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KINETICS AND MECHANISMS OF RELEASE OF SERUM PROTEINS OF INTOXICATED RAT LIVERS IMPLANTED INTO GUINEA PIGS

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Abstract: We examined the organic chemistry, kinetics, and mechanisms of release of rat serum proteins by aseptic rat donor livers (DL) pathologically intoxicated by CCl_4, alloxan, or cholera toxin implanted into the circulatory systems in parallel with the host livers (HL) of guinea pigs (GP). This implant model was biologically functional as hepatocytes of the DL released rat plasma proteins into the host's extracellular compartment (ECC) as monitored in GP serum samples collected during 4 h of the experiment. Furthermore, rat DL hepatocytes retained an in vivo preimplantation pattern of protein biosynthesis acquired in response to a regulatory mechanism most likely involving leukocytic endogenous mediator (LEM), kinins, or platelet activating factor (PAF), and consequently secreted the entire spectrum of acute-phase reaction (APR) serum proteins into the ECC of the host GP. Therefore, the impetus to synthesize and release APR proteins into GP circulation by intoxicated rat liver originated within and was retained by injured rat hepatocytes.

The primary aim of the present work is to examine the relationships of the organic chemistry, kinetics, and mechanism(s) of the APR of implanted pathological livers to histological examinations of biopsies of transplanted cadaveric livers into patients with terminal liver diseases. With this understanding, the use of anti-rejection drugs with consequent side effects, and the probability of re-transplantation due to rejection of transplanted cadaveric liver to patients undergoing liver transplant operations may then be minimized. In view of the currently successful use in our laboratories of a liver implant model in the rat to examine the viability of liver dialysis as an alternative to liver transplantation,[1] we were prompted to investigate changes in the biological function of chemically injured liver when implanted in parallel into the circulatory system of a healthy liver of a different species. Present data and conclusions therefrom are of significance as liver transplantations are expensive and dominated by rejection episodes and other complications leading ultimately to patient death.

Rats were rendered pathological via either intubation of 2 mL carbon tetrachloride,[2] or iv and ip administration of 50 mg alloxan[2] or 0.16 mg cholera toxin/kg body weight.[3] Under ether, blood was withdrawn at predetermined times corresponding to apexes of the APR of each of the three intoxicated models[2,3] (i.e., at 2 day, 36 day and 3 day post-intoxication, respectively). While under ether the rats were

terminated. As described earlier,[3] serum was separated for two-dimensional-immunoelectrophoresis (2-D-IEP) against anti-rat serum proteins prepared in rabbits, to determine the relative increase or decrease of APR proteins. Pathological livers were surgically removed and immediately perfused for 9-12 minutes with oxygenated L-15 medium supplemented with gentamicin sulphate to wash them free of rat plasma proteins and to minimize hepatic ischemia.[4] Thus, probabilities of early rejection of implanted rat DL into the circulatory system in parallel with the liver of a healthy GP[4] with consequent pathological complications were minimized. During this period of time, the host GP was surgically prepared for implantation of the donor rat liver. Sera (~10 μL) were separated from GP blood collected at 1, 2, 3, and 4 h post implantation and were 2-D-IEP assayed for released rat serum proteins against anti-rat serum proteins as described.[3] Triplicate determinations for each toxin model and a control are presented (Table).

In the control experiment, healthy rat liver implanted into GP circulatory systems released de novo biosynthesized rat serum proteins that had a 2-D-IEP spectrum whose proportions of protein immuno-precipitates were closely similar to those of healthy rat serum proteins, within an experimental uncertainty of 9-15%. Healthy DL initially released amounts of rat serum proteins into GP circulation at a rate of 29.43 $\pm$ 8% hepatic efficiency which decreased to 16.12 $\pm$ 2% after 4 h (Table). However, rates of release of de novo rat plasma proteins did not follow zero-order kinetics usually observed for artificial conditions (i.e., in vitro monolayer culture of rat hepatocytes in a serum-free L-15 medium).[5] This observation may suggest concomitant catabolism of de novo rat plasma proteins at the mitochondrial-lysosomal (ML) levels of either DL or HL and/or increased necrosis of rat hepatocytes due to rejection. When L-15 medium, of rat hepatic monolayer cultures or isolated perfused rat liver was enriched with ^{3}H-radiolabelled serum or iv introduced into rats, dialyzable radiolabelled amino acids were detected in the culture, perfusion medium or serum, indicative of in vitro or in vivo degradation of plasma proteins.[5] Thus, hepatic efficiency of DL and HL is most likely partitioned between biosynthesis and ML degradation[4] of plasma proteins alien to each liver. Once the GP immune system is alerted to the presence of rat hepatic tissue releasing plasma proteins into the ECC, GP hepatic ML enzymes that are most likely released in response to signals of inflammatory mediators, would indiscriminately degrade both GP and de novo rat plasma proteins. As well, necrosis of rat hepatic tissue initiated by rejection would lead to decreased biosynthesis of rat plasma proteins and to the release of rat ML enzymes into its own hepatic cytosolic milieu with consequent indiscriminate degradation of both rat and GP plasma proteins (Scheme). Thus, the observed level of released rat serum proteins in the ECC is a net result of biosynthesis and catabolism. This rational is supported by observation of other laboratories.[4] Similarly, during the post implantation period of 4 h, intoxicated rat livers secreted serum proteins into the GP

Table: Kinetics of hepatic release of de novo rat serum proteins into GP ECC. Healthy or pathological rat livers were implanted *ex-vivo* in GP and APR to inflammatory mediators and PR toxin.

