

Impact of corticosteroids on late growth of radiofrequency lesions in infant pigs: histopathological and electroanatomical findings

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Aims

Corticosteroids attenuate late growth of radiofrequency (RF) lesions in the thigh muscle of infant rats. We sought to assess the impact of these drugs on the late growth of RF lesions in immature swine myocardium and to determine the electroanatomical mapping (EAM) characteristics of these lesions.

Methods and results

Radiofrequency (60°C; 60 s) lesions were created in the right atrium ($n = 2$) and ventricle ($n = 2$) of 14 piglets (age 65 days; weight 5 kg) and 3 adults. Piglets were divided into: controls ($n = 7$) and treated ($n = 7$), receiving hydrocortisone (10 mg/kg iv after RF) and prednisone (1 mg/kg/day) for 29 days. After 8 months, animals were sacrificed for histological analysis. In four piglets, endocardial and epicardial voltage EAM were performed.

In infant groups, the dimensions of atrial (11 ± 5 vs. 13 ± 7 mm) and ventricular (12 ± 3 vs. 11 ± 3 mm) lesions were similar. In adults, atrial (6 ± 1 mm) and ventricular (6 ± 1 mm) lesions were smaller. In controls, ventricular lesions depicted dense fibrosis and multiple strands of fibrous tissue extending from the lesion into normal muscle. Treated piglets revealed scars exhibiting less dense fibrosis with predominance of fibroadipose tissue and less collagen proliferation. Large atrial and ventricular low-voltage areas corresponding to the macroscopic lesions were identified in all animals.

Conclusion

Radiofrequency lesions in infant pigs reveal late growth and invasion of normal muscle by intense collagen proliferation. Corticosteroids do not prevent late enlargement of the lesions but modulate the fibrotic proliferation. The expressive growth of the lesion may generate low-voltage areas detectable by EAM.

Keywords

Arrhythmias • Radiofrequency ablation • Electroanatomical mapping • Corticosteroids

Introduction

Since its introduction, catheter ablation with radiofrequency (RF) energy has proved to be safe and effective and is currently used routinely, also for the paediatric population, for the treatment of cardiac tachyarrhythmias.^{1–3} However, the effects of RF ablation on the myocardium are not limited to the time of energy application but may occur hours to months after the procedure.^{4,5} The real reason for this behaviour is unknown, but it has been

postulated that these late effects may result from the growth of the lesion during healing due to progression of the inflammatory response, to microcirculatory injury or to ultrastructural damage to surrounding tissue.⁵

These findings seem to be more marked in the immature myocardium. In a study of the behaviour of RF lesions produced in the ventricular myocardium of young lambs, Saul *et al.*⁶ demonstrated a marked growth of the lesions after 8.5 months of follow-up, associated with the invasion of normal myocardium by fibrotic and elastic

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tissue. This suggested that late pro-arrhythmic effects may occur in children subjected to RF, also leading to sudden death.⁷ Since the inflammatory process seems to contribute significantly to the late extension of the lesion,⁵ we formulated the hypothesis that corticosteroids may be effective in preventing this occurrence.⁸ On the basis of this hypothesis, we recently demonstrated that corticosteroids attenuate the late growth of RF lesions in the thigh muscle of infant and pubescent rats.^{9,10} However, that study was conducted in skeletal muscles and needed to be validated for the myocardium.

Thus, the objective of the present study was to assess the impact of corticosteroids on the late growth (8 months) of RF lesions in the immature atrial and ventricular myocardium of pigs, with emphasis on macroscopic and histopathological aspects. In addition, since electroanatomical mapping (EAM) is being increasingly used for the definition of atrial and ventricular substrates in various diseases,^{11,12} we mapped the substrate in a subgroup of animals to determine the electroanatomical characteristics of these lesions.

Methods

The study was approved by the Research Ethics Committee of the Federal University of São Paulo and was conducted according to institutional norms.

Experimental design

Sample

Seventeen pigs of the minipig line were studied: 14 recently weaned piglets aged 65 ± 24 days and weighing 5.2 ± 0.71 kg and 3 adults aged 8 months and weighing 40 ± 5 kg. The age ranges of the minipiglets and of adult animals correspond to approximately those of human infants and young adults, respectively.

