Stability of elastin in the developing mouse aorta: a quantitative radioautographic study

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Abstract. Elastic lamina growth during development and the ultimate stability of elastin in the mouse aortic media was investigated by light and electron microscopic radioautography. Following a single subcutaneous injection of L-[3,4-3H]valine at 3 days of age, animals were killed at 9 subsequent time intervals up to 4 months of age. One day after injection, radioautographic silver grains were primarily observed over the elastic laminae; however, silver grains were also seen over the smooth muscle cells and extracellular matrix. By 21 to 28 days of age, the silver grains were almost exclusively located over the elastic laminae. From 28 days to 4 months of age, the distribution of silver grains appeared relatively unchanged. Quantitation of silver grain number/µm² of elastin showed a steady decrease in the concentration of silver grains associated with the elastic laminae from 4 to 21 days of age. After this time, no significant difference in silver grain concentration was observed. Since the initial decrease in grains/µm² of elastin corresponds to a period of rapid post-natal growth, the decrease is likely to be a result of dilution of the radiolabel due to new elastin synthesis. With the assumption that little or no significant turnover occurs during this time, a constant growth rate of 4.3% per day was predicted by linear regression analysis. Since no significant difference in the concentration of silver grains was observed from 28 to 118 days of age, no new growth or turnover of elastin can be said to occur during this time period. This is supported by the observation that animals injected with radiolabeled valine at 28 days and 8 months of age showed no significant incorporation of radiolabel into the elastic laminae. The results from this study present the first long-term radioautographic evidence of the stability of aortic elastin and emphasize that initial deposition of elastin and proper assembly of elastic laminae is a critical event in vessel development.

Introduction

Elastin accumulates rapidly in the aortic wall during perinatal development in response to changing hemodynamic factors (Bendeck and Langille 1991). In the rat aorta, elastic fibers are first observed at a gestational age of 13 days (Nakamura 1988). These fibers continue to develop until complete elastic laminae are formed at a post-natal age of approximately 4 weeks (Gerrity and Cliff 1975). Similarly, a rapid synthesis of elastin during early development has been reported for human (Berry et al. 1972), pig (Davidson et al. 1986), chick (Lee et al. 1976; Keeley 1979), quail (Lefevre and Rucker 1980) and rat aortae (Looker and Berry 1972). Following maturation, however, little new elastin synthesis is apparent (Davidson et al. 1986; Keeley 1979; Lefevre and Rucker 1980). These results imply that little or no turnover of elastin occurs in the mature vessel. Thus, elastin synthesized early in development must remain intact and functional throughout adult life.

Most previous estimates of elastin turnover in the aorta have been based on measurements of the specific activity of radiolabeled elastin isolated from vessels at various times following injection. The investigations have produced conflicting reports of turnover times that range from several weeks (Fischer 1971; Fischer and Swain 1978) to several years (Slack 1954; Walford et al. 1964; Lefevre and Rucker 1980; Rucker and Tinker 1977; Dubick et al. 1981). The discrepancy in turnover times appears to be due, in large part, to contamination of elastin fractions with collagen. Other difficulties encountered in an accurate prediction of elastin turnover have been due to inappropriate labeling techniques and inadequate experimental time periods.

The present study was designed to investigate the turnover of elastin in the aorta by radioautography and thus circumvent the problems encountered by biochemical methods. Using light and electron microscopic radioautography, Ross and Klebanoff (1971) demonstrated the uptake of 3H-labeled proline into aortic smooth muscle cells and subsequent incorporation and deposition...
of the radiolabel in the elastic laminae of prepubertal rats. Similar radioautographic results were obtained by Gerrity and colleagues (1975); however, since both studies were primarily concerned with the synthesis of connective tissue proteins, the longest time points examined were in the order of hours and the fate of the radiolabel was not determined. In theory, radiolabeled amino acids that become incorporated into the elastic laminae during development should remain evident throughout life if little or no turnover of elastin occurs. Based on this premise, aortae were investigated from animals injected early in development and killed at subsequent time points up to 4 months of age. The radioautographic observations presented in this report provide both qualitative and quantitative evidence for the long-term stability of elastin in the aorta.

