Continuous Extracorporeal Immunoadsorption

A Tool for the Selective Removal of Plasma Components.
Low Density Lipoprotein (LDL) Depletion of a Hypercholesterolemic Patient

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A concept and its experimental realization in a collaborative study are presented which permit the highly selective removal of any blood plasma component from endogenous or exogenous sources, provided they have antigenic or haptenic properties.

Our primary goal was the selective removal of low density serum lipoprotein which would lead to the reduction of plasma cholesterol, an event most desirable in homo- and heterozygous familial hypercholesterolemia. So far, surgical intervention, such as ileal bypass [1] and terminal portacaval shunt [2-4], and nonsurgical procedures such as plasmapheresis [5, 6] or the discontinuous venous blood removal, adsorption of VLDL and LDL to heparin sepharose and reinfusion of the filtered blood [7] are being used. In the latter experiments, plasma-cholesterol levels were lowered by 15 to 20% in the rather expensive plasma-exchange experiments of Thompson et al. [5] and in Apstein et al. [6] a decrease of 60% was achieved. The present cost of a plasma exchange in the U. S. amounts to $800. The disadvantage due to these economic considerations and the necessity of continued treatment for an indefinite period are apparent.

The concept is as follows:
(1) The procedure for the removal of a plasma component must be highly specific.

(2) The method must be capable of continuously depleting the plasma of the component under study.
(3) The method must be rapid and efficient, e.g. eventually leading to a complete removal of the component.
(4) The physical status of the individual should remain unimpaired.
(5) A repetitive use should make the system financially feasible.

The example reported here, namely the LDL removal from swine
plasma, should prove the potential of the procedure, the methodology of which will be reported in details elsewhere [8].

The highly specific removal is achieved by immunoadsorption:

Serum-LDL was purified exhaustively for (a) the covalent coupling to sepharose CL-4B, and (b) for the immunization of sheep.

Sheep anti-LDL was adsorbed by the LDL-Sepharose and desorbed with glycine buffer, pH 2.8, as monospecific antibodies after thorough elution of the non-immunoadsorbed plasma proteins with PBS (phosphate-buffered saline). $3^{1/2}$ g of monospecific anti-LDL-IgG were isolated and covalently cross-linked to CNBr-activated Sepharose CL-4B. This anti-LDL-Sepharose-4B bed was used as a selective adsorber of LDL from the plasma.

Pigs develop a form of arteriosclerosis somewhat resembling that of humans [9].

It can be induced experimentally by a number of methods [10]. Therefore a swine is the most rewarding experimental animal, particularly under the conditions reported in this cooperative study.

We proceeded as follows:

Silicon tubings of 2 mm internal diameter were introduced into the common carotid artery, the jugular vein, or the femoral artery and vein of the 25 to 40 kg deeply anesthetized pigs.

Into the arteriovenous or venous-venous shunt a continuous-flow blood centrifuge (model Aminco celltrifuge) was interposed to separate the plasma from any corpuscular elements and the erythrocytes, white

Fig. 1. Schematic diagram of the arrangement of continuous-flow centrifuge (Aminco celltrifuge) and immunoadsorbent column in the shunt.

Fig. 2. Kinetics of LDL removal from normal pig plasma in vivo by adsorption to anti-LDL-Sepharose CL-4B.

Curve (a): Protein concentration as marker for dilutional effects
Curve (b): LDL concentration during an immunoadsorption experiment

Continuous Extracorporeal Immunoabsorption 21

Stoffel/Greve/Lange/Borberg 22

Fig. 3. LDL concentration of a normal pig after repeated immunoadsorption treatment. Op: operation of arterio - venous or venous - venous shunt. Numbers above arrows: LDL (mg) desorbed from immunoadsorption column after experiment.
cells, and thrombocyte concentrate. The plasma was passed over a Sepharose 4B-LDL-antibody column (fig. 1). Its capacity is sufficient to adsorb about 5 g total LDL-cholesterol corresponding to 11 g of low-density lipoproteins.

The LDL-depleted plasma eluting from the LDL-adsorber is recombined with the erythrocyte-concentrate flow before entering the venous side of the shunt.

In more than 35 experiments with normal pigs we were able to demonstrate
(1) that our approach selectively removes the apo-B-containing lipoproteins LDL and VLDL;
(2) that the repeated application of this highly selective immunoadsorption method is tolerated without any physical impairment.