Preparation of the experiment

The pigs were medicated with 1.0% acepromazine (1.0 mg/kg/im) and 5 mg/mL midazolam (1.0 mg/kg/im) and anesthetized 15 min later with 3.0 mg/kg propofol iv in bolus followed by 12–14 mg/kg/h for maintenance. The animals were kept under spontaneous ventilation while receiving 1–2 L/min oxygen through a nasal catheter under electrocardiographic monitoring and pulse oximetry. Body temperature was kept at $\sim 37^\circ\text{C}$ with the use of a thermal mattress. After trichotomy and antisepsis, an indifferent electrode plate was placed on the left dorsal region of the minipigs, and the external jugular vein was dissected and catheterized under sterile surgical conditions.

Ablation protocol

A standard 6 French quadripolar ablation catheter with a deflectable tip and a 4 mm distal electrode (Biosense Webster, Diamond Bar, CA, USA) were introduced and guided by fluoroscopic vision up to the right atrium and right ventricle (RV), respectively (Figure 1). The local electrograms and the electrocardiogram were filtered (30–500 Hz) with a TEB SP32 polygraph

(Tecnologia Eletrônica Brasileira, São Paulo, Brazil). Using the RF generator (RF-100 TEB), four lesions, two atrial and two ventricular ones, were created (60°C , 50 W; 60 s) under temperature control in each animal at distinct anatomical sites: right atrium (appendage and upper lateral wall) and RV (apex and free wall). During each application, the power (in W), impedance (in Ω), and temperature of the catheter tip were monitored, and the mean value was recorded for analysis. At the end of RF applications, the ablation catheter was removed, the vessel tied, and the wound sutured. The procedures were performed by the same operator under sterile conditions, with no need for prophylactic antibiotics.

Animal groups

The piglets were divided into two groups of seven animals each, one of them receiving no drugs (control) and the other receiving hydrocortisone (10 mg/kg iv) immediately after ablation and prednisone (1 mg/kg po) for 29 days. The prednisone dose was adjusted weekly according to weight gain. Three adult animals receiving no drugs were also studied. The piglets were followed up for 8 months and the adults for 60 days under veterinary care.

Macroscopic analysis

At the end of follow-up, the animals were weighed for the assessment of weight gain, re-anesthetized as described above, and sacrificed with a 19.1% iv KCl bolus for heart removal. The epicardial surface of the heart thoroughly inspected for the presence of lesions, and the heart was opened for the localization of RF scars in the cardiac chambers according to the applications. Other characteristics of the lesion such as geometry and transmural-ity were also analysed. The lesions were dissected and their largest endocardial and epicardial dimensions (width and length) were measured with a millimetre ruler.⁸ In infant animals, but not in adults, all atrial and ventricular lesions were transmural. Thus, the depth of the lesions was evaluated in adults only.

Histological analysis

The lesion was cut into various histological sections throughout its extension. The slides were stained with haematoxylin–eosin and Masson trichrome for the evaluation of connective tissue and analysed qualitatively by a pathologist (S.A.) who was blind to the study groups. By definition, the late growth of the lesion was characterized by an increase in RF lesions compared with adults, whereas the late extension of the lesion was defined as the occurrence of multiple extensions of fibroelastic tissue which, starting from the central fibrotic area, invaded muscle tissue and surrounded the chronic RF lesions.^{9,10}

Electroanatomical mapping

At the end of follow-up, voltage mapping with the EnSite NavX 8.01 system (St. Jude Medical, St. Paul, MN, USA) was performed only in the last four piglets for the assessment of RF lesions, since the equipment was not available at the beginning of the protocol. The animals were anesthetized with propofol iv, intubated, and mechanically ventilated. The right femoral vein and the right external jugular vein were dissected and cannulated. A quadripolar catheter was positioned in the coronary sinus as the anatomical

