# EFFECT OF PORTACAVAL ANASTOMOSIS ON HEPATIC HMG-CoA-REDUCTASE ACTIVITY IN NORMAL RATS

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## 1. Introduction

The plasma cholesterol lowering effect of a portacaval anastomosis (pca) in experimental animals has been known since 1941 [1] and was confirmed in several different species. On the basis of these observations Starzl et al. [2] created a pca in man for the treatment of otherwise intractable hyperlipoproteinaemia type IIa in its homozygous form. In this and subsequent cases a reduction of serum cholesterol was demonstrated [3–5]. The patho-mechanism of this effect has remained until now unclear.

The establishment of pca in rats results in ultrastructural alterations, especially in a reduction of the smooth endoplasmic reticulum (Terlunen et al. unpublished data). In parallel a reduction of the microsomal cytochrome *P*-450 concentration was observed [6,7]. HMG-CoA-reductase, considered to be the rate limiting enzyme of cholesterol synthesis, is of microsomal origin, at least in the liver. Thus, we were interested in studying the influence of a pca on the hepatic HMG-CoA-reductase activity.

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## 2. Materials and methods

### 2.1. *Treatment of rats*

Male Wistar rats (Versuchstieranstalt Hannover, FRG) weighing 230–310 g were used. The animals were kept in metabolic cages receiving a practically cholesterol-free rat chow (Altromin C 1045, Atromin GmbH, Lage, FRG) in pair-feeding technique and water ad libitum. At a constant room temperature of 22°C, an electrical timer provided 12 h periods of light and darkness. Dark periods lasted from 4 a.m. to 4 p.m. in order to achieve maximal enzyme activity about 9.00 a.m.

Under light ether anaesthesia portacaval anastomosis were performed using an end-to-side technic [8,9]. Sham-operation consisted of laparatomy, mobilization of the vena portae after ligation of the vena pylorica and clamping the portal vein for a corresponding time. Control animals remained without any surgical treatment.

### 2.2. *Assay methods*

After a postoperative period of 14 days the rats were killed by decapitation at 9.00 a.m. All obtainable blood was collected for serum assays. Livers were rapidly removed, weighed and homogenized in a cold solution containing 0.3 mol sucrose and 10 nmol mercaptoethanol/liter. Liver microsomes were prepared according to Shapiro and Rodwell [10].

Table 1
Effect of portacaval anastomosis (pca) on total body weight and liver weight

| | pca (A) | Sham (B) | Control (C) | Significance |
|---|---|---|---|---|
| Initial body weight (g) | 282 ± 17 | 278 ± 19 | 271 ± 28 | n.s. |
| Terminal body weight (g) | 296 ± 19 | 297 ± | 303 ± 21 | n.s. |
| Liver weight (g) | 7.4 ± 1.1 | 11.4 ± 2.0 | 12.1 ± 2.0 | $p < 0.001$ A versus B/C |

The microsomal pellet was resuspended in the homogenizing medium and made up to contain about 1 mg protein/ml. Microsomal protein was determined by the method of Lowry [11].

The assay of HMG-CoA-reductase activity was performed according to Shefer et al. [12]. An internal standard of [$^3$H]mevalonic acid was carried through the entire procedure. Results were expressed in terms of nmol HGM-CoA converted to mevalonolactone/min/mg microsomal protein. Serum lipoproteins were separated by ultracentrifugation according to Havel et al. [13]. Each lipoprotein fraction was assayed for its protein and lipid content. Serum cholesterol, triglycerides and phosphatides were determined as described by Zöllner, Zöllner and Eberhagen, and Eggstein, respectively [14–16]. The significance of differences between the mean values was tested by Student's *t*-test.

## 3. Results

An initial post-operative weight loss in shunted animals was overcome within the 14 day test period.

Table 2
Effect of portacaval anastomosis (pca) on serum lipids and lipoproteins in the rat (all data in mg/100 ml)

| | pca (A) | Sham (B) | Control (C) | Significance |
|---|---|---|---|---|
| Cholesterol | 41 ± 15 | 57 ± 16 | 71 ± 25 | A versus C $p < 0.001$<br>A versus B $p < 0.05$<br>B versus C n.s. |
| Triglycerides | 91 ± 19 | 149 ± 48 | 179 ± 38 | A versus C $p < 0.01$<br>A versus B $p < 0.05$<br>B versus C n.s. |
| Phosphatides | 191 ± 54 | 570 ± 55 | 685 ± 291 | A versus C $p < 0.001$<br>A versus B $p < 0.001$<br>B versus C n.s. |
| VLDL protein | 41 ± 17 | 72 ± 40 | 111 ± 43 | A versus C $p < 0.001$<br>B versus B $p < 0.05$<br>B versus C n.s. |
| LDL protein | 58 ± 24 | 55 ± 25 | 60 ± 30 | n.s. |
| HDL protein | 97 ± 26 | 155 ± 65 | 188 ± 79 | A versus C $p < 0.01$<br>A versus B $p < 0.05$<br>B versus C n.s. |
| *n* | 10 | 8 | 8 | |

There was no significant difference in body weight in the pair-fed rats of groups A (pca), B (sham-operated) and C (control). In contrast liver weight of the shunted animals was significantly diminished as compared to group B and C (table 1).

