Published in final edited form as:

J Surg Res. 2008 June 15; 147(2): 200–205. doi:10.1016/j.jss.2008.02.004.

Activation of Peroxisome Proliferator-Activated Receptor- γ During Hepatic Ischemia is Age-Dependent

Thomas Shin, M.D., Satoshi Kuboki, M.D., Nadine Huber, M.D., Thorsten Eismann, M.D., Elizabeth Galloway, M.D., Rebecca Schuster, B.S., John Blanchard, M.S., Timothy A. Pritts, M.D.Ph.D., and Alex B. Lentsch, Ph.D.

The Laboratory of Trauma, Sepsis & Inflammation Research, Department of Surgery, University of Cincinnati, Cincinnati, OH 45267

Abstract

Hepatic ischemia/reperfusion (I/R) injury is a complication of liver surgery, transplantation and shock and is known to be age-dependent. Our laboratory has recently shown that peroxisome proliferator-activated receptor- γ (PPAR γ) is downregulated during hepatic ischemia and that this exacerbates injury. Here we examined whether activation of PPAR γ during ischemia was age-dependent. Male mice of different ages (young: 4–5 weeks; adult: 10–12 weeks; old: 10–12 months) were subjected to up to 90 minutes of hepatic ischemia. PPAR γ activation occurred throughout ischemia in young mice, whereas activation in adult and old mice was lost after 30 minutes. No significant differences were noted in PPAR γ ligand expression amongst the age groups. However, in young mice we observed a predominance of PPAR γ 1 in the nucleus, whereas in old mice this isoform remained largely in the cytoplasm. Finally, the degree of PPAR γ activation was associated with autophagy in the liver, a mechanism of self-preservation.

Conclusion—PPAR γ activation is prolonged in young mice as compared to older mice. This appears to be mediated by a selective retention of PPAR γ 1 in the nucleus and is associated with increased autophagy. The data suggest that PPAR γ activation is an important component of the age-dependent response to hepatic I/R injury.

Keywords

liver injury; transcription; autophagy; hepatocytes

Introduction

Hepatic ischemia/reperfusion (I/R) injury is a complication of a number of diverse clinical settings such as trauma, vascular surgery, liver resection and transplantation(1). Hepatic I/R injury has been modeled in rodents and consists of initial phase mediated by reactive oxygen species released by Kupffer cells (2). The later phase of injury is dependent upon the recruitment of activated neutrophils that cause direct damage to hepatocytes by release of oxidants and proteases (2). We have shown that there is a distinct difference in the injury response to hepatic I/R in mice of different ages (3). These studies demonstrated that mice 10–12 weeks of age had significantly less liver injury than mice 9–12 months of age. These ages

Correspondence: Alex B. Lentsch, Ph.D., University of Cincinnati, Department of Surgery, 231 Albert Sabin Way, Cincinnati, OH 45267-0558, Phone: (513) 558-8674, Fax: (513) 558-8677, alex.lentsch@uc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

correspond approximately to 10–15 year old and 40–50 year old humans, respectively (3). Interestingly, trauma and critical care physicians have long noted a significant divergence in the responses of pediatric and adult populations to severe trauma (4). There is also evidence that age is an important factor in liver transplantation as well as liver resection with or without ischemic-preconditioning (5,6). The underlying mechanism(s) by which age alters the injury response to liver injury is unknown.

Peroxisome proliferators-activated receptors (PPARs) belong to the hormone nuclear receptor superfamily and consist of three isoforms, PPAR α , PPAR β/δ and PPAR γ . Of these, we and others have recently shown that PPAR γ is an important regulator of post-ischemic liver injury (7,8). We found that activation of PPAR γ was suppressed during the ischemic period and that treatment with a synthetic PPAR γ ligand restored activation and reduced injury (7). In the present study, we examined whether PPAR γ activation in the liver during ischemia was age-dependent. Our data provide direct evidence that PPAR γ activation is preserved in animals corresponding in age to a pediatric human population, and may represent a significant age-related regulatory mechanism of I/R injury in the liver.

Materials and Methods

Hepatic ischemia/reperfusion injury model

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were used. The project was approved by the University of Cincinnati Animal Care and Use Committee and was in compliance with the National Institutes of Health guidelines. Three ages of mice were utilized: “young” mice were 4–5 weeks old, “adult” mice were 10–12 weeks of age, and “old” mice were 10–12 months old. Mice underwent either sham surgery or partial hepatic ischemia as described elsewhere (9). Briefly, mice were anaesthetized with pentobarbital (60 mg/kg, i.p). A midline laparotomy was made and an atraumatic clip was placed at the portal confluence to interrupt the blood supply to the left and median lobes of the liver which consists about 70% of the total liver. Animals underwent either 30, 60, or 90 minutes of ischemia after which time ischemic lobes of the liver were harvested. Sham control mice underwent the same operation without vascular clamping. Core body temperature was maintained between 34–36 °C from time of anaesthesia to sacrifice. Some adult mice were pretreated orally with methylcellulose (vehicle) or 10 mg/kg rosiglitazone dissolved in methylcellulose 30 minutes prior to the induction of ischemia.

Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts of liver tissue were prepared by the method of Deryckere and Gannon (10). Double-stranded consensus oligonucleotides to PPAR (Santa Cruz Biotechnology, Santa Cruz, CA) was end labeled with γ [32 P] adenosine triphosphate (3000 Ci/mmol at 10 mCi/mL; GE health). Binding reactions (total of 15 μ L) containing 20 μ g of protein extract and 35fmol of oligonucleotide were incubated for 30 minutes at room temperature. Binding reaction products were separated in a 4% polyacrylamide gel.

Western Blot Analyses

Liver nuclear or cytosolic extracts containing 50 μ g of protein were separated in a denaturing polyacrylamide gel. Proteins were transferred to a 0.45 μ m pore polyvinylidene difluoride membrane and blocked for 1 hour at room temperature with 5% nonfat dry milk in tris-buffered saline with 0.1% Tween 20. Membranes were then incubated with antibodies for PPAR γ (Santa Cruz Biotechnology, sc 7273) and LC3B (Cell Signaling #2775) according to the manufacture protocol. Secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) was detected by chemiluminescence (Denville Scientific Inc., South Plainfield, NJ). Protein loading was confirmed by staining for β -actin.

Tissue Analysis

Levels of 15-deoxy-delta-12,14-prostaglandin J₂ (15d-PGJ₂) in the liver were measured by ELISA using reagents obtained from Assay Designs Inc. (Ann Arbor, MI). Methods for extracting samples for tissue ELISA are described elsewhere (7). The measurement of liver fatty acid composition was performed by gas chromatography at the Mouse Metabolic Phenotyping Center at the University of Cincinnati.

Statistical analysis

All data are expressed as mean $\pm$ SEM. Data were analyzed with a one-way analysis of variance with subsequent Student-Newman-Keuls test. Differences were considered significant when $P < 0.05$.

Results

PPAR γ activation decreases with ischemia but is prolonged in young mice

In order to assess the activation of PPAR γ during hepatic ischemia, liver nuclear extracts were assayed by EMSA. In sham-operated controls, there was constitutive activation of PPAR γ which was similar in all age groups (Figure 1). After 30 minutes of ischemia, activation of PPAR γ did not change from sham levels in young mice, but almost completely disappeared in adult and old mice. After 60 minutes of ischemia, PPAR γ activation was reduced, but still present in young mice, but lacking in adult and old mice. After 90 minutes of ischemia, there was limited activation of PPAR γ in young mice and none in adult or old mice (Figure 1). The specificity for PPAR γ binding was verified by unlabelled oligonucleotide competition and PPAR γ antibody supershift assays as previously described (7).

Activation of PPAR γ is not due to age-dependent differences in ligand expression

15d-PGJ₂ is thought to be a primary endogenous ligand of PPAR γ (11). We have previously shown that in adult mice, 15d-PGJ₂ levels progressively decrease with ischemia reaching a nadir at 90 minutes of ischemia (7). Here we show similar results, and that there are significant reductions in hepatic levels of 15d-PGJ₂ in old mice (Figure 2). Interestingly, the level of 15d-PGJ₂ in young mice was lower than in the older groups and did not significantly change during the ischemic period. We also investigated the hepatic levels of other known PPAR γ ligands (12), including arachidonic acid, linoleic acid, and petroselenic acid as well as possible ligands, cervonic acid, palmitic acid and stearic acid. We observed no differences in the hepatic levels of these mediators (Figure 2).

Divergent compartmentalization of PPAR γ 1 in young and old mice

To further evaluate the nature of PPAR γ in liver cells, we examined the composition of the PPAR γ in cytoplasmic and nuclear extracts from livers of young, adult and old mice during ischemia. PPAR γ has two isoforms, PPAR γ 1 and PPAR γ 2, generated by alternative promoter usage and splicing (13). PPAR γ 2 is 30 amino acids longer than PPAR γ 1 in the N2-terminus and therefore has a lower mobility by gel electrophoresis. In the cytoplasm, expression of PPAR γ 2 was similar amongst age groups at all times examined (Figure 3). Interestingly, PPAR γ 1 was abundant in the cytoplasm of old mice during hepatic ischemia (Figure 3). In nuclear extracts from sham-operated mice, PPAR γ 1 was the predominant isoform present and had similar expression amongst the age groups (Figure 3). After 30 minutes of ischemia, the expression of PPAR γ 1 was maintained in the young but was significantly reduced in the older mice. After 60 minutes of ischemia, there was further loss of PPAR γ 1 expression but still with greater level in the young mice. By 90 minutes of ischemia, there was limited expression of PPAR γ in all age groups.