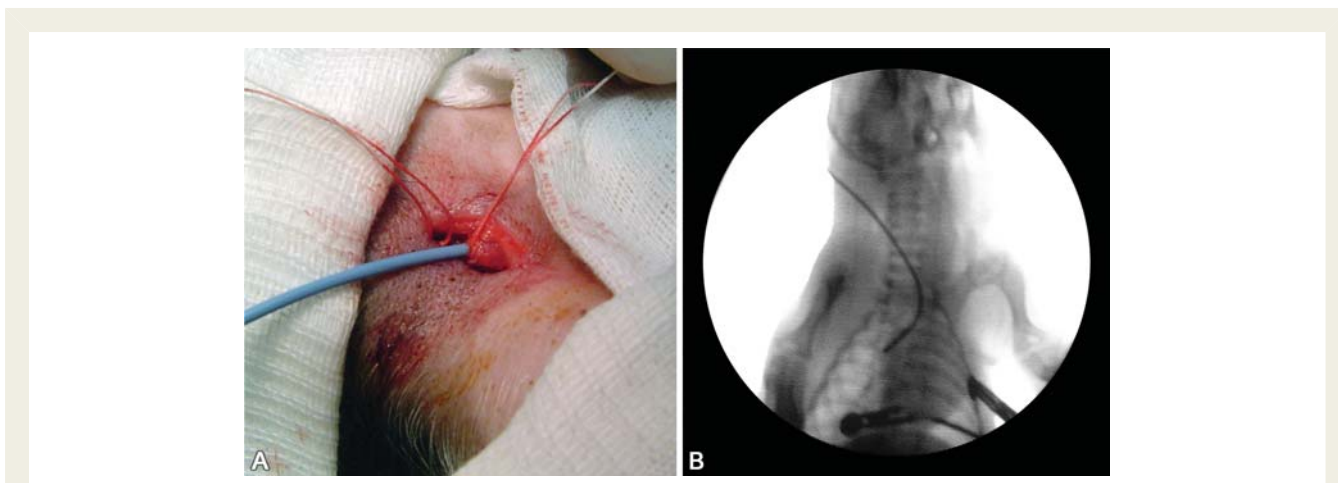


Figure 1 The ablation procedure is illustrated. After trichotomy and antisepsis, the external jugular vein was dissected, and the 6 French ablation catheter was introduced under fluoroscopy guidance (A). Antero-posterior view showing the catheter positioned in the right atrium (B). Note the small size of the animal.

reference electrode, impedance was calibrated, and the respiratory movements were compensated in a constant manner. The three-dimensional geometry of the cavities and the voltage maps were obtained in sinus rhythm by the same operator using the distal electrode pair of the 7 French deflectable ablation catheter with a 4 mm tip. The atrial and RV endocardial surface was first mapped, followed by epicardial mapping of the respective cardiac chambers by means of a subxiphoid puncture of the pericardial space.¹³ After the acquisition of geometry, the voltage map was obtained by catheter navigation in the virtual cavity. At least 200 points with interpolation programmed for 15 mm were used for map construction. Areas with bipolar electrogram amplitude >1.5 V (purple colour) were considered to be normal tissue, areas with bipolar amplitude ≤ 0.5 V (grey) were considered to be a dense scar, and areas with bipolar amplitude between 0.5 V and 1.5 V were considered to be the margins of the scars.^{12,13} After the EAM was obtained, the animals were sacrificed and their hearts removed for analysis as described above.

Statistical analysis

Data are reported as means \pm SD. The continuous variables were analysed by analysis of variance or by the Student *t*-test, and the categorical variables were compared by the χ^2 test or Fisher exact test, with the level of significance set at $P < 0.05$.

Results

Radiofrequency ablation

Four RF lesions (two in the right atrium and two in the RV) were created in each animal. As shown in Table 1, there were no differences between groups regarding the biophysical parameters evaluated. Pigs are highly susceptible to the induction of ventricular fibrillation (VF) by RF. A total of 25 episodes of VF occurred during the applications of RF to the RV in 16 of the 17 animals (6 control piglets, 7 treated piglets, and 3 adults; $P = \text{NS}$). Mean

time to the beginning of VF was 12 ± 4.9 s after the beginning of the applications. In all cases, the 60 s of application were completed before electrical defibrillation.¹⁴ All animals recovered well.

