HISTOPATHOLOGICAL STUDY OF MYOCARDIAL INFARCTION (II)

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In previous study, various stainings of experimentally produced myocardial infarction were examined and considered about their specificity, reliability and practical use. HBFP and PTAH staining methods were useful to detect the early myocardial infarction.

In this report, these two staining methods were examined about the usefulness for human autopsy cases. In nine fresh myocardial infarction, six of them showed the regional similarity in PTAH and HBFP positive areas, the similarity in remainders were partially. In the earlier lesion, HBFP method stained stronger than PTAH. As lesion becomes older, PTAH positive area become to be stronger. In operation materials positive findings were variable. In control, false positive areas were found at intermediate zone. These findings were mostly due to unskillfull decolorization technique.

From the results of our study, it was thought that the early myocardial infarction could be successfully diagnosed by the combination of HBFP and PTAH staining methods.

INTRODUCTION

As is well known, fresh myocardial infarction within 5-6 hours is difficult to recognize. In infarcted case of more than 12 hours duration, infarcted area is intensely eosinophilic, and inflammatory infiltration and congestion become apparent. In such a case, histological diagnosis of myocardial infarction is easy.

To recognize recent myocardial infarction within 5-6 hours, HBFP (Hematoxylin-basic fuchsin picric acid) staining method by Lie et al. (1971) was reported. Myocardial fibers damaged by ischemia retained the fuchsin staining as early as 60 minutes after infarction in our experimence.

From the result of our experimental data, HBFP and PTAH (Phosphotangs-

tic Acid Hematoxylin) methods were thought to be very useful to detect the effect of early myocardial infarction.

We will discuss about utilization of theses taining methods to human autopsy materials.

MATERIALS AND METHODS

We used 28 human autopsy cases; recent myocardial infarctions within 5 or 6 days (9 cases), old infarctions (7 cases) and cases died after cardiac operation (12 cases). As controls, autopsy materials that had died by the causes except for cardiac diseases were used. Slices of the hearts were fixed in 10% formalin solution and the comparative study was performed about ischemic areas under various staining methods (H.E, Azan, PTAH, HBFP).

In the canine hearts with myocardial infarction, HBFP and PTAH stainings demonstrated early ischemic areas early as 1 hour after the attack. The assessments of these stainings are as follows :

1. Recent myocardial infarction
(9 cases)

This group consisted from the cases of few hours to 5-6 days after attack of myocardial infarction. Grossely, ischemic areas of few hours duration was not grossly recognized but areas of long period more than 4-6 days duration were dark-red in colour, hemorrhagic and had irregular yellowish necrotic foci.

Microscopic findings : Early ischemic myocardiums were HBFP positive and were stained red intensely against brilliant yellow background of cardiac muscle fibers (Fig. 1). The extent and the intensity of fuchsin staining varied among individual myocardial cells. In PTAH staining, ischemic myocardiums showed deep blue in colour (Fig. 2), PTAH positive findings of the ischemic myocardium did not appear before fuchsin positive staining by the HBFP staining. In such an occasion there were not detectable changes in the HE stained sections. At the time when

contraction bands and destruction of myofibriles by PTAH method became apparent, intensity of HBFP staining was patchy and became somewhat weak.

Contraction bands were stained with fuchsin staining of HBFP and PTAH stainings. When the myocardial necrosis and inflammatory infiltration were obvious in HE staining, fuchsin staining of HBFP method was almost disappeared and fuchsin-stained fibers were scattered among the necrotic fibers. As the ischemic necrosis, advanced fuchsin stained areas had completely disappeared.

2. Relation with PTAH and HBFP stainings in recent myocardial infarction

The correlation of staining intensity by these both staining methods shown on Table 1. In 6 of 9 cases in recent myocardial infarction, PTAH staining was corresponded with HBFP, but in the other 3 cases PTAH was partly correlative to the HBFP.

It was suggested that both HBFP and PTAH would exhibit early ischemic lesions. Both staining methods can be adopted for the human cases as well as for experimental animals.

Fig. 1. Recent myocardial infarction (No. 119) Ischemic myocardium revealed positive areas in HBFP.

Fig. 2. Recent myocardial infarction (No. 119) Myocardium was stained deep blue colour in PTAH.