Materials and methods

Experimental design

Two experimental groups were designed to investigate elastin kinetics in the mouse aorta (Fig. 1). The first experimental group consisted of 18 C57/BL mice given a single injection of radiolabel at 3 days post-natal age. Two mice were subsequently killed at 4, 7, 10, 14, 21, 28, 54, 84, and 118 days post-natal age. This experimental design allowed the fate of the radiolabel incorporated into the elastic laminae to be followed over the course of development. The second experimental group consisted of 20 C57/BL mice; 4 mice at each of the following post-natal ages: 3 days, 14 days, 21 days, 28 days, and 8 months. Each animal received a single injection of radiolabel. Two mice were subsequently killed from each age-group 1 day after injection. The remaining two mice from the first four age-groups were killed at 4 months of age and the two mice from the last age group were killed at 12 months of age. This experimental design allowed the synthesis and deposition of elastin to be investigated at different developmental ages. As a control, two additional animals were injected at 3 days post-natal age with buffer containing no radiolabel. One mouse was killed at age 4 days and the other at age 4 months.

Preparation and administration of radiolabel

All radiolabeling experiments were performed using L-[^3,4-^3H]valine in 0.01 N hydrochloric acid purchased at a concentration of 1 mCi/ml (NEN Research Products, Du Pont, Mississauga, Ontario, Canada). Immediately prior to use, radiolabel samples were concentrated and buffered with 1.5 M sodium chloride and 0.5 M phosphate buffer (pH 7.5). Each mouse, at the appropriate age for injection, was individually weighed and given a single subcutaneous injection on the back with radiolabel at a level of 50 μCi/gm body weight.

Preparation of tissue for radioautography

Tissues prepared for radioautography were embedded in Epon as previously described (Davis 1993). Briefly, mice aortae were fixed by cardiac perfusion with 3% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) and post-fixed sequentially in 1% osmium tetroxide, 2% tannic acid, and 2% uranyl acetate prior to methanolic dehydration and Epon infiltration. Care was taken to ensure that both cross sections and longitudinal sections were prepared from each aorta. Tissue from the two different animals killed at one time point were kept separate.

Light microscope radioautography

Four blocks from each animal were selected for radioautography based on the quality of the tissue segment and plane of section: two cross sections and two longitudinal sections. From each block selected, sections 0.5 μm thick were cut with a diamond knife on a Reichert ultracut microtome and placed in a single row on untreated glass slides. Light microscope radioautographs were prepared as previously described (Kopriwa and Leblond 1962). Briefly, slides were heated to 85° C and the sections subsequently stained with iron alum solution followed by hematoxylin for 7 min each. To enhance the blue tones of the stain, the slides were covered with tap water for 3 min. Slides were coated with Kodak NTB-2 emulsion and stored for 12 weeks at 4° C. Radioautographic slides were developed in Kodak D-170 developer (pH 7.1) for 6 min at 18° C and fixed in 24% sodium thiosulfate (pH 6.7) for 3 min. The radioautographs were photographed with an Ultraphot II camera-light microscope (Zeiss, Toronto, Ontario, Canada) for qualitative analysis.

Electron microscope radioautography

Electron microscope radioautographs were prepared as described by Kopriwa (1973). From the blocks previously chosen for light microscopic radioautography, 80 nm thin sections were cut and placed on celloidin coated slides in three groups of several sections each per side. Prior to the application of Ilford L4 emulsion, the sections were coated with a thin layer of carbon to prevent displacement of the silver grains following development, using an Edwards 306 carbon evaporator modified to permit control of carbon deposition. After 6 months exposure, radioautographic slides were de-