Protective effect of Coupled Plasma-Filtration Adsorption (CPFA) on Bile-associated Cast Nephropathy and tubular injury through direct adsorption of bilirubin and Liver-type Fatty Acid Binding Protein (L-FABP)

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Background

Bile-associated cast nephropathy and consequent tubular damage are relevant causes of acute kidney injury (AKI) during severe liver dysfunction. The mechanisms of bilirubin-associated AKI are mainly due to tubular cell apoptosis consequent to mitochondrial dysfunction following cast formation.
Liver-type Fatty Acid Binding Protein (L-FABP) is a 15 KDa peptide belonging to free fatty acid family able to bind hydrophobic molecules including bilirubin. During liver failure, the increase of L-FABP plasma levels enhances bilirubin uptake and consequent apoptosis of tubular cells through a mechanisms dependent on megalin, the endocytic receptor located on the luminal surface of tubular cells.

Objectives

To investigate the protective role of Coupled Plasma Filtration Adsorption (CPFA) on bile cast nephropathy through L-FABP and bilirubin adsorption by the hydrophobic polystyrene resin.

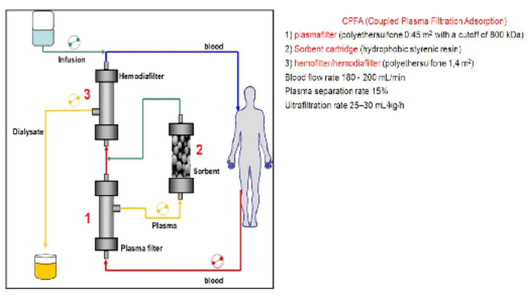


Fig. 1: Main operative characteristics of Coupled Plasma Filtration Adsorption (CPFA).

2012
November
• Kidney transplantation from deceased donor: ESRD for chronic pyelonephrithis • Delayed graft function (DGF) with need of RRT
December
• First kidney biopsy: acute tubulo-interstitial and vascular rejection: start Thymoglobulin • Septic shock due to Legionella infection with MOF
2013
January
• serum creatinine 5,2 mg/dl, oliguria • Start again RRT
February
• Bilirubin 42 mg/dl, plasma L-FABP 52 ng/ml, presence of tubular cells and intense positivity for bilirubin at urinary sediment, urine NGAL 356 ng/ml. • Liver biopsy: marked cholestasis • Second kidney biopsy: bile cast nephropathy and severe tubular injury • Start CPFA
March
• Increase of urine output, decrease of bilirubin (< 15 mg/dl), plasma L-FABP (9 ng/ml) and urine NGAL (82 ng/ml)

Fig. 2: Time course of the clinical events occurring in a kidney transplanted patients with sepsis and MOF including severe AKI requiring RRT and liver dysfunction.

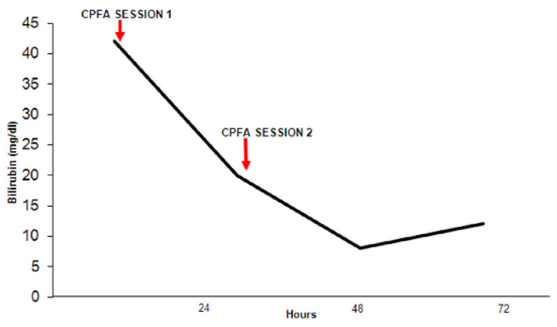


Fig. 3: Time course of plasma levels of bilirubin (mg/dl) after CPFA treatment.

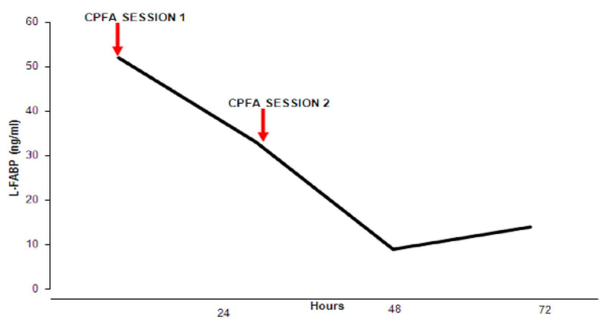


Fig. 4: Time course of plasma levels of L-FABP (ng/ml) after CPFA treatment.

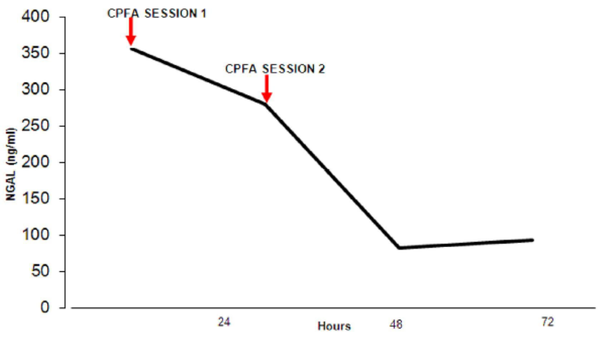


Fig. 5: Time course of urine levels of NGAL (ng/ml) after CPFA treatment.

