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Porcine alanine transaminase after liver allo-and xenotransplantation

Burcin Ekser^{1,2}, Bruno Gridelli^{1,3}, and David K.C. Cooper¹

¹Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

²Department of Surgery, Transplantation and Advanced Technologies, Vascular Surgery and Organ Transplant Unit, University Hospital of Catania, Catania

³Mediterranean Institute for Transplantation and Advanced Specialized Therapies, Palermo, Italy

Abstract

Aspartate transaminase (AST) and alanine transaminase (ALT) are measured following liver transplantation as indicators of hepatocellular injury. During a series of orthotopic liver allo-and xenotransplants, we observed that there was an increase in AST in all cases. The anticipated concomitant rise in ALT did not occur when a wild-type (WT) pig was the source of the liver graft, but did occur when a baboon or a genetically engineered (1,3-galactosyltransferase gene-knockout [GTKO]) pig was the source of the graft. We hypothesized that the cience of Gal 1,3 Gal in GTKO pig livers may render pig hepatocytes similar to human and baboon hepatocytes in their response to hepatocellular injury. Reviewing the literature, after WT pig liver allotransplantation or xenotransplantation, in the majority of reports, although changes in AST were reported, no mention was made of changes in ALT, suggesting that there was no change in ALT. However, Ramirez et al. reported two cases of liver xenotransplants from hCD55 pigs, following which there were increases in both AST and ALT, suggesting that it is not simply the cience of expression of Gal 1,3 Gal that is the cause. We acknowledge that our observation is based on a small number of experiments, but we believe it is worth recording.

Keywords

alanine transaminase; allotransplantation; aspartate transaminase; liver; pig; xenotransplantation; 1,3-galactosyltransferase gene-knockout

Introduction

Aspartate transaminase (AST) and alanine transaminase (ALT) are commonly measured in the days following liver transplantation in the evaluation of hepatocellular injury. Although AST and ALT activity can be associated with injury of other organs (e.g., heart, brain, skeletal muscle for AST, and heart, skeletal muscle for ALT), they are both considered good indicators of hepatocyte integrity [1,2].

During a series of orthotopic liver allo- and xenotransplants carried out at our center [3], we observed that, within 48 h after the transplant, there was an increase in AST (anticipated

from ischemia-reperfusion injury) in all cases (Fig. 1A). However, the anticipated concomitant rise in ALT did not occur whenever a wild-type (WT) pig was the source of the liver graft, but did occur whenever a baboon or a genetically engineered pig (1,3-galactosyltransferase gene-knockout [GTKO; n = 1] or GTKO transgenic for hCD46 [n = 5] pig) was the source of the graft (Fig. 1B). Differences in ALT between genetically engineered pig-to-baboon and WT pig liver allotransplantation were significant at 5 h, 1, 2, 3, and 4 days after transplantation ($P < 0.01$), after which ALT values normalized. The transient increase in ALT after genetically engineered pig-to-baboon liver xenotransplantation and baboon-to-baboon liver allotransplantation was similar to that seen after clinical liver allotransplantation.

We considered possible reasons for this observed difference. We suggest that WT pig hepatocytes may be more resistant to hepatocellular damage than GTKO pig hepatocytes, possibly because of the expression of Gal 1,3 Gal. The absence of Gal 1,3 Gal in GTKO pig livers may render pig hepatocytes more similar to human and baboon hepatocytes in their response to hepatocellular injury.

We searched the literature for evidence to support this observation.

Literature review-experimental liver transplantation

Medline and Pubmed were searched for articles published in pig liver allo- and xenotransplantation. Search terms included “xenotransplantation”, “xenograft”, “allotransplantation”, “allograft”, and “transplantation”, in combination with “large animals”, “pig”, “porcine”, “swine”, “baboon”, “monkey”, and “nonhuman primate”. Those considered relevant included reports in which the measurement of AST or ALT or both were reported after liver transplantation.

Several groups used pig-to-pig allotransplantation models to study the effect of different preservation solutions [4,5], cold and/or warm ischemic damage [6,7], and different medications on graft survival [8–11]. After WT pig liver allotransplantation, we could not find any report of increase in ALT; in the majority of reports, however, no mention was made of changes in ALT (although changes in AST were reported), suggesting to us that there was no change in ALT, and therefore, it was not considered worthy of reporting. Not surprisingly, there are no reports of allotransplantation between GTKO pigs.

Terblanche et al. [12] reported a slight rise only in AST, but did not mention ALT, in seven cases of WT pig liver xenotransplantation in baboons which were followed for only 2 to 10 h. In the same model, Powelson et al. [13] reported survival of up to 75 h, but only commented on AST at three time-points (1, 2, and 3 days) when increases were seen; there was no mention of ALT.

With the exception of our study [3], there was only one study that reported transplantation of liver from genetically engineered pigs into baboons with reported liver function [14]. Ramirez et al. reported two cases of liver xenotransplants from hCD55 pigs. Both AST and ALT displayed the same trend as baboon and human liver allotransplantation. This suggests that it is not the absence of expression of Gal 1,3 Gal that is the cause of the difference from WT pigs.