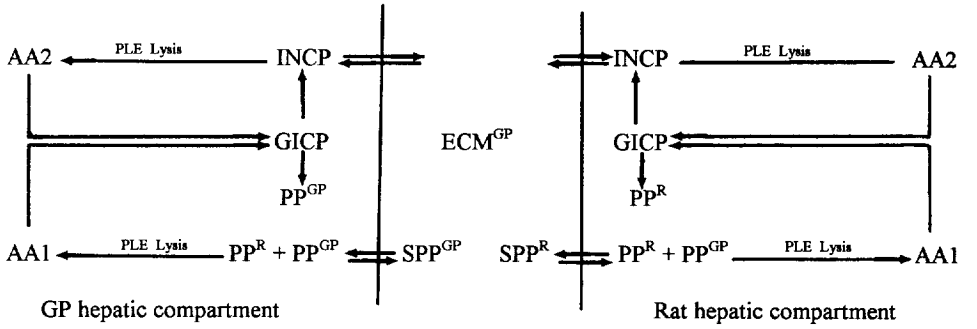
Protein	CRS	Hours Post Healthy Implant				Hours Post Intoxicated Implant						%increase/decrease in acute-phase protein Hours post innoculation*								
												PR	Histamine				Bradykinine			
		1	2	3	4	2:A	2:B	2:C	4: A	4: B	4:C	46	20	48	72	96	20	48	72	96
Alb	31.56	9.52	16.9	16.9	21.42	6.67	5.3	7.8	14.37	11.66	15.53	- 9	-15	-18	-25	- 6	-15	-23	-26	-11
α-Lip	2.59	0.78	1.44	1.69	1.64	0.31	0.45	0.58	0.68	0.9	1.31	24	-18	-31	-27	37	-24	-44	-32	-29
Hg	9.87	2.8	4.38	5.72	5.88	6.8	9.3	9.1	14.05	20.5	18.2	- 9	68	63	78	47	67	57	99	63
Hpx	4.96	1.49	2.68	3.04	3.13	3.17	pna	pna	6.97	pna	pna	-60	12	10	21	- 3	12	-31	18	0.2
Peak X	10.47	2.91	5.22	5.94	6.31	5.1	2.15	4.48	11.27	4.72	6.72	- 5	30	43	39	34	22	15	63	45
Tf	17.0	5.13	8.39	9.46	11.69	4.9	2.87	5.69	10.87	5.81	12.52	2	32	42	46	36	33	39	68	35
α-At	13.65	3.9	6.24	7.95	8.39	5.46	5.25	6.24	11.4	11.28	12.27	3	59	55	62	59	97	59	86	42
α-1-Ag	2.9	0.8	1.39	1.63	1.68	9.9	0.42	1.92	21.28	0.91	4.25	305	86	290	178	55	188	148	335	69
Ig G		pna	pna	pna	pna	pna	pna	pna	pna	pna	pna	12	-67	-55	-64	-22	-53	-43	-43	-42
C3		pna	pna	pna	pna	pna	pna	pna	pna	pna	pna	-25	8	13	0	11	2	- 7	29	2
Efficiency**		29.43	25.21	20.12	16.12															

PR, toxin isolated from penicillium roqueforti; Alb, albumin; α-Lip, α-lipoprotein; Hg, haptoglobin; Hpx, hemopexin; peak X, unidentified acute-phase plasma protein; Tf, transferrin; α-At, α-1-antitrypsin; α-Ag, α-1-acidglycoproteins; IgG, immunoglobulin G; C3, C3 globulin; pna, immunoprecipitate peak not apparent; A, B, and C denote cholera, alloxan and CCl4 intoxicated rat livers harvested at maximal APR and implanted ex vivo into GP. CRS, control rat serum. Experimental uncertainty ranges between ± 5-8%.

