbits and in the distal region of one, and a few slight changes in the proximal region of 3 rabbits. AG67 caused slight loss of venous endothelial cells only in the proximal region of one rabbit and no changes in the other 5. The difference between AG63 and AG67 was significant in loss of

Fig. 1. Infusion technique and sampling regions for histopathological examination.

Table 3. Criteria for histopathological examination.

Histopathological findings	Grade
Loss of venous endothelial cells	
None	0
Less than 1/3 of vein in cross section	1
1/3 to 2/3 of vein in cross section	2
More than 2/3 of vein in cross section	3
Inflammatory cell infiltration	
None	0
Few inflammatory cells in venous wall or perivascular tissue	1
Many inflammatory cells in venous wall or perivascular tissue	2
More diffuse and denser inflammatory cells in perivascular tissue	3
Edema	
None	0
Localized in perivascular tissue	1
More diffuse edema	2
Edema throughout the whole area	3
Thrombus	
None	0
Less than 1/3 of vein in cross section	1
1/3 to 2/3 of vein in cross section	2
More than 2/3 of vein in cross section	3

venous endothelial cells in the proximal region ($p < 0.05$).

24-hr infusion at 5 mL/kg/hr

The results of the 24-hr infusion are shown in Table 6. Similar to untreated veins (Photo 4), no phlebotic changes were observed in the veins of the 3 rabbits infused with DAG65 for 24 hr (Photo 5).

DISCUSSION

Acidity of infusion solutions is an important risk factor for infusion phlebitis (Lewis and Hecker, 1985; Hesselov, 1985; Nordenstrom, 1992). There are many reports concluding that infusion phlebitis can be reduced by neutralizing the pH (Elfving and Saikku, 1966; Fonkalsrud *et al.*, 1968a and 1968b; Eremin and Marshall, 1977; Kauste and Rantakallio, 1962). On the

Fig. 2. Cumulative grades of histopathological findings after 6-hr infusion at 10 mL/kg/hr.

Table 4. Histopathological findings (grade*) after 6-hr infusion at 10 mL/kg/hr.

Test solution	Examined region †	Loss of venous endothelial cells				Inflammatory cell infiltration				Edema				Thrombus				
		Grade*	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
AG45	Proximal				1	5			4	2			4	2	2		2	2
	Distal				3	3			1	3	2			3	3	2	2	2
	Untreated vein ‡	6					6					6				6		
AG59	Proximal			3	3		1	4	1		1	1	4		6			
	Distal		2	4			4	2			4	1	1		6			
	Untreated vein ‡	6					6				6				6			
AG65	Proximal		5	1			5	1			6				6			
	Distal		6				6				6				6			
	Untreated vein ‡	6					6				6				6			

Values are number of rabbits; six rabbits were examined in each group. * See Table 3. † See Fig. 1; Proximal, specimen from a region 3 - 10 mm downstream from the catheter tip; Distal, specimen from a region 20 - 30 mm downstream from the catheter tip. ‡ Specimen from the contralateral ear.

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other hand, there are some reports showing that increasing the pH of intravenous nutrient solutions may result in calcium phosphate precipitation, causing lethal complication (Driscoll *et al.*, 1994; Hill *et al.*, 1996). Therefore, increasing the pH of infusion solutions is conditioned by the stability of solutions. In the present study, we determined the pH that the peripheral vein can tolerate by using a histopathological evaluation. The commercial 2.72% amino acid/7.5% glucose solution with electrolytes caused obvious phlebitis in rabbit ear veins after 6 hr of infusion because of its low pH (4.52) and high titratable acidity (22 mEq/L). This phlebitis was significantly reduced when pH of the solution was increased to 5.93, and was almost eliminated when it was increased to 6.49. Because the peripheral vein could not tolerate pH 5.93 in all 6 rabbits and pH 6.30 in 5 of 6 rabbits, changes to pH 6.0 or 6.3 are not considered to be sufficient.