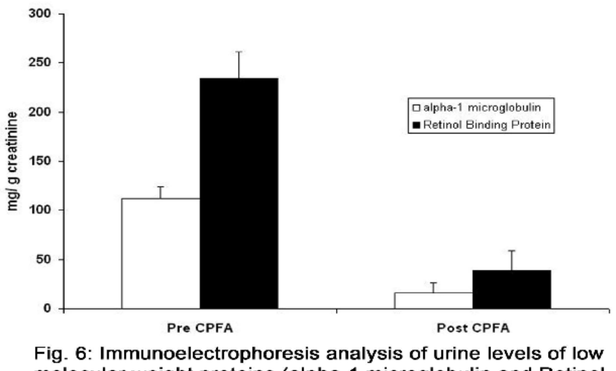


Fig. 6: Immunoelectrophoresis analysis of urine levels of low molecular weight proteins (alpha-1 microglobulin and Retinol Binding Protein) before and after CPFA treatment (data are expressed as mg of proteins in respect to g of urine creatinine).

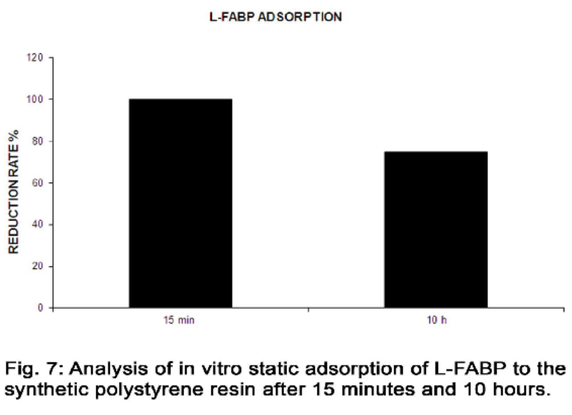


Fig. 7: Analysis of in vitro static adsorption of L-FABP to the synthetic polystyrene resin after 15 minutes and 10 hours.

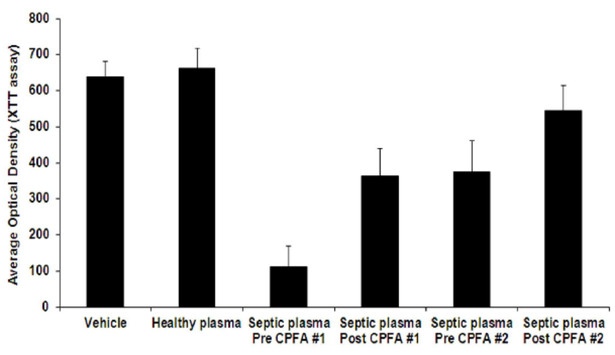


Fig. 8: Evaluation of in vitro cytotoxicity (XTT assay) exerted by septic plasma drawn from the kidney transplanted patients of the case report on cultured human kidney-derived tubular epithelial cells. CPFA significantly reduced plasma-induced cytotoxicity ($p<0.05$). Plasma from healthy subjects (n=3) were used as experimental control.

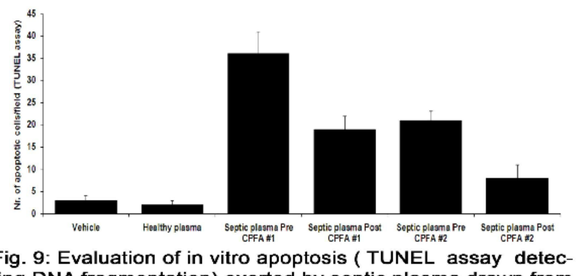


Fig. 9: Evaluation of in vitro apoptosis (TUNEL assay detecting DNA fragmentation) exerted by septic plasma drawn from the kidney transplanted patients of the case report on cultured human kidney-derived tubular epithelial cells. CPFA significantly reduced plasma-induced apoptosis ($p<0.05$). Plasma from healthy subjects (n=3) were used as experimental control.

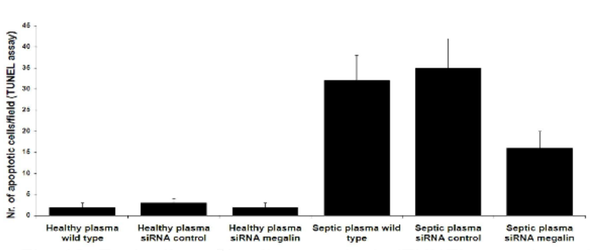


Fig. 10: Evaluation of in vitro apoptosis (TUNEL assay) exerted by septic plasma drawn from the kidney transplanted patients of the case report on wild type tubular cells or on tubular cells transfected with a control siRNA or with a siRNA directed to knock-down megalin, the L-FABP receptor. The pro-apoptotic effect of septic plasma on siRNA megalin-engineered tubular cells was significantly reduced ($p<0.05$). This effect was not observed in wild type and in control siRNA-transfected tubular cells.