TABLE 1
Recent myocardial infarction

No.	PTAH Staining	HBFP Staining
4545	posterior	posterior
4543	anterior, posterior	anterior, posterior
4501	septum~posterior	posterior
4470	anterior	anterior
4378	anterior	anterior lateral
4332	posterior	posterior
4060	posterior	posterior
3692	anterior~lateral	lateral~posterior
119	posterior	posterior

Fig. 3. Old myocardial infarction (No. 4359) Myocardial cells surrounding infarcted scar revealed positive area in HBFP.

3. Relation between PTAH and HBFP for old infarcted scar

Comparative study between the PTAH and HBFP stainings of old myocardial infarction was done. All seven cases of old myocardial infarction had positive findings in PTAH staining such as contraction bands, destruction of myofibrils and detachment of myofilaments but in the HBFP few myocardial cells surrounding infarcted scar were faint positive (Fig. 3). Most of surviving myocardial cells did not stain with basic fuchsin.

4. Relation between PTAH and HBFP for myocardial cells after cardiac surgery

The myocardial cells after cardiac surgery which performed to congenital and acquired heart diseases showed more or less damage. Staining features of both methods varied in each cases had no constant correlation with post operative hours.

Control

We used 18 hearts of the autopsied materials died of other diseases except of heart diseases. In HBFP staining few cases had faint positive findings at the intermediate muscular layer of

the heart. In PTAH staining, specimens of the similar region there was no abnormal changes. Faint positive (HBFP) staining was considered to be false positive. False positive areas were completely disappeared by longer decolorization steps in acetone fluid.

COMMENTS

Morphologic diagnosis of early myocardial infarction, before 6 hours after onset of infarction, is difficult. Because of this reason, many methods have been reported (Brody et al, 1967 ; Cox et al, 1968 ; Fine et al, 1966 ; Hack and Helmy, 1967 ; Hudson, 1965 ; Nachlas and Shnitka, 1963 ; Sybers et al, 1972 ; Zugibb et al, 1965). However, the number of methods of them were not practical and reliable. Enzyme histochemistry and electron microscopy have been used and many findings of early myocardial infarction has been discussed. These methods were apt to be limited in cases of experimental material. Non-enzymatic practical histochemical methods (PAS, diastase resistant PAS positive material, phospholipid and so forth) have been studied until now. But in human materials the positive findings were not constant. Lie et al.

reported in 1971 HBFP staining as a new histochemical method for early myocardial ischemia (Lie et al., 1971). This method is not affected by post-mortem change, unlike enzymic methods, and the result are reproducible. Experimentally positive reaction was apparent as early as 30 minutes after coronary ligation in dogs. Autopsy materials with infarction of less than 6 hours duration showed positive in the ischemic lesions by this staining method. However, its origin of staining materials was not apparent.

Positive finding of PTAH staining was appeared later than that of HBFP staining. In PTAH staining, ischemic area of two hs duration was stained more densely and the heart muscle surrounding the infarcted are showed contraction band and interstitial edema. Ischemic areas for three hs or longer are stained a homogenous bluish black color and transverse striation disappeared. They were clearly demarcated from intact muscle fibers. In HBFP and PTAH stainings, histological findings were considerably corresponded. When the both stainings were used in human autopsy cases, the staining results revealed considerable similarity of lesional areas. However, normal heart muscles as the control were often stained positive, especially in intermediate layer of heart muscle by HBFP staining. As described by Lie et al, the decolorization step with picric acid acetone and following acetone step within limited time was important. False HBFP positive areas were frequently produced in human cases. Because of the unstability of HBFP, especially in decolorization step, HBFP staining was unreliable in human case (Zugibe et al., 1973).

Based on our results in experiments (Tanimura, A. et al., 1977) and human

autopsy materials, fuchsinophilia in ischemic area seemed persistent more than in normal heart muscles. False positive finding in normal hearts showed diffuse faint staining in individual fibers, compared with strongly, perinuclear or scattered staining in ischemic areas.

Though the appearance of positive effect in PTAH is later than that of HBFP, comprehensive histological interpretation of lesions could be given from this method (increased density, granular degeneration and appearance of contraction band). So the combination of PTAH and HBFP stainings was thought to be the best to appreciate cardiac ischemia accurately.

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