Because the peripheral vein could tolerate pH 6.49 and pH 6.72, we estimated the tolerance pH to be about 6.5. We found previously that the tolerance osmolality of peripheral veins falls as the infusion duration increases

(Kuwahara *et al.*, 1998a), so we tested whether tolerance pH is also affected by prolonged infusion. We found no detectable changes in the veins of 3 rabbits infused for 24 hr with solution of pH 6.49, suggesting that the tolerance pH does not change when infusion duration increases.

Since solutions for peripheral intravenous nutrition are sometimes more acidic than pH 6.5 (Nordenstrom *et al.*, 1991; Palmer *et al.*, 1996), phlebitis may be caused not only by hyperosmolality, but also by acidity. However, acidity of infusion solution was not taken into account for phlebitic factors in recent clinical studies on peripheral intravenous nutrition (Timmer and Schipper, 1991; Madan *et al.*, 1991). To accurately evaluate the phlebitic potential of infusion solutions, osmolality, pH, and titratable acidity should all be considered.

We conclude that the tolerance pH for the peripheral vein is about 6.5, and that a solution does not cause phlebitis due to acidity if the pH is not lower than the tolerance pH.

Therefore, to eliminate the acidic factor causing phlebitis, the pH of peripheral intravenous solutions

Table 5. Histopathological findings (grade*) after 8-hr infusion at 15 mL/kg/hr.

Test solution	Examined region †	Loss of venous endothelial cells				Inflammatory cell infiltration				Edema				Thrombus				
		Grade*																
			0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
AG63	Proximal		1	5			3	3			4	2			6			
	Distal		5	1			6				6				6			
	Untreated vein ‡		6				6				6				6			
AG67	Proximal		5	1			6				6				6			
	Distal		6				6				6				6			
	Untreated vein ‡		6				6				6				6			

Values are number of rabbits; six rabbits were examined in each group. * See Table 3. † See Fig. 1; Proximal, specimen from a region 3 - 10 mm downstream from the catheter tip; Distal, specimen from a region 20 - 30 mm downstream from the catheter tip. ‡ Specimen from the contralateral ear.

Table 6. Histopathological findings (grade*) after 24-hr infusion at 5 mL/kg/hr.

Test solution	Examined region †	Loss of venous endothelial cells				Inflammatory cell infiltration				Edema				Thrombus				
		Grade*	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
DAG65	Proximal		3				3				3				3			
	Distal		3				3				3				3			
	Untreated vein ‡		3				3				3				3			

Values are number of rabbits; three rabbits were examined. * See Table 3. † See Fig. 1; Proximal, specimen from a region 3 - 10 mm downstream from the catheter tip; Distal, specimen from a region 20 - 30 mm downstream from the catheter tip. ‡ Specimen from the contralateral ear.

Photo 1. Proximal region of vein infused with AG45 (pH 4.52). Loss of venous endothelial cells is observed on most parts of the venous wall, although a few endothelial cells (arrowhead) remain (small dense humps are nuclei of venous endothelial cells). Many inflammatory cells (arrow), perivascular edema (*), and thrombus (T) are observed. HE, Bar = 50 μ m.

Photo 2. Proximal region of vein infused with AG59 (pH 5.93). Partial loss of venous endothelial cells, some inflammatory cells (arrow), and perivascular edema (*) are observed. HE, Bar = 50 μ m.

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Photo 3. Proximal region of vein infused with AG65 (pH 6.49).
No changes are observed. Endothelial cells (arrowhead) line the whole
venous wall.
HE, Bar = 50 μ m.

Photo 4. Untreated ear lateral vein.
HE, Bar = 50 μ m.

Photo 5. Proximal region of vein infused with DAG65 (pH 6.49) for 24 hr. No changes are observed. HE, Bar = 50 μ m.

should be 6.5 or greater as long as the stability of the solution is not affected.

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