〔ORIGINAL〕

Tartrate-resistant acid phosphatase activity induced by pre-incubation with tartrate in mouse embryonic mandibles

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Abstract

The present study used mouse embryonic mandibles to examine the characteristics of tartrate in tartrate-resistant acid phosphatase (TR-ACPase) histochemistry. Short-term incubation (30 min) in substrate-containing reaction medium showed intense and specific activity of TR-ACPase only in a small number of mononuclear cells, presumably pre-osteoclasts, present around a population of differentiating osteoblasts. Pre-incubation with tartrate and subsequent incubation of reaction medium resulted in slightly increased intensity in the pre-osteoclasts and also weak enzyme activity in other cells such as oral and dental epithelia, osteoblasts, and chondrocytes of Meckel's cartilage. Pre-incubation with tartrate and subsequent incubation with reaction medium may result in overestimation of the histochemical products. Therefore short-term incubation is important to estimate the enzyme activity exactly in TR-ACPase histochemistry, especially in osteoblasts.

Key words: Tartrate-resistant acid phosphatase, Histochemistry, Pre-osteoclast, Osteoblast, Mandible

Introduction

From tartrate-resistant acid phosphatase (TR-ACPase) was identified in osteoclasts[1−3], this enzyme has been employed as a marker for cytochemical identification in osteoclast differentiation and cell lineage[4−6]. In osteoblasts and osteocytes as well as osteoclasts, TR-ACPase activity has been found when sections were incubated with high concentrations of tartrate and/or incubated for longer periods[2,7]. No TR-ACPase activity has been detected in osteoblasts incubated for 30 min in reaction media containing low concentrations of tartrate[8]. The inhibition of tartrate-sensitive ACPase by tartrate has only been identified when osteoblasts

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were exposed to tartrate before incubation in the reaction medium[5]. Thus, TR-ACPase activity in osteoblasts may be positive or negative, depending upon the histochemical procedures, including the tartrate concentration, whether pre-incubation with tartrate took place, and the incubation time of tissue sections. The present study focused on the inductive effect of tartrate on TR-ACPase activity of osteoblasts, and examined the localization of TR-ACPase activity following pre-incubation with tartrate in routinely paraffin-embedded mouse embryonic mandibles.

Materials and Methods

Tissue preparation

Embryonic mandibles from ddY pregnant day 14 mice were fixed in 4% paraformaldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) overnight at 4°C. The fixed specimens were dehydrated in 70-100% ethanol following a brief rinse with 0.1 M cacodylate buffer (pH 7.4), then cleared with xylene and embedded in TissuePrep paraffin (melting temperature 58°C, Fisher Scientific, Fair Lawn, NJ, USA). The infiltration of paraffin into the tissues took place over 90 min (three times 30-minutes). The paraffin-embedded tissues were stored at -20°C until use. Sections were cut at 5 μm in thickness.

Enzyme histochemistry

The TR-ACPase activity in the deparaffinized sections was detected by using an incubation medium containing 50 ml 0.1 M sodium acetate buffer, pH 5.2, 0.25 ml N, N-dimethylformamide, 5 mg naphthol AS-MX phosphate (Sigma, St. Louis, MO, USA) as a substrate, 30 mg fast red violet LB salt (Sigma) as a coupler, and 50 mM L(+)-tartaric acid (Wako, Osaka, Japan) as an inhibitor of tartrate-sensitive ACPase. The sections were pre-incubated in the buffer for 15 min. The incubation media were prepared and filtrated immediately before use, and the sections were incubated for 30 min at 37°C in a moisture chamber. After incubation, the sections were thoroughly rinsed in the 0.1 M acetate buffer and distilled water, and then mounted in aqueous mounting medium (AQUATEX, Merck, Germany). The effects of 50 mM tartrate, added to the same buffer as above, on the TR-ACPase activity in sections pre-incubated for 30 min at room temperature were examined. The pre-incubation medium was prepared immediately before use and then applied to the sections. The pre-incubation medium had a pH of 5.3 when the sections were immersed. Incubated sections were not counterstained to compare the intensity of reddish staining products of each section.

