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ORIGINAL ARTICLE

Histopathological Evaluation of Horse Serum-Induced Immune Complex Vasculitis in Swine: Implication to Coronary Artery Lesions in Kawasaki Disease†

Saji Philip [a,b,c,*], Wen-Chuan Lee [c], Mei-Hwan Wu [d], Cherian Kotturathu Mammen [a,b], Hung-Chi Lue [d]

[a] Department of Pediatric Cardiology, St. Gregorios Cardio-Vascular Center, Parumala, Kerala
[b] Department of Cardiothoracic Surgery, Fontier Lifeline Hospital, Dr. K. M. Cherian Heart Foundation, Ambattur, Chennai, India
[c] Division of Biotechnology, Cardiovascular Research Center, Animal Technology Institute, Miaoli 350, Taiwan
[d] Division of Pediatric Cardiology, Department of Pediatrics, National Taiwan University Children's Hospital, National Taiwan University, Number 7, Chung-Shan South Road, Taipei 100, Taiwan

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Key Words
coronary artery lesions;
histopathology;
immune complex vasculitis;
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two-dimensional echocardiography

Background: Immune complex (IC) vasculitis can be experimentally induced in animal models by intravenous injection of horse serum (HS), and the findings of HS-induced IC vasculitis in swine were very similar to that of Kawasaki disease (KD). The IC mechanism may be involved in the pathogenesis of vasculitis in KD. Here, we studied the two-dimensional (2D) echocardiographic and histopathological findings of acute, subacute, and healing phases of vasculitis induced by two different types of HS, and the reproducibility of IC vasculitis in swine.
Methods and results: Our study group consisted of 24 pure-bred landrace male piglets of 1.5–3 months of age. They were divided into three HS groups ($n = 17$), namely, Group A ($n = 8$) receiving gamma globulin-free HS, and Group B ($n = 6$) receiving donor herd HS, three doses at 5-day intervals, and Group C ($n = 3$) that received only one dose of donor herd HS on Day 1, and the saline group ($n = 7$) that received three doses of intravenous normal saline (NS) at 5-day intervals. The 2D echocardiography was performed every 3–4 days, and all piglets were killed for histopathological studies at different dates from Days 2 to Day 60. All the HS groups developed rashes and demonstrated significant dilation (54–150%) of coronary arteries in Groups A and B; when compared ($p < 0.02$) with 9–53% dilation in Group C and the saline group. Histopathological changes of test groups were asymmetric coronary vasculitis in various stages, whereas none of the piglets in the control group developed vasculitis. No significant

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* Corresponding author. Dr. K M. Cherian Heart Foundation, Parumala, Pathanamthitta District, Kerala 689626, India.
E-mail addresses: tfcsaji@yahoo.co.in, sajitfc@hotmail.com (S. Philip).

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difference in the echocardiographic and histopathological findings was observed among the piglets that received two types of HS.
Conclusion: HS can induce IC vasculitis in swine. The rashes and 2D echocardiographic and histopathological studies of the acute to healing phases showed close similarities with KD, and it is concluded that swine may serve as a unique experimental model for IC vasculitis and for various therapeutic trials.

1. Introduction

Kawasaki disease (KD), an acute febrile disease with a systemic vasculitis, has become a leading cause of acquired heart disease other than rheumatic heart disease in many developed countries.[1] Coronary artery lesions (CALs) with aneurismal dilation, thrombosis, and/or stenosis, leading to myocardial infarction and death have been recognized as the most severe complication.[2] Circulating immune complexes (ICs), triggered by infectious agents, bacteria, viral, or other unknown causes, have been detected in the early phase of KD patients, suggesting that immunopathological mechanisms might be involved in the pathogenesis of vasculitis in KD (Table 1).[3–22] Attempts to produce coronary vasculitis in animal models have been tried in mice, weanling rabbits, and guinea pigs by injecting infectious agents, foreign proteins, *Lactobacillus casei* cell walls, and horse serum (HS).[23–26] Swine is a unique and promising animal for biomedical research, especially in the field of cardiovascular diseases.[27] IC vasculitis induced in swine showed rashes, and significant dilations of echocardiographic CA. In addition, the histopathological changes in the subacute stage of vasculitis were closely related to the lesions in KD, and thus we postulate that IC-mediated mechanisms may play a significant role in the pathogenesis of CALs in KD.[26–28] Here, we further evaluated the HS-

Table 1 Summary of studies on detection of immune complexes in KD patients.

