Acta histochem. 72, 253—254 (1983)

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Short original communication

Elimination of iron pigments and background staining which mask immunoperoxidase reactions

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(Received October 20, 1982)

Summary

When immunohistochemical stainings are applied to demonstration of antigens in histopathological specimens, the ferrous pigments which may be present in the tissues usually mask the final precipitates of the reaction. These pigments can be eliminated with oxalic acid or sodium dithionite after the immunohistochemical staining. These treatments also help in the bleaching of unspecific backgraund stain.

Introduction

During the course of an investigation on the presence of muramidase in tumor biopsy specimens by applying the immunoperoxidase method, we have found that the great amount of hemosiderin present in some tumors was a drawback in the interpretation of results. The colour of the pigment, which is similar to that of the final precipitation of oxidized diaminobenzidine (DAB), masks the antigenic reaction. The usual comparison with control sections without specific reaction is not enough when the pigment content is much higher than the immunoperoxidase reaction.

In this study we report the elimination of ferric pigments by oxalic acid or sodium dithionite, without affecting the DAB precipitate. Moreover, it was found that these treatments help in the bleaching of unspecific background stain.

Material and methods

Adjacent sections from conventional specimens of non-ossifying fibroma, having a great content of hemosiderin, and eosinofilic granuloma with lower content of ferric pigment, were stained with:

1. Immunoperoxidase reaction (Sternberger et al. 1970) for demonstration of muramidase, using rabbit antihuman muramidase antiserum in the 1st step and DAB in the final step to reveal sites of peroxidase binding.
2. Perls' (1867) reaction for iron demonstration.
3. Immunperoxidase technique followed by Perls' reaction.
4. 5 % aqueous solution of oxalic acid during 10 min, 1 h, 24 h and 48 h at room temperature to remove hemosiderin, followed by Perls' reaction.
5. 1 % sodium dithionite in acetate buffer $pH = 5$ for 10 min, 1 h and 24 h at room temperature, followed by Perls' reaction.
6. Immunoperoxidase reaction followed by treatment with oxalic acid or dithionite as in point 4 and 5.

Results and discussion

The immunoperoxidase reaction in both types of tumor showed strong but isolated points of reaction localized in the wall of some histocytes. (The meaning of this reaction is reported elsewhere.) Hemosiderin usually present in these tumors masked the DAB specific precipitates specially when they co-existed in the same cell.

Perls' reaction in an adjacent section revealed the characteristic blue colour of ferric ferrocyanide precipitates.

Immunoperoxidase reaction followed by Perls' reaction showed the brown and blue colours in the same section, but produced a very strong and mixed staining in some cells which made the interpretation very difficult. As was stated by Lillie (1948) and Lillie et al. (1963) pre-treatment with 5 % oxalic acid or 1 % sodium dithionite completely avoided the positive ferocyanide reaction. Oxalic acid requires 1 to 24 h for complete elimination of hemosiderin. Sodium dithionite removes all iron deposits after 5 min exposure.

When oxalic acid or dithionite treatments were applied after the immunoperoxidase reaction, they eliminated the iron pigment leaving the DAB precipitates intact even after 48 h of treatment. Moreover, it was evident that the treatment eliminates to a great extent the background staining which is an undesirable effect produced in formalin-fixed, paraffin processed tissue sections due to the non-immunologic binding of peroxidase to collagen (Fan 1980).

Literature

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