The following figures analyse the biochemical parameters:

Figure 2 depicts the kinetics of the removal of LDL during the experiments.

Figure 3 outlines some features characteristic for the experimental animal:

Continuous Extracorporeal Immunoadsorption 23

(a) The transient influence of the shunt operation on plasma LDL-concentration of the animal which normalizes within 2-4 days.
(b) In some experiments an overshoot phenomenon was observed, following the establishment of the pre-experimental LDL levels after immunoadsorption, which occurs within 4-8 days [3-6].
(c) Repetitive experiments show identical kinetics.
(d) Serum enzymes, electrolytes, urea, creatine, glucose, and several hematological parameters remain unaltered with the exception of the lowered hematocrit, due to the removal of several blood samples for analysis during the experiment.

Hypercholesterolemic swines with total serum LDL-cholesterol of 600 mg/dl due to a 2 % cholesterol diet over a period of almost one year were used. For this LDL-adsorption two anti-LDL-columns were arranged in series and used alternately. The column completely saturated with LDL was regenerated with glycine, HC1, pH 2.8 buffer, and neutralized.

Fig. 4. Kinetics of LDL removal from hypercholesterolemic swine in vivo by adsorption to anti-LDL-Sepharose CL-4B.
A: Change to regenerated anti-LDL-column
Curve (a): Protein concentration
Curve (b): LDL concentration during experiment
Stoffel/Greve/Lange/Borberg 24

Fig. 5. LDL concentration of a hypercholesterolemic pig after repeated treatment to immunoadsorption.
Op: Shunt operation. Numbers above arrows: LDL (mg) desorbed from immunoadsorption column.

with PBS, while the second column was in function. As demonstrated in figure 4, LDL levels were lowered from 600 to 120 mg/dl. The kinetics of the replacement of the LDL-concentration was comparable to that of a normal swine (fig. 5).

Approved by the ethic commission of our universitys medical faculty we then started an immunoadsorption treatment of a 29-year-old hypercholesterolemic volunteer.

A venous-venous shunt between the cubital veins of the patient was used for the extracorporeal blood centrifugation and the immunoadsorber.

Table I. Results of three LDL-immunoadsorption treatments of a 29-year-old hypercholesterolemic male volunteer

Continuous Extracorporeal Immunoadsorption 25

Table I summarizes the results of three treatments all of which occurred without the slightest physical impairment to the patient. Figure 6 outlines the kinetics of the patients LDL-concentration in these successive experiments.

Figure 7 summarizes the results of this new approach for the removal of a plasma component; in this case the apo-B-containing lipoproteins are positively correlated to the pathogenesis of arteriosclerosis.

(1) LDL can be specifically removed during a short-time (2-4 h) application by our set-up.

(2) The LDL-concentration returns to the original level within 2-3 weeks. The method is, therefore, acceptable for repeated and routine application, particularly in homozygotes.

Fig. 6. Kinetics of LDL removal from a hypercholesterolemic male volunteer by adsorption to anti-LDL-Sepharose CL-4B.

Curve (a): Total cholesterol concentration
Curve (b): LDIWLDL cholesterol concentration
Curve (c): LDL/VLDL cholesterol concentration (sample after column).

Stoffel/Greve/Lange/Borberg 26

Fig. 7. LDL depletion in extracorporeal combined plasma separation, immunoadsorption
and kinetic of LDL replenishment of plasma LDL in man.
Curve (a): Total cholesterol
Curve (b): LDL/VLDL cholesterol

(3) No overshoot-synthesis was observed in man.
The potential of the method is obvious
(a) for biochemical questions such as: Is the gradual increase of LDL
after its removal to the original level due to a de novo synthesis of the
lipid and apoprotein components of LDL or are cholesterol deposits
mobilized by the dramatic changes of serum-cholesterol concentra-
tions?
(b) since the principle of this approach appears to be adaptable to other
clinical problems, basically to the selective removal of plasma components
with antigenic sites.
Finally returning to the conceptual basis of our approach outlined in
the beginning. We are confident that this method may be of great benefit
to removing the main-risk factor of coronary heart disease of homo- and
heterozygous hypercholesterolemic patients.
The treatment of a female homozygous familial hypercholesteremic
patient is in progress.

Continuous Extracorporeal Immunoadsorption 27

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