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The use of microwave irradiation for immunohistochemistry: a new methodological proposal

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Summary. We developed a rapid immunohistochemical method using a microwave oven in formalin-fixed, paraffin-embedded sections from normal and pathological tissues. The strongest immunoreactivity was obtained for actin, Ca 125, CEA, pan-cytokeratin, chromogranin A, EMA, GFAP, thyroglobulin, kappa and lambda chains. In control tissues, processed with conventional immunocytochemical procedure, the reactivity was found to be qualitatively and quantitatively similar. Dako EPOS kits were also assayed with good staining intensity, shortening the original technique to 16 min. Our microwave immunohistochemical method is simple, rapid and it may be recommended for use in routine laboratories.

Key words: Microwave irradiation, Immunohistochemistry, Paraffin sections

Introduction

In diagnostic and investigative studies, microwave irradiation has been utilized for rapid tissue fixation (Hopwood et al., 1984; Leong et al., 1985; Leong and Gilham, 1989; Reed et al., 1991), for antigen retrieval from formalin-fixed tissues (Shi et al., 1991; Cattoretti et al., 1993) and also to allow more rapid immunocytochemical staining (Leong and Milios, 1986, 1990; Chiu, 1987; Boon et al., 1989; Flokis et al., 1991). However, the microwave oven in the practical application of immunohistochemistry is not yet largely used.

In the present study we report our experience to develop and standardize a rapid method for immunostaining for formalin-fixed paraffin sections, using a cheap domestic microwave oven, in which a wide panel of polyclonal and monoclonal primary antibodies as well as the most important detection systems were assayed.

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Materials and methods

Surgical or bioptic specimens, taken from several normal and pathological tissues, were fixed in 10% neutral-buffered formalin, at room temperature for 6-18 hrs, embedded in paraffin and from each block 5 μm serial sections were cut.

All immunohistochemical procedures were realized in a domestic microwave oven with a rotary tray (Philips, Mod AVM 600/WH, maximum output 900 W). A moist chamber (18 cm in diameter), equipped with applicator sticks as described by Flokis et al. (1991), was used; five slides at a time were horizontally placed in the centre of the moist chamber, prewarmed in the microwave oven for 1 min at 160 W. During the microwave irradiation a plastic container with 200 ml tap water was placed in a corner of the oven. The temperature of all incubation solutions, measured immediately after irradiation with a thermometer equipped with a thermocouple probe (Hanna Instruments, USA), was found to be 37-38 °C.

The sources of polyclonal and monoclonal antibodies and working dilutions used are given in Table 1. On parallel sections for each case, the following detection systems were employed: StrAviGen Super Sensitive (BioGenex, San Ramon, CA), ABC Elite (Vector, Burlingame, CA) and PAP (Dakopatts, Copenhagen, Denmark). Both horseradish peroxidase (HRP) with 3,3'-diaminobenzidine (DAB) chromogen and alkaline phosphatase with Fast Red chromogen were used.

Dako Epos anti-human cytokeratin/HRP and anti-human kappa and lambda chains/HRP kits were also assayed without proteolytic predigestion.

Microwave immunostaining method

On the basis of the preliminary experiments, considering a minimal non-specific staining and the possibility of working with higher dilutions, a power output of 160 W and an irradiation time of 2 min were selected for microwave immunostaining. During the

incubation in the microwave oven the sera should not boil and dry.

Deparaffinized and rehydrated sections were treated as follows:

1. Covered with 200 µl of 3% H_2O_2 in methanol for 2 min at 160 W, to block endogenous peroxidase.
2. Washed in Tris-Buffered Saline (TBS) (0.05M, pH 7.6) for 5 min, at room temperature.
3. Incubated with 200 µl of normal serum for 2 min at 160 W.
4. Incubated with 200 µl primary antibody for 2 min at 160 W.
5. Washed in TBS for 6 min at room temperature.
6. Treated with 200 µl of secondary antibody for 2 min at 160 W.
7. Washed in TBS as step 5.
8. Incubated with 200 µl of immune complexes for 2 min at 160 W.
9. Washed in TBS as step 5.

After microwave irradiation, about 150 µl were still present on each tissue section.

Finally, peroxidase deposits were revealed by DAB, prepared as conventional procedure (Weir et al., 1974), incubating the sections in the microwave oven for 2 min at 160 W. Using alkaline phosphatase/anti alkaline phosphatase (APAAP) complexes, slides were developed with naphtol AS-BI phosphatase (Sigma) as substrate and Fast Red TR salt (Sigma) as coupler (To et al., 1983); levamisole (1 mM, Sigma) was added to inhibit endogenous phosphatase activity. The incubation time in the oven was 4 min at 160 W. The amplification of the reaction was obtained by repeating steps 6-9.

The slides were counterstained with haematoxylin and mounted in Eukitt (Kindler, Freiburg, Germany) or in glycerin jelly.

Dako Epos kits, in which primary antibody and horseradish peroxidase were coupled to an inert polymer backbone, were used in the microwave oven without proteolitic enzyme predigestion. The slides were: 1) incubated with 3% H_2O_2 in methanol for 2 min at 160 W; 2) rinsed in TBS for 5 min at room temperature; 3) incubated with Dako Epos antibody (200 µl) for 2 min at 160 W; 4) rinsed in TBS for 5 min at room temperature; and 5) incubated with DAB solution for 2 min at 160 W.