No of studies	Authors	Year	No of KD cases	Positive for IC	Methods of detection
1.	Fossard and Thompson[3]	1977	01	Strongly+	Platelet aggregation test
2.	Sawa[4]	1979	15	06 (41%)	Raji cell method Inhibition latex agglutination
3.	Weindling et al[5]	1979	01	01 (100%)	Inhibition latex agglutination
4.	Eluthesen et al[6]	1981	81	48 (59%)	C1q solid phase array
5.	Furuse and Matsuda[7]	1983	16	50% and 26.6% (25 samples)	In acute and remission phase C1q binding assay
6.	Yanase et al[8]	1984	30	100%	No significant positive titer
7.	Miyata et al[9]	1984	32	11 (34.4%)	C1q binding assay
8.	Takiguchi et al[10]	1984	35	26%	Antibody inhibition test
9.	Mason et al[11]	1985	42	29 (69%)	Raji cell method C1q solid phase array
10.	Ono et al[12]	1985	32	11 (34%)	C1q binding assay
11.	Levin et al[13]	1985	19	13 (68%)	Polyethylene glycol/ Precipitation method
12.	Pachman et al[14]	1987	06	6 (100%) 5 (83%)	Deposition in coronary artery in the myocardium (IHS)
13.	Levin et al[15]	1987	19	19 (100%)	(Platelet interaction study)
14.	Lin and Hwang[16]	1987	20	70%	Polyethylene glycol method 60% Raji cell method
15.	Fujimoto et al[17]	1987	67	50 (75%)	C1q enzyme immune assay
16.	Ohshio et al[18]	1987	43	22 (51%)	ELISA solid phase Anti-C3 assays
17.	Salcedo et al[19]	1988	01	01 (100%)	IC deposition in kidney (IF)
18.	Salo et al[20]	1988	27	99%	C1q binding assay
19.	Li et al[21]	1990	17	8 (47%)	Polyethylene glycol method
20.	Koike[22]	1991	11	11 (100%)	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

ELISA = enzyme-linked immunosorbent assay; IC = immune complex; IF = immunofluorescent sections; IHS = immunohistochemical staining; KD = Kawasaki disease; +, positive.

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induced vasculitis in swine during the acute, subacute, and healing phases, from 2 days to 60 days of follow up, by two-dimensional (2D) echocardiography and histopathological studies. In addition, we also attempted to establish the reproducibility of IC coronary vasculitis in swine with two different kinds of HS infusions. The implications to CALs in KD are discussed in this study.

2. Methods

2.1. Experimental animal

A total of 24 pure-bred castrated piglets, weighing 20—39 kg, aged 1.5—3 months, which were randomly selected from a certified farm of the national nuclear herd of the Animal Technology Institute of Taiwan were induced in this study. They were equivalent to human age of 4 months to 1 year according to the percentage of maturation and metabolic age chart.[29] The Institutional Review Board of the Animal Technology Institute of Taiwan approved the study design; the care and handling of piglets followed the guidelines of the Animal Protection Law, Council of Agriculture.[30] The HS group consisted of 17 piglets, aged 1.5 months ($n = 3$), 2.5 months ($n = 8$), and 3 months ($n = 6$), and the saline (NS) group consisted of seven piglets, aged 1.5 months ($n = 1$), 2.5 months ($n = 4$), and 3 months ($n = 2$), respectively (Table 2).

2.2. Procedures

Piglets were anesthetized by either azaperone, 8—10 mg/kg intramuscularly, or thiamylal sodium, 5—8 mg/kg intravenously or in combination. We used two types of HS, namely, HS1 and HS2 to detect any differences in the induction of vasculitis in swine model. The HS1 type was negative for virus, mycoplasma, and cytopathogenic agents with a total protein content of 5.2 g/dL and <5% gamma globulin content (catalog number 16270-035, Lot no. 1026238; Gibco BRL Life Technologies, Gaithersburg, MD, USA); and the HS2 was donor herd heat-inactivated, virus- and mycoplasma-free HS, with a total protein content of 6.6 g/dL and 1.49 g/dL gamma globulin content (catalog number 29211Z0, Lot no. R15383 ICN; MP Biomedicals, Santa Ana, California, USA). The serum was slowly infused at around 10 drops/minute and then increased to 20—40 drops/minute for subsequent doses to avoid severe immediate reactions such as chills and cyanosis, respiratory distress, convulsions, shock.

In Group A ($n = 8$), 10 mL (0.52 g protein)/kg of HS1 was intravenously infused and in Group B ($n = 6$), 10 mL (0.7 g protein)/kg of HS2 was infused intravenously three times at 5-day intervals. In Group C ($n = 3$) only one dose of 10 mL (0.7 g protein)/kg HS2 was infused intravenously. The piglets were killed on Day 2 and Day 3, respectively, to study the histopathological changes during the early phase of HS infusion. In seven piglets in the saline group, 10 mL/kg of NS was administered intravenously three times at 5-day intervals.

