

BRIEF REPORT

Galactose binds to focal segmental glomerulosclerosis permeability factor and inhibits its activity

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Focal segmental glomerulosclerosis (FSGS) is associated with circulating permeability activity (P_{alb}) and recurs after transplantation in about 30% of patients. The FS permeability factor (FSPF) consists of anionic low-molecular-weight protein(s) that might be excluded by the anionic filtration barrier. We postulated that FSPF may interact with sugars of the glycocalyx, and we tested its affinity for sugars using column chromatography. FSPF showed high affinity for galactose; P_{alb} activity was absent from unbound material and present in eluate after dialysis to remove galactose. In parallel studies, P_{alb} activity of serum was lost after adding galactose $\geq 10^{-12}$ M. To determine whether galactose also abolishes plasma P_{alb} activity *in vivo*, a patient with posttransplant FSGS was given galactose and serum samples were collected. Intravenous infusion of galactose decreased P_{alb} from 0.88 before infusion to undetectable levels postinfusion and at 48 hours. Oral galactose diminished P_{alb} activity; P_{alb} reached a nadir after 2 weeks and remained low for at least 4 weeks after galactose was discontinued. We conclude that FSPF has high affinity for galactose based on chromatography. Additionally, galactose inactivates FSPF and may lead to its clearance from plasma. The interaction between FSPF and glomeruli may depend on FSPF binding to galactose, and the FSPF–galactose complex may be susceptible to uptake by galactose-binding proteins and to catabolism. We propose testing galactose as a novel nontoxic therapy for nephrotic syndrome in FSGS to determine whether galactose slows progression and whether pretransplant therapy decreases rates of recurrence and graft loss. (Translational Research 2008;151:288–292)

Abbreviations: BSA = bovine serum albumin; FSGS = focal segmental glomerulosclerosis; FSPF = FS permeability factor; MW = molecular weight; NPP = normal pooled plasma; PP = plasmapheresis; PPF = PP fluid

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Focal segmental glomerulosclerosis (FSGS) leads to steroid-resistant nephrotic syndrome and to renal failure.¹ Transplantation is followed by recurrence in 30% of patients, and graft loss is common.² The observations that proteinuria may occur within hours after transplantation³ and that remission of proteinuria often follows plasmapheresis (PP)⁴ or immunoabsorption⁵ provide evidence that a circulating factor causes recurrence. PP before transplantation may reduce the incidence and severity of posttransplant recurrence.⁶ We have shown that glomerular capillary permeability (P_{alb}) is increased by sera from patients with recurrent FSGS⁶ and that administration of plasma fractions causes proteinuria in rats.^{7,8} P_{alb} is reduced by PP and returns after several weeks.⁶ FS permeability factor

AT A GLANCE COMMENTARY

Background

Focal segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome and chronic renal failure. It recurs in about 30% of patients, and recurrence seems to be caused by a circulating permeability factor. Treatment with plasmapheresis and immunosuppressive agents is empiric and often ineffective.

Translational Significance

Galactose, a simple nontoxic sugar, binds to and inactivates the permeability factor during *in vitro* testing. Administration of galactose to a patient during transplant recurrence markedly decreased serum permeability activity. We propose that it will be important to test the effect of oral or intravenous galactose in a prospective study of FSGS.

(FSPF) consists of low-molecular anionic proteins or proteins that alter phosphorylation of several glomerular proteins; phosphatase inhibition blocks the permeability activity.⁹ We postulated that the FSPF may have a lectin-like interaction with sugars of the podocyte glycocalyx and may affect cell signaling pathways. We tested the affinity of FSPF for galactose and studied the effect of adding galactose during P_{alb} testing *in vitro*. We also compared the effect of PP and of galactose administration on P_{alb} activity in posttransplant FSGS recurrence.

METHODS

Effect of PP on P_{alb} . Thirty paired samples from 22 FSGS patients before and after PP were studied. In each case, therapeutic PP was performed as ordered by the patients' physicians. Repeated samples from 27 patients who did not receive plasmapheresis were also studied. Clinical and demographic characteristics of most patients are unknown to us.