Duplicate sections from each case were used as positive controls, immunohistochemical procedures being performed according to conventional methods. Control slides were also made using Dako Epos kits according to manufacturer's instructions.

In each specimen and for each antibody the immunoreaction, obtained in the microwave oven as well as by conventional methods, was rated on a three-point scale and expressed as an average value over all the tissues tested; the sections were scored blindly and were evaluated for labelling results and background staining.

Results

Immunostaining results obtained in the microwave oven in comparison to the conventional technique are summarized in Table 2. In general, the intensity of immunostaining for most of the primary antibodies assayed showed a variable degree of positivity in relation to the type of tissue tested.

High-intensity staining and absence of background were obtained with the following antisera: pan-CK (Fig.

Table 1. Antibodies, clones, dilutions and sources used in the microwave method.

ANTIBODY	CLONE/SERUM	DILUTION	SOURCE
CEA	Polyclonal	1/300	Dakopatts
Chromogranin A	Polyclonal	1/300	Dakopatts
GFAP	Polyclonal	1/500	Dakopatts
HPV	Polyclonal	1/900	Dakopatts
Insulin	Polyclonal	Prediluted	Dakopatts
NSE	Polyclonal	1/400	Dakopatts
Thyroglobulin	Polyclonal	1/2000	Dakopatts
Actin	HHF35	Prediluted	Ortho
LCA	PD7/26-2B11	1/200	Dakopatts
L26	L26	1/200	Dakopatts
CD68	KP1	1/50	Dakopatts
CD45R	4KB5	1/50	Dakopatts
Factor VIII	F8/86	1/100	Dakopatts
Q/Bend-10	Q/Bend-10	1/200	Ylem
Vimentin	V9	1/50	Dakopatts
Pan-cytokeratin	MNF116	1/50	Dakopatts
EMA	E29	1/100	Dakopatts
Ca125	08B	Prediluted	Histocis
Kappa chain	A8B5	1/100	Dakopatts
Lambda chain	N10/2	1/100	Dakopatts

Table 2. Comparison of immunoreactivity obtained by microwave irradiation and conventional method.

ANTIBODY	MICROWAVE METHOD	CONVENTIONAL METHOD
Actin	+++	+++
Ca125	+++	+++
CD45R	-	++
CD68	-	+
CEA	+++	+++
Pan-cytokeratin	+++	+++
Chromogranin A	+++	+++
EMA	+++	+++
Factor VIII	-	+
GFAP	+++	+++
HPV	+	++
Insulin	+	+++
Kappa chain	+++	+++
Lambda chain	+++	+++
LCA	-	+++
L26	-	++
NSE	++	+++
Q/Bend-10	-	++
Thyroglobulin	+++	+++
Vimentin	++	+++
Epos kits	+++	+++

The intensity of immunostaining is graded as +++, ++, +, - for strong, moderate, weak and negative results, respectively.

1), CEA (Fig. 2), actin (Fig. 3), EMA, thyroglobulin, chromogranin A, Ca 125, GFAP, and kappa and lambda chains. On the contrary, with LCA, L26, CD45, CD68, Q/Bend 10 and Factor VIII-related antigen, no reactivity was encountered.

In any positive cases excellent results were obtained, with a higher intensity stain than PAP or ABC, when StrAvigen Super Sensitive (BioGenex) was used as the detection system. The time required for the complete procedure was 35 min with DAB and 53 with APAAP, respectively.

Using microwave exposure optimal results were also obtained with Dako Epos anti-human cytokeratin (Fig. 4) and Dako Epos anti-human kappa and lambda chain kits, shortening the procedure to 16 min.

Discussion

In formalin-fixed paraffin sections, the present immunocytochemical method in the microwave oven has been shown to yield reproducible and qualitatively similar results; the intensity of immunostaining was well evident in all positive cases, without significant presence of background. Therefore, it appears that microwave irradiation during immunocytochemical procedures induced acceleration of incubation of antibodies. However, employing cytochemical markers for endothelium (Factor VIII and Q/Bend 10) as well as antibodies against LCA, L26 CD45 and CD 68 no immunoreaction was seen, suggesting that specific tissue antigens or immune complexes can be altered with our microwave irradiation method.