2.3. Echocardiography

The 2D echocardiographic examinations were performed using Hewlett Packard Sonos 100CF, color ultrasound system, using 3.5 mechanical cardiac probe, CA, USA. The diameters of the left CA (LCA) and right CA (RCA) were checked and measured at 4—5-day intervals prior to and after the HS or NS infusion up to 14 days and then weekly until the autopsy. For the comparative study of the changes in diameter, measurements of the diameter were taken 5 mm from the orifice of the RCA and LCA. All measurements were taken on the modified parasternal long axis and

Table 2 Flow chart on experimental study design on immune complex vasculitis in piglets treated with horse serum and saline.

Total no. of piglets studied ($n = 24$)

Horse serum group ($n = 17$)		Saline group ($n = 7$)	
Group A	Group B	Group C	Saline group
GG-free HS Three doses (10 mL/kg) ($n = 8$)	DH HS Three doses (10 mL/kg) ($n = 6$)	DH HS One dose (10 mL/kg) ($n = 3$)	Saline Three doses (10 mL/kg) ($n = 7$)
Results Rashes ++ Persisted for 4–5 days ($n = 2$) CA changes 40–74% ($n = 3$) >75–100 ($n = 5$)	*Results* Rashes ++ Persisted for 4–5 days ($n = 3$) CA changes 50–74% ($n = 1$) >75–100 ($n = 5$)	*Results* Few rashes disappeared after 3–4 h ($n = 3$) CA changes 12–55% ($n = 3$)	*Results* No rashes CA changes 12–53%

CA = coronary artery; DH = donor herd; GG = gamma globulin; HS = horse serum;

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short axis, and on the modified apical four-chamber views in both right and left lateral positions with the probe at the opposite side, close to the posterior axillary fold. All piglets were carefully observed prior to and after infusions until the day of autopsy. Intraobserver and interobserver measurements were tested.

2.4. Tissue collection and histopathology

Autopsies were performed at 2 days, 3 days, 4 days, 10 days, 14 days, 24 days, 34 days, 41 days, or 60 days after the first dose of HS and at 10 days, 14 days, 24 days, or 41 days after the first dose of NS infusion, respectively. Gross appearance and histopathology of the LCA, RCA, left anterior descending, and left circumflex coronary arteries, and of the myocardium and systemic arteries, such as the aorta, and subclavian, iliac, and femoral arteries, were checked and studied. The liver, kidney, spleen, ear, and biopsy of skin lesions during infusions were also studied. All tissue specimens were perfused and put in 10% phosphate-buffered formaldehyde. All materials were serially sectioned into segments of 2—3 mm thickness and slides were prepared in hematoxylin and eosin stain. Other special stains, such as Masson's trichrome and Van Gieson's stains, for collagen and ground substances, and Verhoeff's stain for internal elastic membrane, were also obtained. For the convenience of recording histopathological changes of specimens in the acute, subacute, and healing phases, the samples were further grouped as follows: 2—4-, 5—13-, 14—24-, and 25—60-day-old samples.

2.5. Statistical analysis

Statistical analysis was carried out using the SPSS version 7.5 (SPSS Inc., Chicago, IL, USA) for Windows. Mean values were used for comparison. Results are expressed as mean $\pm$ standard deviation (SD). A comparison between HS and NS groups was performed using paired and two sample *t* tests. A p value < 0.05 was taken to be significant.

3. Results

Within 20—45 minutes following the first, second, or/and third HS infusion, all piglets developed rashes and showed immediate severe systemic reactions. Cyanosis, chills, respiratory distress, and convulsions were seen in four piglets, shock in four piglets, which were revived by resuscitation; another five piglets showed flushing and mild chills.

3.1. Erythematous rashes

Rashes appeared during or immediately after the HS infusion at the perineal and perianal regions in 12/14 (86%), over the legs in 9/14 (64%), over the chest in 8/14 (57%), over the ears in 8/14 (57%), and on the mouth, lips, and perioral areas in 8/17 (35%) piglets as seen in Figure 1A—C. No significant changes were noted between Groups A and B in terms of rashes, which were less frequent/severe in Group C that received only one dose of HS. Piglets between 2.5 months and 3 months of age developed more skin rashes than the younger ones. The rashes faded and disappeared in 3—6 hours from all piglets, except for two in Group A and three in Group B that had rashes which persisted for 4—5 days after the second dose of HS. None of the piglets in the NS group developed the skin rashes or systemic reactions.