Follow-up

All pigs evolved well throughout the experiment. From the beginning of the procedure to sacrifice, control piglets (5.14 ± 0.88 vs. 46.35 ± 10 kg; $P < 0.05$) and treated animals (5.25 ± 0.55 vs. 39.92 ± 4.6 kg; $P < 0.05$) gained weight in an equal manner (Table 1). The adults also gained weight (27.83 ± 0.76 vs. 54.33 ± 2 kg; $P < 0.05$), but, whereas the weight gain of piglets was predominantly due to the increase in animal length and body mass, the weight gain of adults was the consequence of fattening.

Macroscopic analysis

Of the 56 lesions created in the 14 piglets, 51 (94%) were detected (24 of them ventricular and 27 atrial) and included in the analysis. The prevalence of identified lesions did not differ between groups (Table 1). Of the 12 lesions created in adults, 7 (58%) were identified (4 of them ventricular and 3 atrial). Macroscopically, the lesions of the piglets were large, whitish, and with well-defined margins and contours. All atrial and ventricular lesions were transmural. The atrial lesions were similar in their endocardial and epicardial aspects, but the ventricular ones were more extensive on their epicardial surface, located in regions with no epicardial fat (Figure 2A). As shown in Table 1, the dimensions of atrial and ventricular lesions did not differ between the control and treated groups. Macroscopically, the characteristics of the atrial and ventricular lesions of the adults were similar to those of the piglets, but their dimensions were notably smaller, although only for ventricular lesions this difference reached statistical significance. The three atrial lesions, as well as the three lesions of the RV apex, were transmural, but not the two lesions of the free RV wall, which had a depth of 2 mm.

Table 1 Biometric data of the pigs, biophysical parameters of the applications and dimensions of the radiofrequency lesions

Pigs	Group	Weight (kg)		Follow-up (days)	RF ablation		Biophysical parameters			Complications	Lesions found, <i>n</i>	Epicardial dimensions (mm)		Endocardial dimensions (mm)	
		Initial	Final		Chambers	Lesions, <i>n</i>	Power (W)	Impedance (Ω)	Temperature ($^{\circ}\text{C}$)			Length	Width	Length	Width
1	CP	4.2	33	197	A	2	29/47	85/85	51/59		2	05/07	05/04	05/07	05/04
					V	2	20/20	86/85	60/60	VF/VF	0				
2	CP	6.5	34	197	A	2	20/27	94/100	60/60		2	07/13	04/05	05/10	04/05
					V	2	16/6	90/105	60/60	VF	2	13/10	07/08	10/07	07/05
3	CP	4.5	58	242	A	2	18/15	93/86	60/60		2	15/05	06/07	15/06	06/07
					V	2	10/09	99/102	60/60	VF/VF	2	15/04	05/04	11/04	05/06
4	CP	6.0	59	242	A	2	45/49	85/90	59/55		2	08/05	08/04	08/05	08/05
					V	2	50/10	87/99	60/60	VF	2	15/11	15/04	10	05
5	CP	4.3	52	242	A	2	33/13	89/90	59/61		2	20/15	10/10	20/11	10/10
					V	1	05	98	60	VF	1	18	18	12	07
6	CP	5.5	45	293	A	2	14/09	140/150	60/60		2	20/10	20/10	20	20
					V	2	06/02	138/158	60/59	VF	2	12/10	07/10	10/07	07/05
7	CP	5.0	43	293	A	2	30/12	125/123	60/60		2	15/12	10/10	15/12	10/10
					V	2	15/09	124/141	60/60		2	12/10	10/10	08/10	08/05
Total		5.14 \pm 0.8	46.35 \pm 10*	243.7 \pm 39.2	A	14	27.71 \pm 14	102.5 \pm 22	58.78 \pm 2	0	14	11.21 \pm 5.29	8.07 \pm 4.25	9.91 \pm 4.85	8 \pm 4.32
					V	14	16.23 \pm 12	107.61 \pm 24	59.92 \pm 0.2	8	11	11.81 \pm 3.62	8.90 \pm 4.41	8.9 \pm 2.37	6 \pm 1.15
1	TP	5.5	42	236	A	2	25/46	83/90	60/56		2	20/11	13/05	18/10	14/05
					V	2	25/49	87/85	60/57	VF/VF	2	10/07	07/07	09	06/04
2	TP	5.5	48	236	A	2	30/29	96/86	60/59		2	25/07	12/07	22/07	12/07
					V	2	15/04	97/101	58/60	VF/VF	2	17/07	13/07	12	07
3	TP	5.5	41	236	A	2	05/44	99/97	58/60		2	25/15	07/05	25/15	05/05
					V	2	05/13	109/96	60/61	VF	2	12/12	12/12	10/08	10/08
4	TP	6.0	36	236	A	2	40/41	97/84	60/61		2	20/15	05/05	15/05	07/05
					V	2	10/04	94/105	59/60	VF/VF	1	14	14	10	05
5	TP	4.3	40	236	A	2	49/41	89/81	45/58		1	08	04	08	05
					V	2	05/49	97/86	61/55	VF/VF	2	20/12	20/12	15/10	05/05

