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Differentiation of Human Phospholipase A_2 Isoenzymes in Serum and Other Body Fluids with Use of Monoclonal Antibodies

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Summary: Elevated phospholipase A_2 activities in serum were measured in patients suffering from acute pancreatitis or various inflammatory diseases. The photometric phospholipase A assay of *Hoffmann & Neumann* (Klin. Wochenschr. *67* (1989) 106–109) was combined with immunoabsorption by different monoclonal antibodies directed against pancreatic phospholipase A_2. Pancreatic phospholipase A_2 was purified from human duodenal juice. Monoclonal antibodies were prepared by fusion of spleen cells from immunized mice with P3X63-Ag8-653 myeloma cells. Samples with phospholipase A_2 activity were incubated in monoclonal antibody-coated microtitre plates. Phospholipase A_2 activities were determined in the monoclonal antibody-treated samples as well as in control samples. The method allows the determination of the fraction of human phospholipase A_2 isoenzymes in various biological materials. For pancreatic phospholipase A_2 the specific binding capacity was about 60–80%, the unspecific binding was 5–30%. Practically no cross-reactivity was seen with partially purified serum phospholipase A_2, with recombinant platelet phospholipase A_2, or with the sera of patients with non-pancreatic diseases. In conclusion, the present study confirmed the presence of pancreatic phospholipase A_2 in human duodenal juice and in the ascites of necrotizing pancreatitis. However, pancreatic isoenzyme was absent in non-pancreatic inflammatory diseases. Therefore, elevated phospholipase activities in non-pancreatic inflammatory diseases cannot be attributed to the pancreas.

Introduction

Serum phospholipase A_2 (EC 3.1.1.4) has long been assumed to originate exclusively from the pancreas (for review see l. c. (1)). More recently, this assumption has been challenged by our group (1–4) and other authors (5–7).

In 1988, *Eskola* et al. (6) demonstrated the existence of two different forms of phospholipase A_2 in human serum using polyclonal antibodies against the pancreatic isoenzyme. Their study design, however, did not unequivocally prove that one of these "isoenzymes" was pancreatic phospholipase A_2; since phospholipases A_2 possess a highly conserved protein structure (8, 9), polyclonal antibodies against the pancreatic enzyme might cross-react with structurally related isoenzymes. Immuno-reactive proteins have been found in serum of patients with non-pancreatic malignant tumours (10) as well as in rat spleen (11). On the other hand, most phospholipases A_2 have a marked tendency to bind to proteins and surfaces in an unspecific manner (3, 12, 13), so that antibody-independent binding artifacts may occur.

The present article describes a practicable combination of activity measurements based on a photometric assay (2) and immunoabsorption by different monoclonal antibodies. The photometric assay has been

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