3.2. CA changes

Results of 2D echocardiography demonstrated 12—53% increase in the diameter of coronary arteries in the NS group and in Group C (Table 3). The HS group showed a more significant dilation of the LCA and RCA (Table 3). The CA dilation was noted from Day 4 to Day 10, which gradually resolved to normal size from Day 14 to Day 20; however, one piglet showed thickening and irregularity of the CA wall. Of the 14 piglets in Groups A and B, eight (50%) showed severe dilation (>100%), three (21%) showed moderate dilation (75—99%), and three (21%) showed mild dilation (54—74%). However, there was no significant dilation of CA in Group C. Groups A and B showed moderate to severe dilation of CA in 11 (78%) piglets. Changes in the diameter of the LCA ($p < 0.003$ in Groups A and B) and RCA ($p < 0.009$ in Group A and $p < 0.003$ in Group B) in the HS group were highly significant when compared with the saline group. There were no significant differences in the changes in the CA diameter in Groups A and B ($p > 0.6$). Mild pericardial effusions were seen in two piglets in HS2. The mean $\pm$ 1 SD of intraobserver measurements of the CA diameter was 0.5 $\pm$ 0.05 mm and that of interobserver

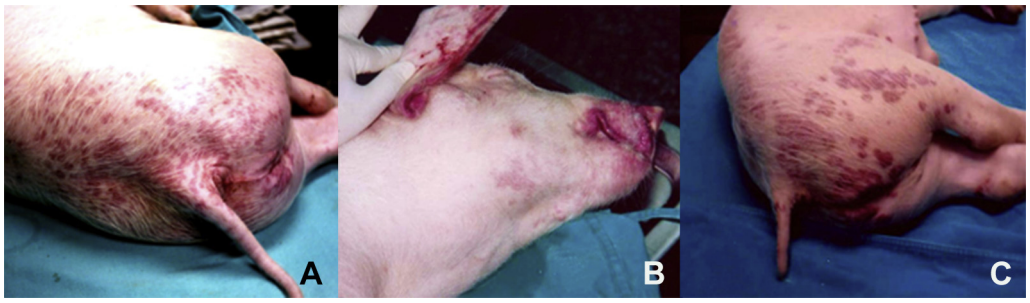

Figure 1 (A—C) Erythematous rashes in test groups after a horse serum infusion. After 1 hour of HS: (A) rashes on buttocks, back, and perianal areas in piglet number 7 of test Group A; (B) redness and rashes at the ear, chin, and lips in piglet number 6 of Group B. After the second dose of HS: (C) rashes on the back, buttocks, and perianal areas in piglet number 4 of Group A. HS = horse serum.

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Table 3 Data summary of coronary artery enlargement in horse serum and control saline groups.

Case no	Age (mo)	Wt (kg)	Left coronary artery			Right coronary artery			Day at autopsy
			Base (mm)	Max (mm)	Enlargement (%)	Base (mm)	Max (mm)	Enlargement (%)	
Horse serum group									
Group A (HS1; $n = 8$)									
1.	2.5	27–36	2.2	4.4	100	2.0	3.1	55	D10
2.	2.5	25–30	2.2	3.7	68	2.3	3.0	30	D10
3.	2.5	13–24	2.0	2.8	50	2.1	3.2	52	D14
4.	1.5	10–12	1.6	2.4	50	2.0	3.3	65	D24
5.	3.0	26–34	2.0	4.5	125	1.6	3.5	118	D24
6.	2.5	24–34	2.8	6.2	121	2.0	2.6	30	D34
7.	2.5	24–35	2.6	5.8	123	2.0	3.3	65	D41
8.	1.5	14–35	2.3	4.2	82	1.7	3.0	76	D60
Group B (HS2; $n = 6$)									
1.	2.5	20–24	2.0	3.0	50	1.5	2.7	80	D10
2.	2.5	26–28	2.2	4.0	81	1.4	2.3	64	D14
3.	3.0	32–39	2.7	5.4	106	2.2	3.2	45	D24
4.	2.5	23–26	2.5	5.0	100	2.3	3.4	47	D34
5.	2.5	15–24	1.7	3.4	100	2.0	3.6	80	D41
6.	2.5	25–32	1.6	4.6	187	2.0	3.8	90	D60
Group C (HS2; $n = 3$)									
1.	2.5	13–14	2.6	2.8	7.6	3.1	3.4	9.6	D02
2.	2.5	21–22	3.2	3.4	6.2	2.6	2.9	11.5	D03
3.	2.5	10–11	1.7	2.0	17	1.6	1.8	12	D04
Normal saline group ($n = 7$)									
1.	3.0	24–28	2.8	3.4	33	2.0	2.9	45	D10
2.	2.5	16–22	1.8	2.4	33	1.9	2.7	42	D14
3.	2.5	22–27	2.8	2.9	3.5	2.2	2.6	18	D24
4.	3.0	27–37	3.0	3.6	20	2.4	3.3	37	D24
5.	2.5	20–31	2.8	3.4	21	2.0	2.6	30	D34
6.	1.5	09–10	1.3	2.0	53	1.6	1.8	12	D41
7.	2.5	26–32	2.4	2.8	20	1.8	2.2	22	D60

HS = horse serum; Max = maximum.

measurements was 0.6 ± 0.08 mm, indicating that the measurements were reproducible.