Galactose affinity chromatography. Affinity chromatography of 25 PP fluid (PPF) samples (P_{alb} activity > 0.5) from 13 FSGS patients was performed. Seven were male; 12 were adults. Plasma from multiple donors at the Blood Center of Southeastern Wisconsin, normal pooled plasma (NPP) (P_{alb} activity undetectable), was used as a negative control. PPF (3-8 L) or NPP (9-18 L) was applied to galactose-agarose beads. Unbound material was collected. Bound material was eluted using galactose solution, 0.1 M. Eluate was extensively dialyzed against galactose-free medium using dialysis membrane, molecular weight (MW) cutoff 1 kDa. After dialysis, the solution was subjected to sequential filtration using 50-kDa and 30-kDa cutoff membranes. An aliquot of unbound

material and of each fraction was added to incubation medium (2% vol/vol) and P_{alb} tested.

Tests of FS serum activity after addition of galactose. Three series of studies were performed to define interactions between FSPF and galactose. To determine whether galactose blocked the effect of FSPF, active serum (P_{alb} activity > 0.7) was added to the isolation medium (2% vol/vol) containing galactose (final concentrations 10^{-19} to 10^{-6} M), and glomeruli were incubated for 10 min. To determine whether galactose reversed the effects of FSPF, glomeruli were incubated in medium with active serum for 10 min and then washed with fresh medium; galactose, 10^{-6} M, was added and incubation was continued for 60 min. To determine whether galactose pretreatment prevented the effect of FSPF, glomeruli were incubated with galactose, 10^{-18} M, 10^{-12} M, or 10^{-6} M, before incubation with FSGS sera. In each series, P_{alb} was measured.

Administration of galactose to patient with recurrent FSGS. The effect of galactose was studied in a 30-year-old male patient with proteinuria and renal failure caused by recurrent FSGS. Pyrogen-free galactose solution, 30 g in 200 mL of saline solution, was infused intravenously over 1 h. Serum was collected before, immediately after, and 48 h after infusion.

Oral administration of galactose solution. Approximately 4 years later, the same patient ingested galactose (15 g galactose in 120 mL of tap water twice a day for 6 weeks) while awaiting cadaveric transplant. Serum was collected and P_{alb} tested after 1, 2, and 4 weeks of galactose ingestion and 4 weeks after discontinuing galactose.

Dialysis of pregalactose and postgalactose sera. To determine whether galactose inactivated FSPF or whether FSPF was permanently removed, sera were extensively dialyzed against galactose-free medium (MW cutoff 1 kDa); P_{alb} was tested and compared with the values before dialysis.

P_{alb} measurement. Methods for measuring P_{alb} ¹⁰ in injured glomeruli and the application of this method to the study of FS permeability activity have been described.^{4,6,7,9,11,12} Glomeruli isolated from the superficial cortex of kidneys of normal rats (250–300 g) in medium containing an isotonic salt solution, and 5-g/dL bovine serum albumin (BSA) were incubated for 10 min with PPF, serum, or test material (2% vol/vol). Videomicroscopic images were recorded before and after changing the medium to 1-g/dL BSA medium. Exchange of medium generated a transcapillary oncotic gradient and resulted in an increase in capillary fluid and of total glomerular volume. In these experiments, the net increase in glomerular volume is proportional to the oncotic gradient and to the solute reflection coefficient σ_{alb} . When the oncotic gradient applied is constant, $\sigma_{alb} = (\Delta V_{\text{experimental}}/\Delta V_{\text{control}})$. P_{alb} was calculated as $1 - \sigma_{alb}$.¹⁰

Studies were carried out according to the principles of the Declaration of Helsinki and with the approval of the Institutional Review Board and Animal Research Committee at the Medical College of Wisconsin.

