STUDIES OF SERUM ISOENZYMES AFTER KIDNEY TRANSPLANTATION

A. L. LATNER, A. W. SKILLEN and J. SWINNEY

University Department of Clinical Biochemistry, Royal Victoria Infirmary, and Department of Urology, Newcastle General Hospital, Newcastle upon Tyne, Great Britain

Many enzymes occur in multiple molecular forms called isoenzymes and the isoenzyme patterns may be tissue and species specific. Serum isoenzyme patterns have proved to have clinical applications in a variety of disease states. The diseased tissues release enzymes into the circulation and in certain cases it is possible to recognise the isoenzyme patterns of diseased tissues in the serum. Lactate dehydrogenase isoenzymes are well established as aids in the diagnosis of myocardial infarction and certain hepatic disorders (Latner, 1964). Alkaline phosphatase isoenzymes are primarily of use in differentiating between certain bone and liver diseases (Latner, 1965).

Renal transplantation is becoming an increasingly more realistic surgical procedure and it is important that surgeons gain as much information as possible on the state of the grafted organ in the post-operative period. When rejection of the graft occurs the enzymes in the rejected organ will be released into the circulation so that in the case of renal transplantation we may expect to recognise the isoenzyme pattern of kidney tissue in the serum when rejection is impending. Isoenzyme patterns are much more useful than total enzyme levels as it is possible to differentiate between different sources of increased enzyme activity and show whether the increased activity comes from the transplanted organ or is due to surgical trauma or drug hepatotoxicity.

In the past two years we have had the opportunity to study two cases of renal transplantation; one which survived 47 days and the other 85 days. In each case we have studied the serum isoenzyme patterns and total serum levels of lactate dehydrogenase and alkaline phosphatases.

Methods

Lactate dehydrogenase was estimated using a colorimetric method (Sigma Chemical Co. Technical Bulletin No. 500) and alkaline phosphatase using the method of King (1951).

Starch-gel electrophoresis was used to separate the isoenzymes in the manner previously described (Latner and Skillen, 1961; Hodson, Latner and Raine, 1962). As well as Tris-HCl buffers, borate buffers and Tris-EDTA-borate buffers have been employed in separations of the lactate dehydrogenase isoenzymes. The lactate dehydrogenase isoenzymes were visualised using the MTT/phenazine methosulphate technique (Latner and Skillen, 1961) and the phosphatase isoenzymes with α-naphthyl phosphate/Fast Red TR (Hodson, Latner and Raine, 1962).

Results

The serum lactate dehydrogenase and alkaline phosphatase activities were higher than normal for most of the post-operative period. Apart from one or two sporadic sharp increases the total levels rose gradually following the transplant operation.

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Fig. 1. Serum lactate dehydrogenase isoenzymes following renal transplantation
A. actual gels; B. diagrammatic representation
(a), (b) and (c) represent the first few weeks after the transplant operation where the lactate dehydrogenase isoenzyme pattern is relatively normal except for a slight increase in LDH-5.
(d), (e) and (f) represent the period 6–9 weeks after the transplant where LDH-5 is increased and LDH-1 and LDH-2 are becoming more prominent.
(g), (h) and (i) represent the final two or three weeks when LDH-1 is most prominent.

Fig. 2. Serum alkaline phosphatase isoenzymes following renal transplantation
A. actual gels; B. diagrammatic representation.
(a) 3 weeks after transplant; (b) 8 weeks after transplant; (c) 12 weeks after transplant.
The highest lactate dehydrogenase activity was detected a few days before death in the first patient and about six weeks before death in the second. In both cases the alkaline phosphatase was at its highest level in the few days just before death.

The lactate dehydrogenase isoenzyme patterns were relatively normal in the immediate post-operative period. In each case a lactate dehydrogenase isoenzyme pattern resembling that of renal tissue could be recognised in the serum two or three weeks before death. This pattern became more marked in the last week before death of the patient. In the second patient where the lactate dehydrogenase activity was very high five or six weeks before death, there was a marked increase in the slowest moving isoenzyme and the faster moving isoenzymes, or those present in the kidney, did not show up until a little later (Fig. 1).

The serum alkaline phosphatase isoenzyme patterns showed a slightly increasing level of the liver isoenzyme (i.e. the normal serum isoenzyme) throughout the period. In both cases two or three weeks after the operation another phosphatase isoenzyme relatively near the insertion could be detected. This was thought to be the slower moving isoenzyme which can be detected in homogenates of renal tissue. This second isoenzyme was at its peak half-way through the post-operative period and became a little less intense in the few weeks just before death when another more diffuse zone with slightly less mobility than the liver phosphatase could be detected. This was most probably the major kidney phosphatase (Fig. 2).

Discussion

Reports on the usefulness of total serum lactate dehydrogenase levels have been contradictory. One group of workers has found that patients with excellent acceptance of the homograft show mean serum and urinary lactate dehydrogenase levels within the normal range, those with functioning renal transplants but with occasional episodes of threatened rejection have elevated lactate dehydrogenase levels and those who finally reject the graft have elevated levels even when the homograft function appears essentially normal (Hume et al., 1964). This group of workers have indicated that the presence of an increase in the slowest moving serum lactate dehydrogenase isoenzyme indicates impending or ultimate rejection (Prout et al., 1964). McLean and co-workers (1965) have found that the serum and urinary levels of lactate dehydrogenase were not reliable indicators of the condition of the homograft and suggested that the urinary alkaline phosphatase levels were more useful in predicting rejection of the graft. The presence of a serum-like alkaline phosphatase has been described in the urine of a patient following renal transplantation (Butterworth et al., 1965).

Our own studies have shown that the total serum lactate dehydrogenase and alkaline phosphatase levels were abnormal in two cases of renal transplantation where the patients rejected the homograft. The total enzyme levels did not suggest a gradual rejection of the graft but episodes of partial rejection before the final one. However, some of the sporadic variations in the lactate dehydrogenase activity may be the result of the trauma of repeated haemodialysis. The increase in the 'liver' isoenzyme noted in the second patient may suggest hepatotoxicity of 'Immuran' (azathioprine), a condition previously described by McLean and co-workers (1965). On the other hand this increase in the slowest moving isoenzyme may be the same phenomenon detected by Prout and co-workers (1964), who found an increase in this isoenzyme in patients who rejected their homografts.

The increase in the faster moving lactate dehydrogenase isoenzymes probably represents the necrosis of the homograft itself.

The serum alkaline phosphatase isoenzymes have not previously been investigated after renal transplantation. The two separate types of pattern which we have detected may represent firstly, an initial partial rejection period indicated by the slower moving isoenzyme and, secondly, actual necrosis of the homograft indicated by the appearance of the faster moving isoenzyme.
REFERENCES