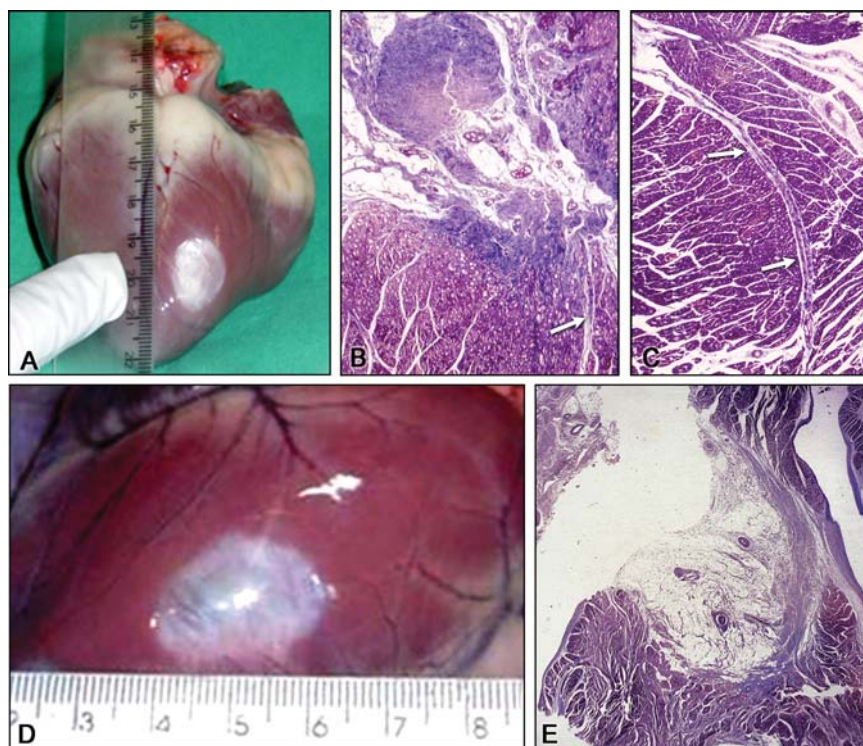


Figure 2 Gross pathological and histological findings of right ventricular ablation lesions in controls (A–C) and treated (D and E) pigs. Macroscopically, the lesions were large, whitish, with well-defined margins and contours, transmural, and more extensive on their epicardial surface. Lesion dimensions did not differ between control (A) and treated (D) animals. Histological analysis of controls depicted extensive lesions with dense fibrosis and also fibroadipose tissue (B) in addition to multiple strands of fibrous tissue (arrows) extending from the lesion into normal muscle (C). In treated piglets, scars were also extensive but exhibited less dense fibrosis with predominance of fibroadipose tissue and less collagen proliferation (E). Slides stained with Masson's trichrome. Magnification: B and E, $\times 20$; C, $\times 40$.