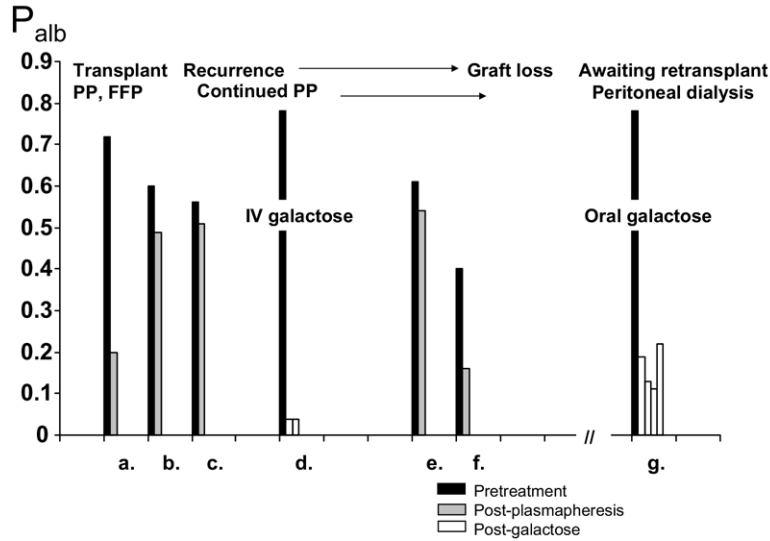


Fig 1. Serial P_{alb} values for patient with posttransplant recurrence of FSGS. Black bars show P_{alb} immediately before PP or galactose therapy. Gray bars indicate P_{alb} immediately after PP, and open bars indicate P_{alb} during or after galactose treatment. Effects of therapy are as shown: a. February 18–22, 2002. A 30-year-old man who had lost a prior cadaveric transplant to recurrent FSGS received 1 PP before transplant and additional PP as well as infusion of fresh frozen plasma (FFP) immediately after transplantation. This intensive therapy at the time of transplantation lowered $P_{alb} < 0.2$. Proteinuria returned within 2 weeks, and additional PPs were performed on multiple occasions, usually weekly. P_{alb} was tested only in the selected specimens shown in columns b–f. (March 6, March 29, December 3, 2002, and January 3, 2003). P_{alb} activity remained in the range of 0.5 and diminished little after each PP. d. Galactose infusion was performed June 6, 2002, about 3 months after transplantation, to attempt to diminish P_{alb} activity and to decrease proteinuria. At that time, serum creatinine was 4.3 and proteinuria was in the range of 8–10 g/g creatinine. P_{alb} decreased immediately after, and 48 h after infusion, it remained unmeasurable as shown in the 2 open bars. However, proteinuria continued and serum creatinine increased; immunosuppression was eventually withdrawn, and the patient returned to peritoneal dialysis. g. Oral galactose was taken during February and March 2006 at the request of the patient while he was awaiting a third transplant. Oral galactose decreased P_{alb} activity to 0.19, less than 30% of pretreatment value, by 1 week (first open bar). P_{alb} was further decreased to 0.13 and 0.11 after 2 and 5 weeks (open bars 2–4), respectively, and was decreased to 0.22 4 weeks after galactose was stopped (final open bar).

Table I. Effect of pretest dialysis of patient serum against galactose-free medium

	P_{alb}	P_{alb} after dialysis	Increase after dialysis
Infusion of galactose, 30 g (0.42 g/kg)			
Pretreatment	0.88	0.67	-0.21
Postinfusion, immediate	0	0.39	0.39
Postinfusion, 48 hours	0	0.15	0.15
Ingestion of galactose, 60 g/day (0.84 g/kg/day)			
28 days after galactose was discontinued	0.22	0.44	0.22

Notes: Dialysis did not alter activity of pretreatment serum, partially restored activity to serum obtained immediately after galactose infusion, and had no significant effect of sera 48 h after infusion or during oral galactose therapy. Of note, based on the experimental variation in the P_{alb} measurement, we have previously defined a change of ≥ 0.3 as a significant difference in P_{alb} . We have used a cutoff of 0.5 as “positive” in clinical evaluation of patients.