Histopathological examinations of coronary and systemic arteries of the NS group showed no significant changes (Figure 2A–C). In the HS group, there were many changes of varying intensities, such as cellular infiltrates, internal elastic membrane disruption, mild to severe intimal proliferative changes, and subintimal change such as coagulation of the cytoplasm, as well as disorientation, separation, cytolysis, vacuolization, degranulation, collagen deposition, and total dissociation and fibrosis of the smooth muscle cells. The histopathological findings of vasculitis in the test group from 2–60 days after the first dose of HS infusion were grouped as follows: 2–4 days (Figure 2D–H; leucocytic and lymphocytic cellular infiltrates in the myocardium, perivenular, and periarterial infiltrates in the heart were seen. Cellular infiltrates were evident in the smooth muscle cells and also around the vasa vasorum of the aorta and in the distal tubular areas of the kidney. There were no significant changes in other vessels and organs); from 5–13 days (Figure 3A–C; intimal thickening, inner smooth muscle cells proliferation, patchy edematous changes, and early smooth muscle cells (SMC) disorganization were noted in coronary arteries. There were a few cellular infiltrates. The iliac artery showed mild intimal thickening. There were no significant changes in other vessels and organs); from 14–24 days (Figure 4A–E; there were intimal and inner SMC proliferations, moderate to severe disorientation of SMC, edematous separation of SMC (moth-eaten appearance), subintimal changes, such as coagulation of the cytoplasm, and disorientation, separation, cytolysis, vacuolization, degranulation, and collagen deposition in coronary arteries. Intimal proliferation was also noted in the intramural artery. No significant changes were observed in other vessels and organs); and from 25–60 days (Figure 4F; patchy areas of fibrosis existed within the SMC with resolving stages and no further progression of proliferation of SMC in the tunica media and intima in piglets that received HS2 infusions on Day 10. No significant changes were observed in other vessels and organs). A morphological examination of the heart showed adhesions and thickening of pericardium (Figure 3D) in two piglets.

Arteritis changes of varying degrees were noted in 79% of the LCA and left anterior descending artery (LAD), and 64% of RCA. Arteritis changes of mild degree, such as disruption of internal elastic membrane, or patchy edematous areas and/or smooth muscle cell proliferation were also noted in systemic arteries with varying percentages: femoral artery, 21%; ascending aorta, 21%; renal artery, 14%; iliac artery, 14%; and subclavian artery, 14%. In the acute stage, diffuse cellular

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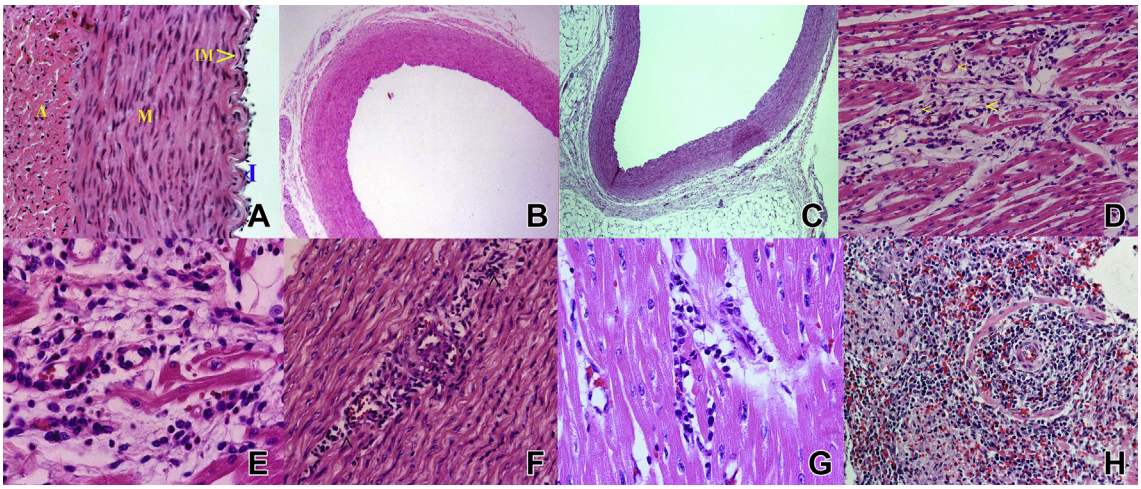