Rats with pca were found to have significantly lowered serum levels of cholesterol, triglycerides and phosphatides. The differences between sham-operated and control animals were not statistically significant. In rats with pca there was a marked decrease of serum VLDL and HDL protein as compared to animals of group B and C. No differences appeared in the LDL fraction. The lipid/protein ratio remained unchanged in all groups (table 2).

Hepatic microsomal HMG-CoA-reductase activity was greatly reduced in rats with pca in comparison to sham-operated and control animals. There was another marked difference between sham-operated and control animals although to a much lesser extent (table 3).

## 4. Discussion

The cholesterol lowering effect of a pca has been demonstrated in several animal species as well as in man [1–5, 17]. In rats Balasubramaniam et al. [18] were unable to show a similar influence of a pca. In contrast our data indicate a significant decrease of serum cholesterol as compared with sham-operated and control animals. This is supported by investigations of Edwards et al. [19]. The mechanism of the cholesterol decreasing effect could be explained by one or more of the following points:

(1) Reduced intestinal absorption of exogenous cholesterol or diminished reabsorption of endogenous cholesterol

(2) Impaired cholesterol synthesis

(3) Enhanced cholesterol catabolism or excretion

(4) Alterations of pool sizes

(5) Qualitative or quantitative changes of lipoproteins.

The initial fall in body weight after performance of a pca is due to decreased food intake. There is no evidence for intestinal malabsorption [20]. In our study the application of cholesterol-free food eliminates the possibility of reduced exogenous cholesterol intake as compared with control animals. Diminished reabsorption of endogenous cholesterol is unlikely because the lipid content of the faeces in rats with pca equals that of untreated animals. [20]. As our results show, the hepatic cholesterogenesis is an important working point for the cholesterol lowering effect of a pca. HMG-CoA-reductase activity is reduced to 26% of pair fed control rats.

Diminished total liver weight and loss of smooth endoplasmic reticulum should intensify the effect of pca on hepatic cholesterol synthesis. This observation is supported by results of Pector et al. [21] using an in vitro technique. In contrast Magide et al. [22] found an increased HMG-CoA-reductase activity. Sham-operated rats with ligated pyloric vein had a hepatic HMG-CoA-reductase activity about 53% of normal controls. Thus, the dominant role of pancreatic hormones, especially pointed out by Starzl et al. [17], might be supported. There is until now little information about intestinal cholesterol synthesis, the other main source of endogenous cholesterol, after pca. Coyle et al. [23] reported investigations in dogs using a [$^{14}$C]acetate incorporation technique

Table 3
Effect of portacaval anastomosis (pca) on hepatic microsomal HMG-CoA-reductase activity in the rat
(nmoles × min$^{-1}$ × mg protein$^{-1}$)

| | pca ($n$ = 6) | Sham ($n$ = 4) | Control ($n$ = 5) |
|---|---|---|---|
| HMG-CoA-reductase | 0.56 ± 0.27 | 1.11 ± 0.33 | 2.11 ± 0.38 |
| Significant differences | $p < 0.02$ | $p < 0.01$ | $p < 0.001$ |

in vitro. They found virtually unaltered intestinal cholesterol synthesis after pca.

The involvement of cholesterol catabolism and biliary excretion remains unclear. On the one hand, the activity of cholesterol-7-α-hydroxylase, which is believed to be the rate-limiting enzyme in the catabolism of cholesterol to bile acids, was shown to be increased after pca in rats [18,24]. On the other hand, bile flow is lower and bile acid excretion is unaltered in rats with pca [25].

No information is available about cholesterol pool changes and disturbances of lipoprotein metabolism after pca. We found decreased serum concentrations of VLDL and HDL. LDL remained unchanged. These findings are in agreement with results of Magide et al. [22]. Thus, an inhibition of HMG-CoA-reductase activity by LDL, as demonstrated by Brown and Goldstein in normal fibroblasts [26], appears unlikely.

## 5. Conclusion

Portacaval anastomosis in rats results in a marked inhibition of hepatic HMG-CoA-reductase activity, the rate limiting enzyme of cholesterol synthesis. Thus, the cholesterol lowering effect of the portacaval anastomosis is due, at least partly, to a decrease in hepatic cholesterol synthesis.

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