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Silibinin, a plant extract with antioxidant and membrane stabilizing properties, protects exocrine pancreas from cyclosporin A toxicity

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Abstract. Silymarin can be extracted from the milk thistle, and silibinin is the main component of the plant extract. Possibly due to their antioxidant and membrane-stabilizing properties, the compounds have been shown to protect different organs and cells against a number of insults. Thus liver, kidney, erythrocytes and platelets have been protected from the toxic effects of ethanol, carbon tetrachloride, cold ischemia and drugs, respectively. The effect of silibinin on endocrine and exocrine pancreas, however, has not been studied. We therefore investigated whether silibinin treatment attenuates cyclosporin A (CiA) toxicity on rat endocrine and exocrine pancreas. Groups of 15 male Wistar rats were treated for 8 days with CiA and/or silibinin. On day 9, endocrine and exocrine pancreatic functions were tested in vitro. At the end of the treatment period, blood glucose levels in vivo were significantly higher in rats treated with CiA, while silibinin did not affect glucose levels. In vitro, insulin secretion was inhibited after treatment with silibinin, but amylase secretion was not affected. After treatment with CiA both insulin and amylase secretion were reduced. Silibinin and CiA had an additive inhibitory effect on insulin secretion, but silibinin attenuated CiA-induced inhibition of amylase secretion. Despite CiA treatment, amylase secretion was in fact restored to normal with the highest dose of silibinin. Thus silibinin inhibits glucose-stimulated insulin release in vitro, while not affecting blood glucose concentration in vivo. This combination of effects could be useful in the treatment of non-insulin-dependent diabetes mellitus. Furthermore, silibinin protects the exocrine pancreas from CiA toxicity. As this inhibitory effect is probably unspecific, silibinin may also protect the exocrine pancreas against other insult principles, such as alcohol.

Key words. Silibinin; cyclosporin A; endocrine pancreas; exocrine pancreas; insulin; amylase.

Silibinin is a water-soluble form of one of the structural isomers of the flavonoid silymarin, which can be extracted from the milk thistle. In a 4-week placebo-controlled clinical trial, silymarin was shown to improve liver function tests and liver morphology in outpatients with increased serum activities of AST or ALT, usually due to regular alcohol intake [1]. Patients with liver cirrhosis have also been reported to benefit from silymarin treatment [2]. In animal experiments silymarin and silibinin have been shown to protect rat or mouse liver against the toxic effects of alcohol, carbon tetrachloride, thallium and phenylhydrazine, to protect rabbit kidneys against the effect of cold ischaemia, and to

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protect erythrocytes and platelets against the toxic effects of phenylhydrazine [3–7]. These beneficial effects have been attributed to the antioxidant and membrane-stabilizing effects of the compounds [5].

The effect of silibinin on endocrine and exocrine pancreatic tissue, however, has not been studied. We therefore investigated in rats whether silibinin therapy affects endocrine and exocrine pancreatic function, and whether silibinin protects the pancreas against cyclosporin A (CiA) toxicity.

Methods

Animals. Male Wistar rats (Versuchstieranstalt Lippe, Lippe, Germany), weighing between 250 and 300 g, were used in this study. Before the start of the experiment, the animals were adapted to the housing conditions over a period of 7 days. The animals were kept in wire-bottom cages in a light-controlled room with a temperature of 21 °C. The rats were fed on a standard diet (Altromin, Altrogge, Germany) with free access to water, as described previously [8].

Study design. In the first set of experiments, rats were treated for 8 days with either silibinin (50, 100 or 200 mg/kg bw × d) or saline, both given as an intraperitoneal injection once daily, and olive oil, given via an intragastric tube once daily. In the second set of experiments, rats were treated with either silibinin (50, 100 or 200 mg/kg bw × d) or saline, both given as an intraperitoneal injection once daily, and CiA (10 mg/kg bw × d), dissolved in olive oil and given via an intragastric tube once daily.

Silibinin ($C_{25}H_{22}O_{10}$) was obtained from Dr. Madaus, Cologne, Germany. It was given as an intraperitoneal injection, because it is water soluble and therefore could not be dissolved in the olive oil that was given as a vehicle via the intragastric tube. Cyclosporin was obtained from Sandoz, Basel, Switzerland. The dose of cyclosporin has previously been shown to impair endocrine and exocrine pancreatic functions in our laboratory [9, 10].