*In vivo % increase/decrease in acute-phase protein synthesis relative to healthy rats.
**Total protein synthesized/h (relative to healthy rats), mean of three determinations.

circulation whose immunoprecipitates had proportions similar to that of serum collected from intoxicated rats immediately prior to surgical hepatectomy. This observation may suggest that toxin stimulated hepatocytes of rat DL have retained a pattern of protein biosynthesis acquired prior to implantation following a response to a regulatory mechanism that may involve LEM,[5] PAF,[6] and/or kinins[7] which continued the secretion of APR rat serum proteins into the GP circulatory system after implantation. We considered as a plausible alternative that injured rat liver cells lyse and release their contents into the GP circulation after transplantation as a result of toxin-induced hepatocyte necrosis. In this model, these are assumed to be pre-existing proteins in DL cells that appear in the host circulation. As injury progresses, additional cells are injured and release their contents just as previously released in the donor animal, regardless of whether the liver is in the donor animal or in the new host animal. Experimental data reported by this group[2,5,8-10] and others working in this field[11,12] support the present thesis. Monolayer cultures of collagenase isolated rat hepatocytes in serum-free medium or hepatocytes in isolated perfused organ with serum-free medium, released radiolabelled de novo biosynthesized plasma proteins[2,8,11] into the extracellular medium when the culture or perfusion medium was enriched with a mixture of ^{3}H-labelled amino acids. As well, measured rates of release of radioactive plasma proteins[2] pointed to the conclusion that hepatic intracellular concentrations of plasma proteins are at all times at a steady state concentration of zero.[12] Furthermore, livers isolated from rats sc implanted with Yoshida sarcoma or ip inoculated with Zajdela ascietes and perfused with L-15 medium enriched with ^{3}H-labelled amino acids, released rapidly increasing amounts of radiolabelled plasma proteins into the perfusion medium in a manner similar to the in vivo release of APR plasma protein.[9] As well, in vitro kinetics of hepatic nuclear proliferative activity and release of APR proteins into culture medium of α-amanitin or phalloidin intoxicated rat hepatocytes[10] or in vivo kinetics of hepatic nuclear proliferative activity and release of APR proteins into the circulation of CCl_4, α-amanitin or phalloidin intoxicated rat[10] pointed to intracellular hepatic nuclear proliferative activity and release of corresponding de novo biosynthesized APR plasma proteins. The present data show that the relative concentration of α-1-antitrypsin to albumin remained almost constant within a given liver implant experiment (Table). α-1-Antitrypsin is an APR substrate that interacts with α-2-macroglobulin and inactivates proteolytic enzymes including kallikrein to limit autodigestion of necrotic tissue. This experimental observation further supports our deduction; namely toxin stimulated hepatocytes of rat DL have retained a pattern of APR protein biosynthesis acquired prior to implantation and continued to secret APR rat serum proteins into the GP circulatory system after implantation. This demonstrated the viability of implanted liver model which suggests that an artificial hepatic dialysis cartridge containing living

Scheme: Proposed mechanism for hepatic internalization, catabolism, biosynthesis and release of plasma proteins of ex vivo perfusion of rat liver by GP.



GICP, general intracellular pool of amino acid/carbohydrate monomers; AA1& AA2, amino acid/carbohydrate pools due to proteolysis of plasma and hepatic intracellular proteins respectively; PP, plasma proteins; SPP; secreted plasma proteins; INCP, intracellular proteins; PLE, proteolysis by ML enzymes; ICC, hepatic intracellular compartment and ECM, extracellular compartment. Superscripts R and GP denote rat and guinea pig respectively.

hepatocytes could assist a patient's cirrhotic liver[13] to recover and resume normal functions. It is interesting that i.p. inoculation of inflammatory mediators such as histamine (37 mg/kg), bradykinine (19 mg/kg), or PR toxin to rats precipitated a moderate APR (Table) which lasted for 96 h in contrast to the more severe APR associated with the administration of various injurious stimuli.[4] These results could indicate that the concentrations of administered mediators did not correspond to the concentrations released following pathological injury and/or that other inflammatory mediators are involved. Even though the current experiments are limited to 4 h post implantation, they suggest further research centered on the identification and elimination of harmful circulating toxins from blood streams of individuals with incapacitated livers via in vivo liver dialysis. Initially, liver dialysis may be achieved by means of connection of an aseptic liver of compatible animals (e.g., baboon or hog, to the circulatory system of the cirrhotic patient). Ultimately such toxins may be extracted from blood by circulation through external devices containing immobilized antibodies to these toxins to reduce the metabolic load on a fatigued hepatic system, and increase the ability of the remaining viable hepatocytes to proliferate. Antibodies attached to resin beds could be regenerated once exhausted. This could be an attractive alternative to liver grafts, which have risks of infection,[14] rejection[15] and retransplantation,[16] as the proposed alternative is a temporary one with minimal chance of infection and which involves neither major surgical manipulations nor risks of rejection. It is our contention, regardless of the mechanism by which APR proteins are released into circulation, that histological examination of liver

biopsies would have greater value if the organic chemistry, and mechanism(s) of the APR of cirrhotic or transplanted livers are considered. Therefore, it is imperative to quantitate hepatic efficiency in terms of biosynthesis and release of APR proteins as the functional efficacy of metabolically burdened hepatocytes of the DL implant is expected to exceed that of healthy ones.[2,4] Finally, cadaveric donor liver should be kept as much as possible in optimal physiological condition with evaluation of its functional viability by measuring corresponding APR released into the perfusion medium prior to transplantation into the recipient. Possibilities of rejection, use of anti-rejection drugs with consequent side effects, and the probability of retransplantation may then be lessened.

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