Figure 2 (A—H). H&E staining of coronary arterial walls of piglets in the saline and HS group: H&E staining of coronary arterial walls of piglets in the saline group (A—C) showing normal-looking walls after three doses of NS infusions. (A) Left coronary artery (400×) in piglet number 2 at Day 41 showing normal intima (I), internal elastic membrane (IM), tunica media (M), and adventitia (A) (B) Left coronary artery (magnification 40×) in piglet number 4 at Day 14. (C) Left anterior descending artery (40×) in piglet number 1 at Day 24. (D—H) H&E staining of coronary arterial walls of piglets in the HS group. (D,E; 2—4 days) Perivenular cellular infiltrates of the coronary vein and vasa vasorum (<) (200×) and diffuse cellular infiltrates in the tunica media (400×) in piglet number 2 of Group C at Day 3. (F) Cellular infiltrates in the tunica media (200×) of the ascending aorta at Day 2 in piglet number 1 of Group C. (G) Diffuse cellular infiltrates in the myocardium (400×) and (H) in the distal tubular areas of the right kidney (200×) at Day 2 in piglet number 1. H&E = hematoxylin and eosin stain; HS = horse serum; NS = normal saline.

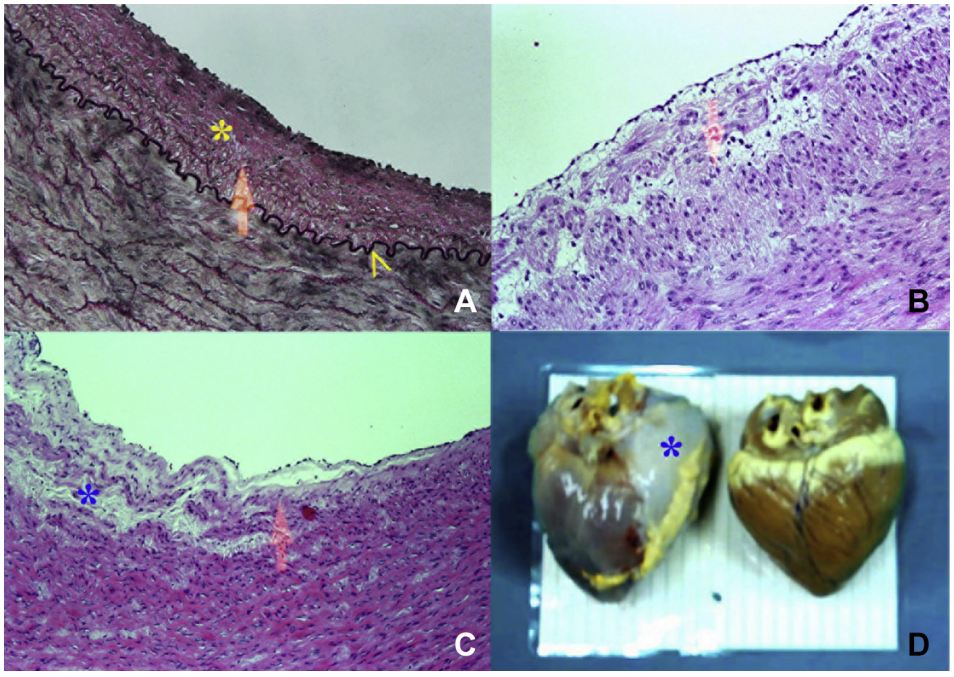

Figure 3 (A—D) H&E staining of arterial walls of piglets after HS infusions in Groups A and B (5—13 days). (A) Verhoeff's staining for internal elastic membrane (^) and intimal thickening (*) of the right iliac arterial wall (200×) of piglet number 2 of Group A; H&E staining of arterial walls of the HS group. (B) Proliferation and thickening (light yellow arrow head) of the intima and inner SMC of the tunica media of the left anterior descending artery (40×) of piglet number 6 of Group A. (C) Patchy edematous areas (*) in SMC of the LCA (100×) in piglet number 3 of Group B. (D) Pericardial thickening (*) and adhesions in the heart of piglet number 3 of Group B at the right side with normal heart of the saline group number 6 on the left side. H&E = hematoxylin and eosin stain; HS = horse serum; LCA = left coronary artery.