Literature review-normal AST and ALT values in humans, baboons, and pigs

We also surveyed the literature and our own data for normal levels on AST and ALT in pigs. Naïve pig AST values were similar between those reported in the literature and obtained in our own studies (WT or GTKO pigs); furthermore, they did not differ significantly from values in healthy humans and baboons. When ALT values were measured in WT and GTKO

pigs (Table 1), there was no significant difference between healthy human, baboon, and GTKO pig values, but WT pig ALT values were higher than GTKO pig values. Naïve pig ALT values varied depending on the breed of the pig (Table 1), with ALT varying significantly [15–17]. Naïve WT pig ALT values in the literature (57 ± 10 IU/l) and obtained from our studies (78 ± 18 IU/l) were significantly different from ALT values measured in genetically engineered pigs ($P < 0.01$) (Table 1). However, mean naïve genetically engineered pig ALT was not significantly different from that of humans and baboons (Table 1).

Comment

We acknowledge that the number of studies on which our observations are made is few and that there are no reports of liver allotransplantation between genetically engineered pigs in the literature. Nevertheless, although we cannot draw a final conclusion on whether the absence of Gal 1,3 Gal in GTKO pigs renders pig hepatocytes similar to human and baboon hepatocytes in their response to hepatocellular (e.g., ischemia/reperfusion) injury or whether the presence of Gal 1,3 Gal on WT pig hepatocytes may make them more resistant to damage, we believe our tentative observation is worth recording.

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Abbreviations

ALT	alanine transaminase
AST	aspartate transaminase
GTKO	1,3-galactosyl-transferase gene-knockout
WT	wild-type

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Fig. 1.

(A) Mean AST values in different models of allo- and xenotransplantation. WT-WT Pig Allo w or w/o IS (WT-WT) = WT pig liver allotransplantation with or without immunosuppression. WT Pig-to-Baboon Xeno w/o IS (WT-Bab) = WT pig-to-baboon liver xenotransplantation without immunosuppression. GE Pig-to-Baboon Xeno w IS (GE-Bab) = genetically engineered (CD55, GTKO, GTKO/CD46) pig-to-baboon liver xenotransplantation with immunosuppression. Baboon-to-Baboon Allo w IS (Bab-Bab) = baboon-to-baboon liver allotransplantation with immunosuppression. Time-point “0” in the *x*-axis of the graph indicates pre-transplant data. Numbers underneath the time-points after transplantation indicate total number of transplants for each group. (P-values were calculated between groups WT-WT and GE-Bab.) Other groups WT-Bab and Bab-Bab were not included in statistical Student’s *t*-test calculation owing to the single transplant in each group. **P* < 0.01. Data are presented as mean ± SD. Significance of the difference between two groups was determined by paired, two-tailed Student’s *t*-test using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Values of *P* < 0.05 were considered significant. (B) Mean ALT values in different models of allo- and xenotransplantation. Time-point “0” in the *x*-axis of the graph indicates pre-transplant data. Numbers underneath the time-points after transplantation indicate total number of transplants for each group. (P-values were calculated between groups WT-WT and GE-Bab.) Other groups WT-Bab and Bab-Bab were not included in statistical Student’s *t*-test calculation owing to the single transplant in each group. **P* < 0.01. (Other abbreviations as for A).

Table 1

Reference AST and ALT values for humans, baboons, wild-type and genetically-engineered pigs

	Human Range (UPitt)	Baboon (Literature) n = 173	Baboon (UPitt) n = 31	WT Pig (Literature) n = 127	WT Pig (UPitt) n = 17	GE Pig (UPitt) n = 10
AST (IU/l)	<40	40 ± 12	39 ± 13	39 ± 23	41 ± 15	39 ± 14
ALT (IU/l)	<40	35 ± 15	31 ± 12	57 ± 10 [*]	78 ± 18 [*]	44 ± 7 [*]

AST = aspartate transaminase, ALT = alanine transaminase, GE = genetically-engineered, UPitt = University of Pittsburgh, WT = wild-type. Baboon data were obtained from Havill et al (18) (n = 106) and Schuurman et al (19) (n = 67). WT pig data were obtained from Rispat et al (17) (n = 127). Different ranges were reported depending on the breed of pig by Bollen (15), Egeli (16), Rispat (17):-

Swine - AST 14–56 IU/l; ALT 5–78 IU/l;

Miniature swine (Yucatan micropig, Gottingen, Sinclair minipig) - AST 13–47; ALT 40–106 IU/l;

Miniature swine (Yucatan, Hanford minipig) - AST 15–53; ALT 20–48 IU/l

Data are presented as mean SD.

^{*} WT pig (literature) vs WT pig (UPitt); WT pig (literature) vs genetically-engineered pig (UPitt); WT pig (UPitt) vs genetically-engineered pig (UPitt) – all P < 0.01.