Results and Discussion

The present study did not detected enzyme activity in differentiating osteoblasts following 30 min incubation (Fig. 1), while pre-incubation with tartrate for 30 min and subsequent incubation with reaction medium showed TR-ACPase activity in osteoblasts (Fig. 2). In previous studies,

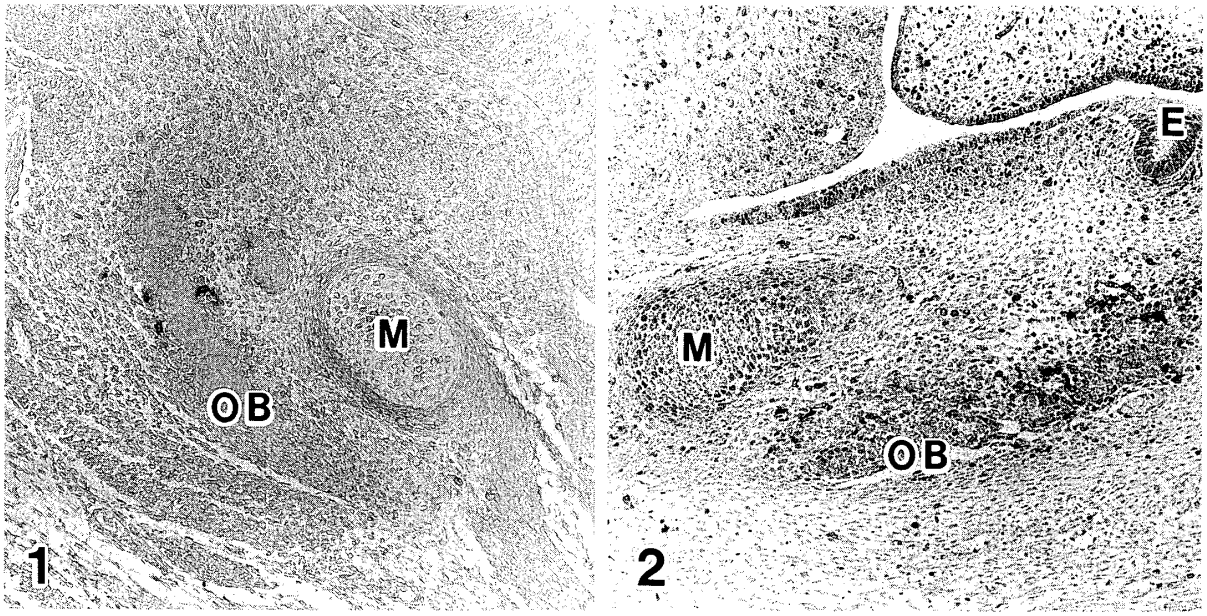

Fig. 1 Mouse mandible stained to detect TR-ACPase activity following pre-incubation without tartrate. Pre-osteoclasts with intense and specific intensity of the enzyme surround a population of differentiating osteoblasts (OB). Note the negative staining in the differentiating osteoblasts and chondrocytes of Meckel's cartilage (M). x100.

Fig. 2 Reaction products after pre-incubation with tartrate and subsequent incubation with reaction medium. The products appear in osteoblasts (OB), chondrocytes of Meckel's cartilage (M), oral and dental epithelia (E), and other cells. x100.

a high concentration of tartrate (100 mM) and/or long-term incubation (up to 3 hours) in tartrate-containing medium also induced TR-ACPase activity in osteoblasts and osteocytes[2,6,7]. Pre-incubation with 100 mM tartrate failed to suppress the enzyme activity in osteoblasts and osteocytes[7]. Chappard et al.[8] demonstrated negative TR-ACPase activity of osteoblasts, and positive activity in osteoclasts, in short incubation (30 min) of reaction mixtures containing low concentration (1 mM) of tartrate. The discrepancy in reaction products in osteoblasts may be explained by Modderman et al.[9] that the end point of the staining reaction is dependent on the amount of enzyme, and also on the staining procedure and the accessibility of the compartment where the enzyme is located. Unexpectedly, however, reaction products were also found in oral and dental epithelia and chondrocytes of Meckel's cartilage (Fig. 2). In addition, the pre-incubation induced a slightly increased intensity of TR-ACPase activity in a small number of mononuclear cells, presumably pre-osteoclasts. The present results suggest that the pre-incubation with tartrate may induce non-specific deposition of reaction products or enhanced visualization of TR-ACPase activity intrinsic to certain cells. Therefore, we concluded that pre-incubation with tartrate and subsequent incubation with reaction medium may result in overestimation of the histochemical products, especially in TR-ACPase histochemistry. Short-term incubation of the sections is important to estimate the TR-ACPase activity in osteoblasts exactly.

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