As observed in the immature lamb myocardium,⁶ in the present study, the atrial lesions presented late growth but not late extension, being histologically well delimited, whereas the ventricular lesions presented both late growth and extension. The increased size of RF lesions in the immature myocardium may be due to passive distention secondary to muscle growth, as is the case for atriotomy scars created in infant dogs,¹⁵ and to cell proliferation of the constituents of the lesion, responsible for the invasion of surrounding tissue by fibrous trabeculae (late extension). In contrast to adult muscle, in this phase, the muscle and interstitial cells are still actively dividing.^{15,16} Our findings suggest that passive growth prevails in atrial lesions, whereas both cell proliferation and passive distention participate in ventricular lesions.

We recently demonstrated qualitatively and quantitatively that RF lesions in the thigh muscle of infant and pubescent rats (corresponding to the beginning of puberty) showed late growth and extension, more marked in the infants, and that the corticosteroids reduced these phenomena.^{9,10} In the present study, the corticosteroids were unable to reduce the macroscopic dimensions of the lesions. These results cannot be attributed to individual variations or to discrepancies in the ablation procedure. The biophysical parameters of the RF applications and the weight gain of the animal during follow-up did not differ between treated animals and controls. Although the volume of the lesions was not

determined, it is unlikely that significant differences in lesion size were not perceived with the methods used in the present study, mainly because the dimension and geometry of the lesions were highly reproducible between groups. The reasons for the discrepant effects of corticosteroids on lesion growth are uncertain, but may be related to different experimental models (rat skeletal muscle vs. porcine myocardium), or to a modification of lesion healing induced by the drug. The reduction of the density of fibrosis and the increase in fibroadipose tissue may have favoured the passive distention of the scar during animal development, partially eliminating the scar retraction secondary to collagen maturation. Supporting this hypothesis, the healing delay caused by the corticosteroids favours the formation of ventricular aneurysms after myocardial infarction.¹⁷

However, in agreement with our observations on the thigh muscle of rats,^{9,10} the corticosteroids attenuated the late extension of the lesions. The histopathological findings were quite consistent in all animals, with better delimited scars with smaller number of fibrous trabeculae and a smaller extension compared with control. The present study did not permit us to identify the mechanisms by which corticosteroids reduce the late extension of RF lesions in the immature myocardium, which is induced by the cell proliferation of the constituents of the lesions. However, these actions may be associated with the complex effects of