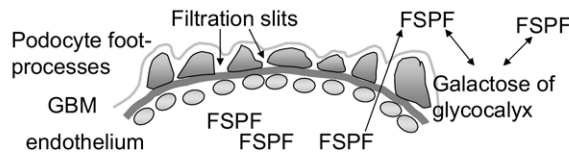
RESULTS

Effect of PP on P_{alb} . P_{alb} before PP averaged 0.63 ± 0.04 . After PP, P_{alb} was reduced to 0.27 ± 0.05 ($P < 0.0001$ vs pretreatment, $N = 30$). In patients who did not have PP, P_{alb} did not change significantly (ΔP_{alb} , 0.05 ± 0.05 , $P > 0.10$, $N = 27$). In the patient who later received galactose, pretransplant P_{alb} was 0.74. P_{alb} decreased to 0.20 after multiple PPs.

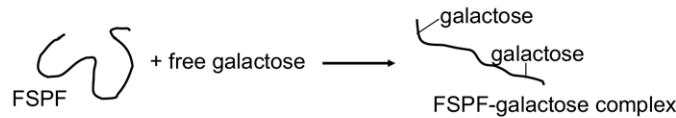
Galactose affinity chromatography. Approximately 0.003% of the total loaded protein was bound to the column. P_{alb} activity was undetectable in the unbound fraction. Bound proteins were eluted by 0.1 M galactose. P_{alb} was measured after dialysis and membrane filtration. P_{alb} activity was present only in the fraction of MW < 30 kDa.

Tests of FS serum activity after addition of galactose. The addition of galactose, 10^{-18} M, to medium with FSGS serum (2% vol/vol) decreased P_{alb} activity; P_{alb} was undetectable at concentrations $\geq 10^{-12}$ M galactose.

1. FSPF is filtered and interacts with galactose of podocyte glycocalyx



2. Free galactose binds to FSPF in plasma and blocks binding sites



3. FSPF-galactose complex is cleared by receptors in liver or other cells

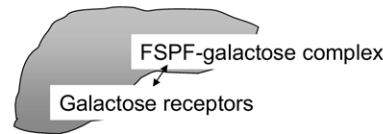


Fig 2. Proposed interactions among FSPF, galactose of the glomerular glycocalyx, and clearance receptors in the liver. We hypothesize that FSPF may have galactose-binding sites that permit it to interact with transmembrane proteins of the glycocalyx and to activate signal transduction in podocytes. Free galactose may block the binding sites on FSPF and/or it may change the FSPF configuration and prevent its binding to the podocyte. In addition, galactose may reverse FSPF binding to the podocyte by competitive inhibition as shown by reversal of increased P_{alb} after incubation in galactose-containing media. The galactose–FSPF complex may be cleared by glucose-specific receptors in hepatocytes or other cells, which accounts for its apparent disappearance from the circulation. Removal from circulation was evidenced by the failure of dialysis to restore activity to serum obtained after galactose treatment (Table I).

Removal of galactose by dialysis restored activity. Incubation with galactose, 10^{-6} M, for 60 min, reversed the effect of FSGS serum. Preincubation of glomeruli with galactose, 10^{-6} M, for 5 min before adding FSGS serum, prevented the increase in P_{alb} ; lower concentrations did not prevent the increase.

Administration of galactose to patient with recurrent FSGS. P_{alb} was 0.88 before infusion; P_{alb} was undetectable immediately after galactose infusion and 48 h later. P_{alb} was 0.80 before oral galactose use. P_{alb} decreased progressively during the 4 weeks of ingestion to a minimum of 0.1 and remained low (0.20) 4 weeks after galactose was discontinued (Fig 1).

Dialysis of pregalactose and postgalactose sera. Activity of sera was abolished by the addition of galactose and was restored by dialysis against galactose-free medium (data not shown). Dialysis against galactose-free medium did not alter P_{alb} of serum obtained before galactose infusion but increased P_{alb} of the immediate post–galactose-infusion serum from 0 to 0.39. Dialysis did not significantly increase activity of 48-h postinfusion serum or serum after oral galactose ingestion (Table I).