In recent years, several authors have investigated the possibility of accelerating immunocytochemical procedures with the use of microwave irradiation. In particular, Leong and Milios (1986) were the first to utilize microwave exposure to accelerate the incubation of the primary antibody, whereas Chiu (1987) and Flokis

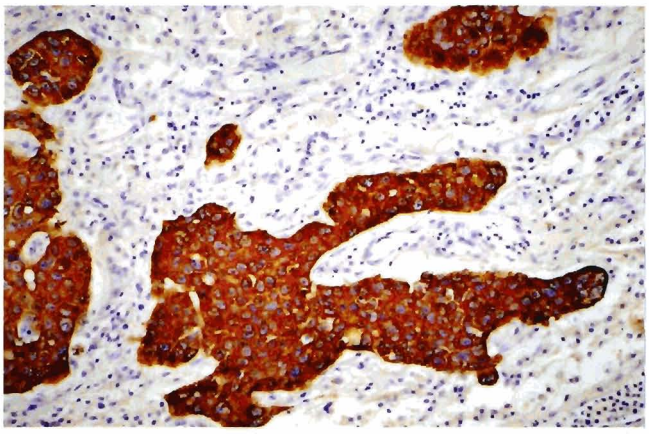

Fig. 1. Pan-CK-positive neoplastic cells in a cerebral metastatis of bronchogenic squamous cell carcinoma. Microwave oven method, StrAviGen Super Sensitive, x 180

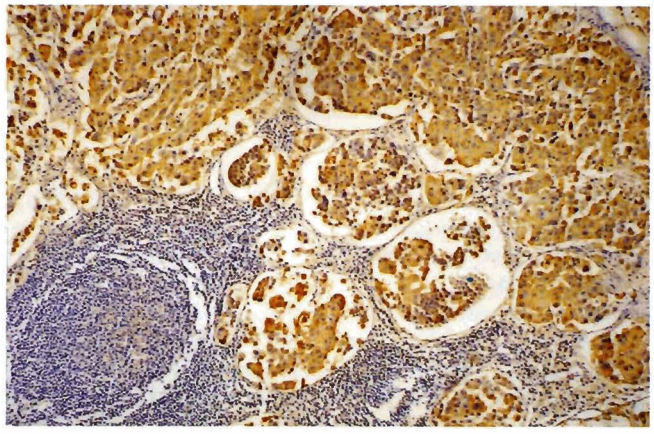

Fig. 2. Metastatic carcinoma of the colon in a lymph node; a large amount of CEA - immunoreactive cells are present. Microwave oven method, ABC, x 90

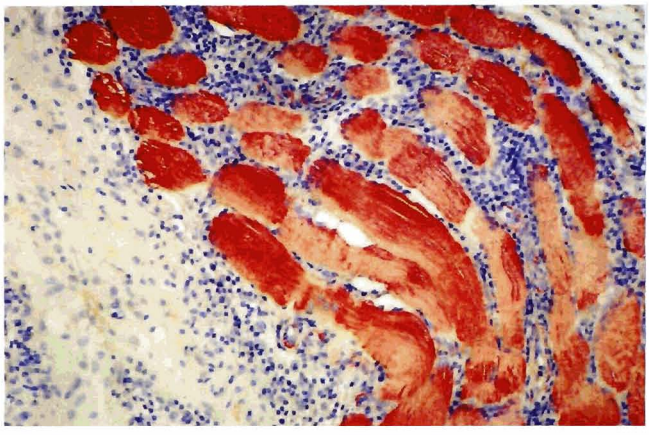

Fig. 3. A section of striated muscle with leukocytic infiltration in a case of polymyositis. Muscle fibres are well stained by actin. Microwave oven method,. APAAP, x 180

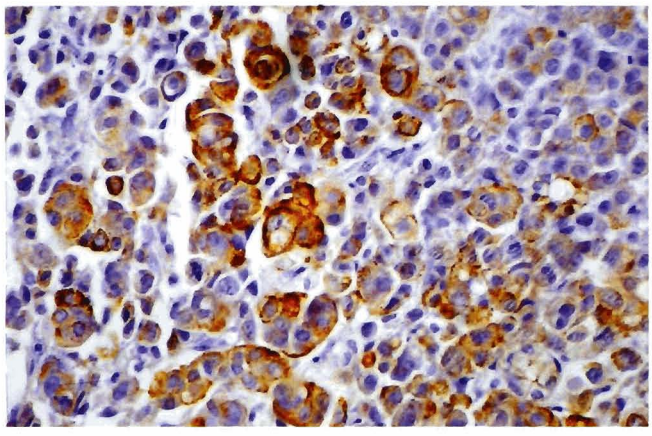

Fig. 4. Strong CK immunoreactive neoplastic cells in a diffuse type of gastric carcinoma. Microwave oven method, Dako Epos, x 260

et al. (1991) accelerated three steps including PAP and APAAP, respectively; a microwave-stimulated immunogold-silver staining, using only ß-HCG as primary antibody, is reported by Boon et al. (1989). In addition, Leong and Milios (1990) have used microwave irradiation for all steps of immunostaining procedure, using increased concentrations of the primary antibody on microwave formalin-fixed and enzymatic pretreated sections.

In the present report, all blocking and incubation steps were performed during microwave exposure, without enzymatic preincubation; the primary antibody was used at the same dilution as in the conventional method. Moreover, we document that the use of StrAvigen Super Sensitive (Bio Genex) appears to be the most suitable approach. Another interesting finding in this study was the optimal immunoreactivity obtained with Dako Epos kits. This is the first time that Dako Epos kits have been utilized in a microwave oven, with a significant shortening of the immunocytochemical procedure, allowing us to complete it in just 16 minutes.

In conclusion, the present immunocytochemical microwave method is simple and reliable and therefore may be of practical value in surgical pathology.

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