Methods

We reported the case of a kidney transplanted patient who developed sepsis, AKI and liver dysfunction treated by CPFA (Fig. 1). We evaluated plasma levels of bilirubin and L-FABP. Renal biopsies, urine sediment, NGAL and immunoelectrophoresis were also performed at different time points.
***In vitro,* we tested:**
1. static adsorption of L-FABP to the polystyrene resin;
2. Cytotoxic (XTT assay) and pro-apoptotic effect (TUNEL assay) of patient's plasma drawn before and after CPFA on cultured human tubular cells. The role of L-FABP was confirmed in tubular cells engineered to knock-down megalin, the L- FABP receptor, by small interfering RNA (siRNA).

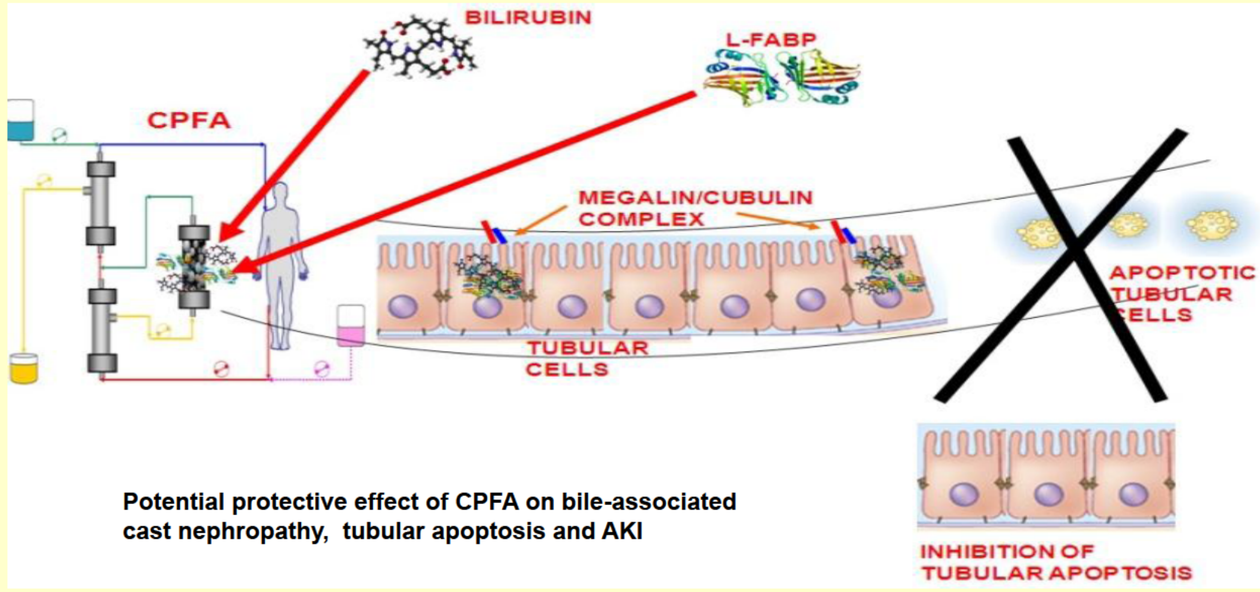
Results

A 50-year-old man was subjected to kidney transplantation with slow recovery of graft function (Fig. 2). Kidney biopsy revealed acute tubulo-interstitial and vascular rejection treated by Thymoglobulin. He then developed septic shock for Legionella with multiple organ failure (serum creatinine 5.2 mg/dl and oliguria requiring RRT; bilirubin 42 mg/dl with liver biopsy showing marked cholestasis; plasma L-FABP 52 ng/ml). Urine analysis showed the presence of tubular cells, intense positivity for bilirubin and presence of low molecular weight proteins such as alpha-1 microglobulin and retinol binding protein: urine NGAL level was 356 ng/ml. A new kidney biopsy showing bile cast nephropathy and severe tubular injury was performed.
After CPFA was started, we observed an increase of urine output and a concomitant decrease of bilirubin (Fig. 3), plasma L-FABP (Fig. 4), urine NGAL (Fig. 5) and low molecular weight proteins (Fig.6).

***In vitro,* the polystyrene resin efficiently adsorbed L-FABP (100% adsorption after 15 minutes, 75% after 10 hours) (Fig. 7). After CPFA treatment, the cytotoxic (XTT assay in Fig. 8) and pro-apoptotic (TUNEL assay in Fig. 9) effect of patient's plasma on cultured human tubular epithelial cells were significantly reduced.**
In addition, plasma-induced apoptosis was dependent on the presence of megalin, the L-FABP receptor located on tubular cell surface (Fig. 10).

Conclusions

CPFA may have a protective role on AKI associated with liver failure through the direct adsorption of bilirubin and L- FABP to the synthetic polystyrene resin. The decrease of bilirubin and L-FABP plasma levels may limit cast formation and tubular apoptosis.



Potential protective effect of CPFA on bile-associated cast nephropathy, tubular apoptosis and AKI

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