In vivo and in vitro measurements. At the end of the treatment period, animals were fasted for 24 h, but they still had free access to water. Rats were anaesthetized with pentobarbital (intraperitoneal application of 60 mg Nembutal/kg body weight; Ceva, Paris, France). In vivo, venous blood was drawn from the right jugular vein to measure concentrations of glucose and CiA. CiA was measured 24 h after the last administration of the compound with an assay using monoclonal antibodies. Subsequently, an isolated perfused pancreas was prepared, as previously described [8]. The preparation consisted of pancreas with a small residue of duodenum. The proximal end of the bile duct was ligated, and every 10 min a calibrated polyethylene tube was inserted into the distal end of the common duct to collect pancreatic juice. The preparation was perfused via the superior mesenteric artery and the celiac trunk at a constant rate of 4 ml/min without recirculation. The perfusate was a modified Krebs–Ringer bicarbonate solution with 0.2% bovine serum albumin (RIA grade) and 3% dextran (T 70). The perfusate was gassed with 95% O_2 and 5% CO_2 to give a pH of 7.4. Glucose concentration in the perfusate was 300 mg/dl. CCK-8 (Sigma) was infused via a side-arm injection using an infusion pump (Braun-Melsungen, Germany) to result in a concentration of 100 pg/ml, which has previously been shown in our laboratory to stimulate exocrine secretion half maximally.

After an equilibration period, we analysed insulin and amylase secretion from the isolated organ during the 30-min period between min 15 and 45. Amylase activity in 10-min samples of pancreatic juice was determined using a standard laboratory method, as described previously [8]. Complete portal vein effluent was collected in 60-s intervals, aliquoted and stored at −20 °C prior to determination of insulin-like immunoreactivity, as described previously [8].

Statistical analysis. A total of 120 experiments were performed, with 15 animals in each experimental group. In the text and in the figures data are given as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by the Scheffés' multiple comparison procedure. F values were considered significant at 95%.

Results

Blood glucose and CiA concentrations in vivo. Silibinin therapy did not affect glucose concentration in vivo in any of the doses tested (108 ± 6/98 ± 8/100 ± 6/117 ± 5 mg/dl; 0/50/100/200 mg silibinin/kg bw × d). Cyclosporin treatment, however, significantly increased blood glucose concentrations (108 ± 6 vs. 128 ± 5 mg/dl). Blood glucose concentrations were not significantly different in rats treated with cyclosporin alone compared with rats treated with cyclosporin and any of the doses of silibinin tested (128 ± 5/125 ± 11/125 ± 11/122 ± 6 mg/dl; 0/50/100/200 mg silibinin/kg bw × d). Silibinin treatment did not affect CiA concentration in vivo (data not shown). Silibinin treatment did not affect body weight of the animals (data not shown).

Insulin and amylase secretion in vitro. Insulin secretion from the isolated perfused pancreas was significantly lower after treatment with silibinin (fig. 1). Amylase secretion, however, was not affected by silibinin treatment (fig. 2). As to be expected [9, 10], CiA treatment inhibited both insulin (24.1 ± 1.3 vs. 11.2 ± 0.6 mU/30 min) and amylase secretion (15.4 ± 2.6 vs. 7.5 ± 1.1 U/min) from the isolated perfused pancreas. Silibinin

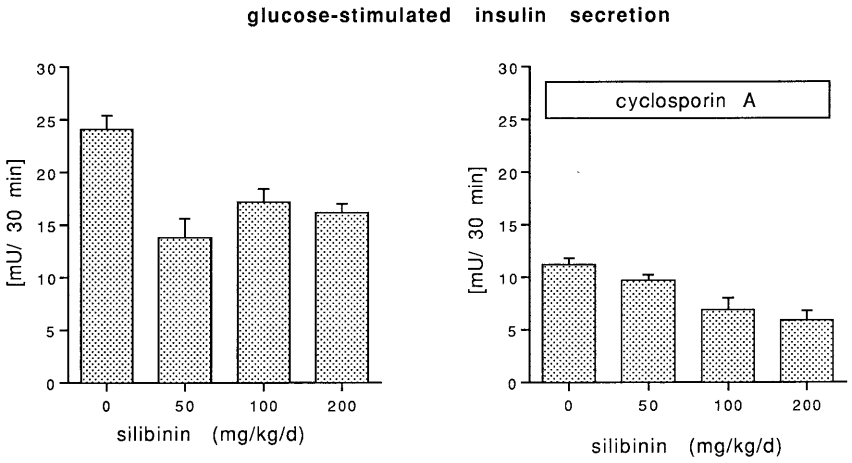

Figure 1. Effect of in vivo treatment with silibinin alone (left panel) or treatment with both silibinin and CiA (right panel) on insulin release from the isolated perfused rat pancreas. Means ± SEM.

and CiA had an additive inhibitory effect on insulin release (fig. 1). However, silibinin-attenuated CiA induced inhibition of amylase secretion (fig. 2). In the series of experiments with cyclosporin, amylase secretion was actually restored to normal with the highest dose of silibinin tested.