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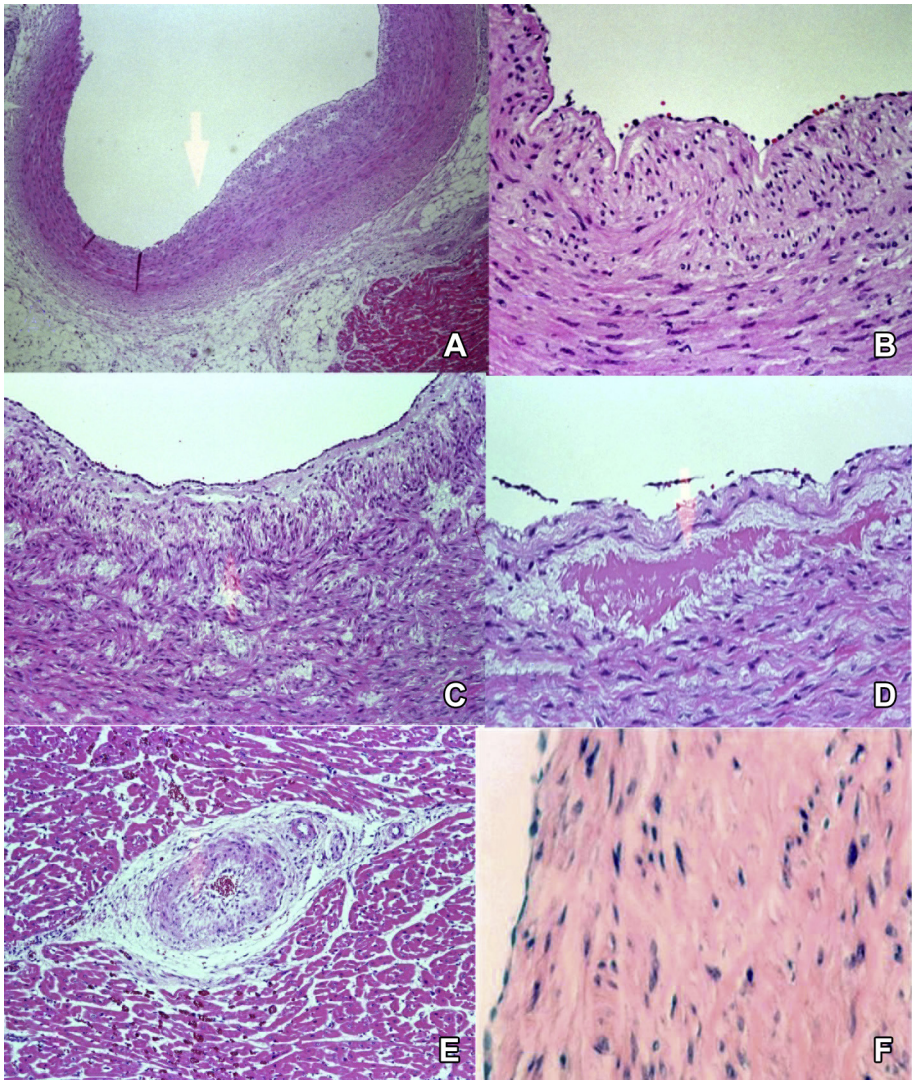

Figure 4 (A—F) H&E staining of arterial walls of piglets after HS infusions in Groups A and B (14—24 days). (A) Intima and inner SMC proliferation in RCA (200×) of piglet number 4 of Group B. (B) Severe disorientation of SMC in the tunica media of the LCA (400×) in piglet number 5 of Group A. (C) Edematous separation (moth-eaten appearance) of the left descending artery (200×) in piglet number 6 of Group A. (D) Collagen deposition at the subintimal area of the LCA (200×) in piglet number 1 of Group A. (E) Intimal and inner media thickening of the intramural artery (100×) (*) in piglet number 5 of Group A; (25—60 days). (F) H&E staining of the LCA arterial wall (400×) showing patchy areas of fibrosis. H&E = hematoxylin and eosin stain; HS = horse serum; LCA = left coronary artery; RCA = right coronary artery.

infiltrates, both neutrophils and lymphocytes, were noted in the myocardium, in the tunica media and peri vasa vasorum areas of the aorta, and also at the distal tubular areas of kidney, which rapidly resolved within 10 days of HS infusions (Figure 2E—H). The histopathology of the skin biopsy taken from the site of rashes showed perivasculitis. Histopathology of other organs and vessels showed no significant changes except for some congestive areas and increased lymphoid follicles in the spleen.

4. Discussion

The pathology of KD has been extensively studied. Although the pathogenesis of the lesions is not well understood, immunopathological mechanisms may play an important role in the genesis of vasculitis in KD (Table 1).[3—22] Circulating ICs in patients with early phase KD have been detected.[5,6] Onouchi et al[26] reported that HS-induced IC vasculitis in rabbits showed similar pathophysiology to CAL in KD. Swine has been used for the study of cardiovascular diseases.[27,28] They are large, omnivorous, and convenient for therapeutic trials.[31,32] The heart and vessels are easy to examine with 2D echocardiography. The CA system of swine is similar to that of humans, and it is applicable for interventional cardiology, cardiac xenotransplantation, and even heart lung transplantation.[33,34] We used piglets for the experimental study, weighing 20—28 kg, and which were equivalent to human age from 4 months to 1 year because more than 80% of the patients with KD are infants and children aged < 5 years.[29]

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The IC coronary vasculitis has been elicited by various agents in mice, guinea pigs, and weanling rabbits, with or without aneurysm of arterial walls.[23–26] The proteins present in the HS can induce acute serum sickness and vasculitis. The pathogenesis of vasculitis postulated is the fixation of compliments by ICs, activation of compliment cascade, and the release of biologically active fragments, notably the anaphylatoxins (C3a and C5a), which increase vascular permeability and yield chemotactic factors for polymorphonuclear leukocytes.[35] Tissue damage may also be mediated by free radicals, which are produced by activated neutrophils.