DISCUSSION

These findings document that FSPF has a high affinity for galactose and that galactose interferes with FSPF activity. All activity was removed by galactose affinity columns, and activity was recovered from eluate with apparent MW < 30 kDa. Activity of sera was blocked by interaction with soluble galactose, and the effect of active sera was reversed by incubation with galactose and was prevented by preincubation of glomeruli with galactose. These results suggest that galactose prevents interaction between FSPF and glomeruli. Both i.v. and PO galactose had a remarkable effect on P_{alb} , causing it to fall to unmeasurable levels immediately after infusion and to decrease within 1 week of oral administration. The effect of IV galactose was comparable with that of multiple PP treatments and persisted for at least 1 month after galactose was discontinued. Prior studies by us and other investigators show that P_{alb} activity is decreased by PP^{4,6} or immunoabsorption therapy⁵ and by chemotherapy for Hodgkin’s lymphoma.¹¹ In contrast, treatment with cryospray ablation does not decrease P_{alb} .¹² The remarkable response to galactose infusion with an immediate decrease in P_{alb} to unde-

tectable levels and the rapid and prolonged effect of oral galactose differed from the effect of PP.

At the time of galactose infusion, the patient had established FSGS with fixed proteinuria (8–10 g/g creatinine). Proteinuria was unresponsive to PP or cyclosporine. His serum creatinine was then 4.3 mg/dL, and transplant biopsy showed FSGS with severe scarring of 11 of 25 glomeruli as well as moderate, diffuse interstitial fibrosis. He had returned to dialysis at the time of galactose ingestion. The dissociation between P_{alb} and proteinuria may represent irreversible injury to the glomerular structure. Similar results were observed in a group of patients with resistant FSGS who were treated with PP and showed a decrease in P_{alb} but no remission of proteinuria.¹³

Our findings support the hypothesis that FSPF interacts with galactose. We postulate that the galactose-binding site on FSPF may be required for interaction with transmembrane signaling proteins on the podocyte and that galactose may prevent this interaction. Podocalyxin has signaling properties¹⁴ and is an example of a glycosylated podocyte protein that might interact with FSPF. Alternatively, galactose binding may alter the tertiary structure of FSPF and prevent its interaction with the podocyte. Blocking the interaction between FSPF and podocyte glycoproteins may prevent it from initiating signaling (Fig 2).

The immediate effect of intravenous galactose infusion is comparable with the *in vitro* loss of activity in the presence of galactose. The long-term decrease in P_{alb} after oral galactose and the failure of extensive dialysis to restore permeability activity are consistent with the clearance of FSPF from the plasma. Clearance of the FSPF–galactose complex may be explained by the finding that hepatic asialoglycoprotein receptors account for rapid clearance of proteins carrying galactose.¹⁵ These proposed mechanisms are illustrated in Fig 2. Galactose has been administered safely in diagnostic testing for glycogen storage disease, as a sonographic contrast agent and in treatment of Fabry's disease.¹⁶

CONCLUSION

We conclude that FSPF has high affinity for galactose and that a trace amount of galactose blocks or reverses the increase in P_{alb} . Galactose inactivates FSPF in the circulation and seems to lead to its clearance from the plasma. The interaction between FSPF and glomeruli may depend on FSPF binding to glomerular galactose. In addition, the FSPF–galactose complex may be susceptible to uptake and catabolism by asialoglycoprotein receptors in hepatocytes or macrophages. We propose that galactose should be tested in a prospective manner for its potential as a novel, non-

toxic, and relatively inexpensive therapy for treatment of nephrotic syndrome in early FSGS. If galactose prevents interaction between FSPF and the glomerulus, it may diminish proteinuria and delay or prevent progression to renal failure. In addition, preemptive therapy before transplantation may decrease the incidence or severity of FSGS recurrence and consequent graft loss.

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