Discussion

This is the first study on the effect of silibinin on endocrine and exocrine pancreatic function, and it has two main implications. First, glucose-stimulated insulin secretion in our in vitro model was significantly reduced after an 8-day treatment period with silibinin in vivo. This was true when rats received silibinin alone and also when CiA was given concomitantly. The fact that the inhibitory effects of silibinin and CiA were additive suggests that both compounds affect pancreatic B cells through different mechanisms. It is most interesting to note that – despite impairment of glucose-stimulated insulin secretion in vitro – blood-glucose concentration in vivo was not signifi-

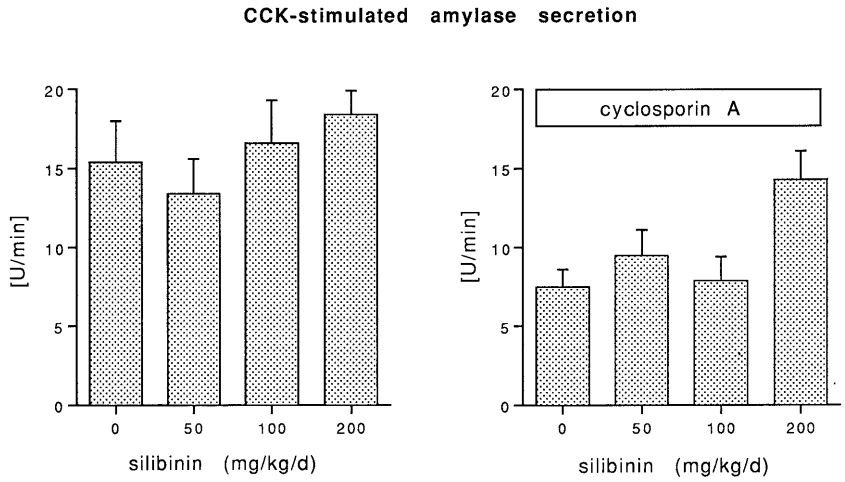

Figure 2. Effect of in vivo treatment with silibinin alone (left panel) or treatment with both silibinin and CiA (right panel) on amylase release from the isolated perfused rat pancreas. Means ± SEM.

cantly different in control animals and rats in the treatment groups. Thus silibinin may reduce insulin secretion without increasing blood-glucose concentration, and this combination of effects could be useful in states of hyperinsulinaemic hyperglycaemia, such as non-insulin-dependent diabetes. Unlike metformin, which is used for this condition [11], serious side effects from silibinin are not known. Therefore we believe that further studies, including clinical trials, are warranted to investigate whether or not silibinin is useful in non-insulin-dependent diabetes mellitus.

The second interesting finding in our study concerns the exocrine pancreas. Silibinin, in the doses tested, did not affect CCK-stimulated amylase secretion from the isolated perfused pancreas. The compound did, however, prevent CiA-induced toxicity.

Besides its well known toxic effects on the kidney and liver, CiA is also known to impair exocrine pancreatic secretion [9, 10] and to alter acinar morphology [12], although the mechanisms involved are not fully understood. It is therefore difficult to speculate how silibinin may have protected ainar tissue from CiA toxicity. The protective effect was definitely not mediated via the so-called islet-acinar axis, which closely links the endocrine with the exocrine pancreas [13, 14]. If the islet acinar axis had been involved, the silibinin-induced inhibition of insulin secretion would have been expected to impair amylase release rather than restore amylase secretion to normal, as seen in our study. Since silibinin protects different organs from a wide range of toxins [3–7], we suggest that the effect is an unspecific one, possibly related to the antioxidant and membrane-stabilizing properties of the compound [5]. This in turn could mean that silibinin might protect acinar tissue not only from CiA toxicity but also against other toxins, such as free radicals or alcohol.

We conclude that the differential effect of silibinin on rat endocrine and exocrine pancreas may have considerable therapeutic potential. By inhibiting glucose-stimulated insulin release in vitro while not affecting blood-glucose concentration in vivo, silibinin could prove to be useful in the treatment of non-insulin-dependent diabetes mellitus. Furthermore, silibinin protects the exocrine pancreas from CiA toxicity. Since this inhibitory effect is probably unspecific, silibinin may also protect the exocrine pancreas against other noxious insults, such as alcohol.

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