Phospholipase-A$_1$ and -A$_2$ in Experimental Acute Pancreatitis in Rats

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Summary. Using a standardized model of bile-induced acute pancreatitis, the reaction of phospholipase-A$_1$ activity was investigated in parallel to that of phospholipase-A$_2$, as well as their relationship to the pathomorphological spread of pancreatitis. While the measurement of the total free fatty acids (FFA) in serum as metabolites of phospholipase-A activity indicated variable reactions with the average remaining the same (0.186 ± 0.15 to 0.192 ± 0.153 mEq/l), phospholipase-A$_2$ exhibited a highly significant increase from 9.6 ± 2.2 to 18.2 ± 5.4 nmol/ml/min ($P < 0.01$). It was demonstrated that in parallel phospholipase-A$_1$ also showed a highly significant increase from 2.5 ± 1.2 to 6.7 ± 3.1 nmol/ml/min ($P < 0.01$). In relation to the pathomorphological score, both phospholipases showed a small increase at a low score (edema to focal necrosis), whereas in groups with a larger spread of the pancreatitis, score 5 or 6 (extended necrosis to retroperitoneal necrosis), the increase was substantially clearer. As a result of these findings, we conclude that phospholipase-A$_1$ could possibly be used as phospholipase-A$_2$ as an indicator of the severity of acute pancreatitis and that it should be taken into consideration in the very promising therapy with phospholipase-A-antagonists.

Key words: Phospholipase-A$_1$ – Phospholipase-A$_2$ – Experimental acute pancreatitis – Pathomorphological spread

Introduction

Experimental and clinical studies have shown the central role of phospholipase-A-activity [1, 4, 5] and particularly that of phospholipase-A$_2$ in serum in the pathogenesis of acute pancreatitis [7, 8, 9]. Schröder et al. [8, 9] showed that the increase in serum phospholipase-A$_2$ correlates closely with the severity of pancreatitis. This led to a series of investigations in which phospholipase antago-
nists were used successfully in the therapy of acute pancreatitis ([10, 12], own experiences in print). On the other side, the formation of lysolecithin by phospholipase-A is seen as the real cytotoxic factor. Both phospholipase-A2 and -A1 are responsible for the splitting of lecithin into lysolecithin and free fatty acids (FFA). However, there has not been a publication so far about the action of phospholipase-A1 and its role in acute pancreatitis.

It was the aim of this experimental study to examine to what extent phospholipase-A1 can be used similarly to phospholipase-A2 as a parameter for acute pancreatitis and how well its increase agrees with the pathomorphological spread of changes in the pancreas.

**Material and Methods**

**Experimental Model**

A rat model of pancreatitis which had been standardized through a series of experiments was used for these investigations. Ten Sprague Dawley rats received a standardized carbohydrate, fat, and protein diet for 3 days which was followed by a 24-h fast. At this time, a catheter was inserted into the v. cava inferior via the v. femoralis to obtain blood samples for analysis of initial values. After laparotomy the common bile duct was proximally clamped, the papilla of Vater transduodenally punctured, and dog bile (0.4 ml) injected intraductally. The abdominal wall was closed using a continuous stitch. Nine hours later the abdominal wall was opened again, the pathomorphological changes caused by the occurrence of pancreatitis were documented, and a blood sample for determination of end values was obtained via the catheter. One animal died before the end of the experiment so that only nine animals were used for the evaluation.

**Determination of FFA in Serum**

The assay was carried out as described previously [2]. FFA were extracted from serum (50 μl) with 5 ml chloroform-heptane-methanol (40:30:1.4 v/v) and mixed vigorously for 5 min with 2 ml Cu(NO3)2 reagents containing 0.5 M Cu(NO3)2, 1 M triethanolamine in ethanol, 1 N NaOH (10:10:3.5 v/v), pH 8.1. The heptane phase was separated, mixed with 0.4% ethanol solution of diphenylcarbazide containing 1 ml 10% triethanolamine solution and incubated for 15 min at room temperature. FFA were then assayed at 546 nm.

**Assay of Phospholipase-A1 and -A2**

Enzyme activities were determined in samples containing 5 μl serum. As substrate 0.005 μCi (0.05 nmol) of 1[14C]palmitoyl-2-acylglycerol-3-phosphoryl plus 50 nmol of nonlabeled phosphatidylcholine in 10 ml of 0.1 M Tris buffer (pH 7.4) containing 2 mM CaCl2 and 0.35% sodium cholate were added, and the mixture was incubated at 37°C for 60 min. The reaction was terminated by adding 0.5 ml methanol, and after 10 min 5 ml chloroform was added. Then the extracts were submitted to lipid extraction [13], applied to silica plates (Kieselgel, H. Merck, Darmstadt, FRG), separated with a solvent system chloroform/methanol/water (65/40/10), and detected with iodine vapor. The amount of the radioactivity recovered from lysophosphatidylcholine and FFA fractions indicated the distribution of degraded fatty acids from positions 1 and 2 of the substrate. The enzyme activity was expressed as nmol FFA (phospholipase-A1) or nmol lysophosphatidylcholine (phospholipase-A2) per minute per milliliter serum.

**Statistical Analysis**

The results are expressed by means + SEM. Data were analyzed using Student’s t-test.