All the piglets receiving HS infusion in our study developed varying degrees of exanthemas, starting mostly from the perineal regions, and then spreading to the trunk, legs, ears, and mouth. The perianal appearance and spread of the rashes we observed were somewhat similar to those of KD described by Friter and Lucky.[36] Indurative edema and peeling of the skin were not observed in our study. Multiple infusions of HS may be better than a single dose because prolonged and continuous exposure to the sensitizing agent may lead to excess antigen and the formation of small to intermediate IC aggregates, which are not easily phagocytosed by the macrophages and circulate widely. These circulating ICs tend to get deposited in the walls of blood vessel. In areas where there is low exposure to the sensitizing antigen, larger IC aggregates are formed, which are easily phagocytosed by the macrophages.[35] There was no significant difference in 2D echocardiographic or histopathological changes between Groups A and B with two types of HS, but the initial reactions such as cyanosis, respiratory distress, convulsions, and shock were more severe with donor herd HS2, which could be due to the higher concentration of total proteins in HS2 when compared with HS1.

To the best of our knowledge, 2D echocardiographic studies on the normal CA diameter and its changes in weanling piglets have not been reported. We interpreted the CA dimension as abnormal when the increase was larger than 9–53% of the baseline diameter, which was observed in Group C and the control group. Our study showed that CA dilations started to occur 4–10 days after the first infusion of HS. All piglets in Group C were sacrificed prior to 5 days for studying the early changes, and therefore, no changes in CA were detected by 2D echocardiography. The echocardiography findings of CALs that were observed in the piglets of this study were similar to those observed in our clinical KD patients.[37]

The histopathological changes of coronary arteries that we induced in piglets by HS infusions were similar to the acute, subacute, and convalescent phases and to the four pathological stages related to the duration of illness of KD.[37–39] In all piglets, the changes were most significant in the tunica media. Similarly, the initial changes in the diameter of coronary arteries in KD occurred in the tunica media at about 7–9 days after the onset of the disease, as reported by Naoe.[40] The pathological stages of IC vasculitis induced by large doses of HS infusions in piglets were shortened to 0–4 days in Stage I, 5–14 days in Stage II, 15–24 days in Stage III, and >25 days in Stage IV when compared with the pathological staging in KD as 0–11 days in Stage I, 12–25 days in Stage II, and >30–40 days in Stages III and IV. Vasculitis changes in piglets were resolved from Day 14 onward. Cellular infiltrations such as mononuclear cells were fewer in the 5–13-day autopsy group. The time span may differ in the human pathology as the swine model would have a shorter course for each stage of vasculitis and the presence of cellular infiltrates and their composition would vary accordingly.[29] Hence, the presence of cellular infiltrations in each stage may change accordingly. The courses and the severity of vasculitis in piglets could be different from the natural course of KD, as it was induced by the large multiple doses of HS. Arteritis changes of varying degrees in piglets were noted more in the LCA than in the RCA, which were similar to the study in KD by Takahashi.[41]

Type III hypersensitivity reaction, induced by antigen–antibody complexes, activates a variety of serum mediators, mainly the compliment system. Both ICs and platelets may have some role to play in the pathogenesis of vasculitis.[14] ICs were identified in the autopsy specimens of KD suggesting that IC might have played a role in producing the coronary arteries changes in KD patients.[14] ICs were also identified in the circulation of the experimental rabbit models with serum sickness.[26] This study documented that systemic type III hypersensitivity reactions and histopathological changes in various stages produced in the HS group were similar to the rashes and histopathological changes of vasculitis in KD; and we suggest, therefore, that a similar mechanism may be involved in the pathogenesis of coronary arteritis in KD.

Type III hypersensitivity reaction in serum sickness is a prototype of IC vasculitis. Induction and reproducibility of IC vasculitis with two different types of HS were possible in piglets weighing 9–39 kg. The rashes and the findings of CALs detected by 2D echocardiography, and histopathological studies in the acute to healed phases of vasculitis showed close similarities to KD. We postulate that IC-mediated mechanisms may play a role in the pathogenesis of CALs in KD and that swine may serve as an experimental model for various therapeutic trials.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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