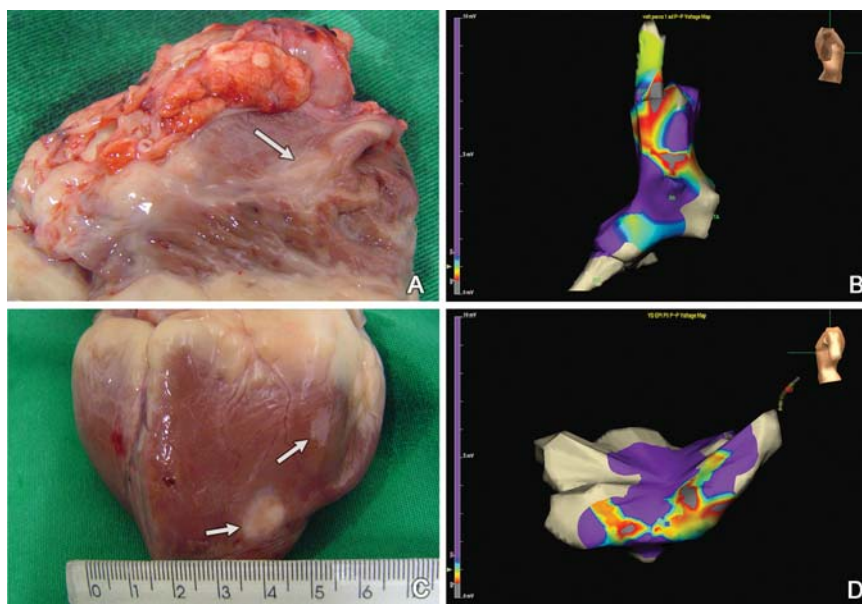


Figure 3 Gross pathological and electroanatomical mapping findings of right atrial (A and B) and right ventricular (C and D) ablation lesions in infant pigs. Right lateral view of the endocardial voltage map of the right atrium (B) showing two low-voltage areas (grey) in the lateral wall, corresponding to radiofrequency lesions (arrows) noted on macroscopic analysis (A). Left lateral view of the epicardial voltage map of the right ventricle (D) showing two low-voltage areas (grey), one in the right ventricular basal lateral free wall and the other at the right ventricular apex, corresponding to the lesions (arrows) found on macroscopic inspection (C). Note that there is no fat close to the epicardial lesions. The geometry shell is colour-coded light grey. Voltages exceeding 1.5 mV are colour-coded purple. IVC, inferior vena cava; RA, right atrium; REF, reference catheter positioned in the coronary sinus; SVC, superior vena cava; TA, tricuspid annulus.

these drugs on the healing process.^{18–20} In addition to delaying healing secondary to the reduced collagen synthesis by the fibroblasts, the corticosteroids inhibit the leukocytes and reduce the release of cytokines and of cell growth factors.¹⁹

In this study, we show for the first time that the late growth of RF lesions in the immature myocardium produces low-voltage areas in the EAM, especially in the epicardium. Despite the limited series ($n = 4$) of animals subjected to substrate mapping, the findings were quite consistent, with a very good correlation between the voltage maps and the atrial and ventricular lesions identified by pathological analysis. It is noteworthy that the EAM systems have proved to be reliable and precise in the definition of myocardial substrates localized in the right atrium¹¹ and in the RV,¹² as was the case for the lesions of the present study.

Clinical implications

The present findings suggest that corticosteroids reduce the late extension of RF lesions in the immature myocardium. If confirmed in other studies, these observations indicate the possibility of limiting the late extension of lesions by means of pharmacological interventions, possibly being useful for the ablation of infants and small children in whom the growth of the lesion may lead to pro-arrhythmic effects.^{5–7} In contrast, chronic RF lesions in adult myocardium have not been associated with pro-arrhythmias because they are characterized by small, well-defined scars with regular borders and invasion of adjacent normal muscle by fibrotic proliferation does not occur.⁸ The fact that the lesions of treated animals were more

homogeneous and had a lesser intensity of fibrous trabeculae compared with controls may suggest a lower propensity to the formation of an arrhythmogenic substrate. On the other hand, the reduction of the density of fibrosis and the marked increase in fibroadipose tissue in the scar of treated animals, resembling the arrhythmogenic dysplasia of the RV in some aspects,²¹ may be pro-arrhythmic. The functional significance of these lesions definitely needs to be evaluated. Finally, the expressive late growth of the lesions detected by pathological examination and in the low-voltage areas in the EAM supports the recommendation of performing RF ablation in small children only when absolutely necessary and with extreme caution,⁷ always avoiding multiple applications.

Limitations

The study was conducted on normal porcine myocardium, and therefore, the results cannot be directly extrapolated to children with arrhythmias. The corticosteroid dose was arbitrary and the effective dose was not determined. Acute lesions were not studied and the adults were followed up for a shorter period of time (2 months) than the piglets (8 months). However, previous studies have demonstrated that acute lesions are similar in infants and adults and that the latter do not show late growth.^{6,8–10} The ventricular lesions were created only in the RV, all of them being transmural. However, in the immature sheep myocardium,⁶ non-transmural left ventricle lesions also showed late growth. Since programmed electrical stimulation was not performed in the animals submitted to EAM, the pro-arrhythmic potential of these lesions remains obscure.

Conclusions

Radiofrequency lesions in the immature porcine myocardium reveal late growth and invasion of normal muscle by intense collagen proliferation. Corticosteroids do not prevent late growth of the lesions but modulate fibrotic proliferation. The expressive growth of the lesion may generate low-voltage areas detectable by EAM. These findings may have implications for paediatric ablation.

Conflict of interest: none declared.

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