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Distribution and Incidence of Calcified Lesions in DBA/2NCrj and BALB/cAnNCrj Mice

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ABSTRACT. DBA/2NCrj and BALB/c AnNCrj mice 4, 8 and 35 weeks of age were examined for calcified lesions. DBA/2NCrj mice showed high incidences of grossly detectable cardiac calcification and focal aortic calcification with arteriosclerotic changes within 8 weeks of age. The incidences in BALB/cAnNCrj mice were low and not age-related. DBA/2NCrj mice can be a useful model for cardiovascular calcification.—**KEY WORDS:** calcification, DBA/2NCrj mouse.

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DBA [1, 3, 5, 7–9] and BALB/c mice [2, 5, 6] show spontaneous cardiac calcification and the incidence and severity of the lesion vary among their substrains [4, 9]. Brownstein [3] reported that cardiac calcification observed in DBA/2 mice had only limited value as a genetic marker because of its polygenic recessive inheritance but it might be useful as model of spontaneous mineralization.

When evaluating the usefulness of DBA or BALB/c mice as a model animal of soft tissue mineralization, it is necessary to examine the systemic distribution and incidence of mineralized lesions at various ages. However, there have been only a few papers describing the systemic histopathology of DBA and BALB/c mice [1, 5, 9]. This paper deals with the distribution, incidence and histopathologic nature of calcified lesions in DBA/2NCrj and BALB/cAnNCrj mice at 3 different ages.

Four-, 8- and 35-week-old DBA/2NCrj (4-week-old; 30 males and 21 females, 8-week-old; 30 males and 21 females, 35-week-old; 12 males and 10 females) and BALB/cAnNCrj mice (4-week-old; 11 males and 11

females, 8-week-old; 29 males and 18 females, 35-week-old; 10 males and 10 females) were obtained from Charles River Japan Inc., Kanagawa, and examined histopathologically. They were fed CRF-1 pellets (Charles River Japan Inc.) and water *ad libitum*.

Mice were sacrificed by exsanguination under ether anesthesia. Tissue blocks were collected from each mice and fixed in 10% buffered neutral formol solution. Paraffin sections were stained with hematoxylin and eosin. Some of them were also stained by Gomori's one step trichrome technique or by von Kossa's procedure.

Gross lesions were found in the heart and tongue. In the heart, white patchy or plate-like lesion was detected at the right ventricular surface. As shown in Fig. 1, the incidence in BALB/cAnNCrj mice was lower than 20% at any weeks of age, and this is similar to that reported in other BALB/c substrain mice [2, 5, 6] excepting female BALB/cCr mice [2]. On the other hand, the incidence in DBA/2NCrj mice reached more than 80% at 8 weeks of age, and this is significantly higher than that in other DBA

Fig. 1. Incidence of macroscopic cardiac calcification in DBA/2NCrj and BALB/cAnNCrj mice.

Fig. 2. Incidence of microscopic calcification in heart and aorta of DBA/2NCrj and BALB/cAnNCrj mice.

Fig. 3. Heart of 8-week-old male DBA/2NCrj mouse. Massive calcification with organization in myocardium under right ventricular epicardium. H-E. $\times 150$.

substrain mice of similar age [1, 4, 5, 7–9].

Macroscopic tongue lesions were observed only in DBA/2NCrj mice. Small white polypoid lesions occurred uni- or bilaterally on the dorsolateral surface of the tongue with the incidence of 40 to 90% (12/30 males and 9/21 females at 8 weeks, and 8/12 males and 9/10 females at 35 weeks of age). Such tongue lesions were first presented in DBA mice by Matsushima *et al.* [10] in 1984 and they described that DBA/2NCrj mice showed the highest incidence among mice of 5 different DBA substrains.

Microscopically, calcification appeared in the heart and aorta of mice of both strains and in the tongue of DBA/2NCrj mice at 4 and 8 weeks of age. The incidence of cardiovascular involvement was significantly higher in DBA/2NCrj mice than BALB/cAnNCrj mice (Fig. 2). Microscopic tongue lesions occurred with almost the same incidence as that of microscopic ones. In addition to these tissues, the femoral muscles (9/12 males and 1/10 females), arterioles in the testicles (5/12) and kidneys (2/10 females), uterine muscles (4/10) and lungs (5/10 males and 10/10 females) were affected by calcification in 35-week-old DBA/2NCrj mice.

In DBA/2NCrj mice, small foci of myocardial calcification were sporadically observed in the right and left ventricular walls and

Fig. 4. Aorta of 8-week-old female DBA/2NCrj mouse. Intimal thickening with calcified and destructed internal elastic membrane. H-E. $\times 200$.

interventricular septum at 4 weeks of age. At 8 and 35 weeks of age, coincided with macroscopic findings, massive calcification with subsequent organization (Fig. 3) was seen in the myocardial layer beneath the right ventricular epicardium, which was also involved in the lesions. In BALB/cAnNCrj mice, slight to moderate calcification was detected in the right ventricular epicardium and/or subepicardial myocardium irrespective of the age.

Aortic changes were somewhat more frequently found near the arterial bifurcation and characterized by focal calcification in the elastic membranes and smooth muscle cells. Moreover, such arteriosclerotic changes as arterial wall thickening due to increase in interstitial matrix or smooth muscle cell proliferation (Fig. 4) were often noted around the calcified and disrupted internal elastic membrane, which have never been pointed up in the previous reports. The incidence of these aortic lesions is apparently higher in DBA/2NCrj mice through 4 to 35 weeks of age (66 to 81%) in comparison with that in other DBA substrain mice (0 to 0.2%) [1, 5, 9].

Although it is not clear what is true cause of such substrain-difference in the incidence of cardiovascular calcification, polygenic nature of inheritance [3, 5] seems to play more important role than environmental factors such as diet [1, 5].

As to the tongue lesions, grossly visible polypoid lesions were microscopically composed of calcified lingual muscles encirculat-

Fig. 5. Tongue of 8-week-old male DBA/2NCrj mouse. Calcified lingual muscles surrounded by inflammatory cells. H-E. $\times 150$.

Fig. 6. Lung of 8-week-old female DBA/2NCrj mouse. A small calcific fleck in an alveolar septum. H-E. $\times 300$.

ed with inflammatory cells and connective tissues (Fig. 5). In the kidneys, calcification affected the arteriolar wall in DBA/2NCrj mice instead of the uriniferous tube in other DBA substrain mice [1, 5, 9]. Furthermore, pulmonary calcification was observed as a small fleck probably formed within a capillary of an alveolar wall (Fig. 6). This lesion was first pointed out in DBA mice in the present experiment and its microscopic feature was the same as that reported by Eaton *et al.* [5] in a small number of C3H mice.

From the above-mentioned findings, DBA/2NCrj mice are considered to be a useful model of spontaneous soft tissue mineralization, especially of cardiovascular calcification. Further studies on the pathogenesis of cardiovascular lesions in DBA/2NCrj mice are now in progress.

要 約

DBA/2NCrj および BALB/cAnNCrj マウスの石灰沈着病変の分布と出現頻度(短報): 土井邦雄・前田尚之・土居千代・伊勢川直久・菅野 茂・光岡知足(東京大学農学部)——4週齢, 8週齢, 35週齢の DBA/2NCrj および BALB/cAnNCrj マウスの石灰沈着病変を病理学的に検索した。DBA/2NCrj マウスでは, 8週齢までに極めて高率に心筋石灰沈着病変および動脈硬化性変化を伴う大動脈石灰沈着病変が認められ, 心血管石灰沈着症のモデルとして有用と考えられた。

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