Extent of autophagy is associated with the degree of PPAR γ activation

Autophagy is a mechanism of self-protection during stress in which energy is produced by the recycling of old proteins and organelles via the lysosomal degradation (14). PPAR γ activation has been previously linked with increased autophagy (15). Therefore, we examined the extent of autophagy in the liver at various times of ischemia. Autophagy was determined by immunoblotting for LC3B, a specific marker of autophagy (16). Sham-operated controls of all age groups showed high levels of constitutive autophagy activity as indicated by strong staining of LC3B-II (Figure 4). With progressive ischemia, autophagy decreased in all groups. However, young mice had no discernable change in autophagy after 30 minutes of ischemia and a less marked reduction in autophagy after 60 or 90 minutes of ischemia, compared to adult and old mice (Figure 4).

Activation of PPAR γ with rosiglitazone increases autophagy during ischemia

To access if PPAR γ activation is directly responsible for the observed differences in autophagy, we pretreated adult mice with 10 mg/kg rosiglitazone 30 minutes prior to ischemia. We have shown previously that rosiglitazone pretreatment increases PPAR γ activation in the liver and is associated with decreased I/R injury (7). Treatment with rosiglitazone increased the autophagy at all ischemic time points when compared to the vehicle treated groups (Figure 6).

Discussion

The present study provides strong evidence that PPAR γ activation is preserved in the livers of young mice and that this may represent an important age-dependent protective mechanism against hepatic I/R injury. Our recent studies have demonstrated that pretreatment of adult mice with rosiglitazone activated PPAR γ in hepatocytes which significantly reduced liver I/R injury (7). In contrast, PPAR γ +/- mice, which have diminished PPAR γ activity, had more severe liver injury than the wild type mice. Interestingly, the rosiglitazone treatment resulted primarily in restoring PPAR γ activation during the early ischemic period only. Thus, the activation of PPAR γ induced by rosiglitazone treatment in adult mice is nearly identical to that observed in young mice in the current study. This suggests that prolonged activation of PPAR γ during hepatic ischemia is an age-dependent protective mechanism that is lost in adult and old mice.

To determine if the differences in PPAR γ activation were due to differences in endogenous ligands, we measured the expression of several ligands during ischemia. In agreement with our previous study, level of PPAR γ ligand 15d-PGJ₂ decreased sharply during ischemia in the adult/old mice. Surprisingly, the level of 15d-PGJ₂ was lowest in the young mice and did not change during ischemia. Furthermore, there was no significant difference in the levels of other PPAR γ ligands between age groups. These data suggest that the differences in PPAR γ activation between young mice and the older groups are not related to ligand expression.

PPAR γ has two isoforms, PPAR γ 1 and PPAR γ 2, generated by alternative promoter usage and splicing (13). PPAR γ 1 is the predominant isoform found in all tissues that express PPAR γ , except in the adipocytes where PPAR γ 2 is the major isoform. In the present studies, we found a differential compartmentalization of PPAR γ isoforms in the liver. In the cytoplasm, expression of PPAR γ 2 is similar in all age groups and does change significantly with ischemia. Interestingly, PPAR γ 1 was observed in the cytoplasm only in the old mice during ischemia. However, in the nucleus there was much stronger expression of PPAR γ 1 compared to PPAR γ 2. In all age groups, nuclear expression of PPAR γ 1 was highest at baseline and gradually diminished during ischemia. In young mice, loss of PPAR γ 1 expression was significantly delayed. PPAR γ 1 expression in the nucleus is strikingly similar to PPAR γ -DNA-binding kinetics, suggesting that PPAR γ 1 may be the active isoform in the hepatocytes. Another notable finding of our study is that in the old mice, the loss of nuclear PPAR γ 1 during ischemia is

accompanied by its appearance in the cytoplasm. These observations raise the possibility that the rapid loss of PPAR γ activity in the old mice may be due to selective trafficking of PPAR γ 1 to the cytoplasm during ischemia in this age group.

Although others have reported that PPAR γ regulates hepatic I/R injury by modulating inflammation (8), our collective studies suggest that other, non-inflammatory mechanisms are important. Our previous study (7) showed that activation of PPAR γ reduced injury without affecting the inflammatory response. Furthermore, that study showed that the majority of PPAR γ -DNA-binding activity in the liver was found in hepatocytes. However, the mechanism by which PPAR γ activation could be directly hepatoprotective was not discerned. Our current study suggests that increased PPAR γ activation is associated with increased autophagy during ischemia. Importantly, we demonstrate that rosiglitazone treatment, which increases PPAR γ activation in hepatocytes, also increases autophagy. This suggests a direct link between PPAR γ activation and induction of autophagy. Autophagy is an evolutionarily conserved cellular process for recycling of old proteins and organelles via the lysosomal degradation (14). It has a constitutive function in resting cells where it is involved in the routine turnover of proteins and organelles. Autophagy can also be activated in stressful stimuli such as nutrient deprivation, I/R and oxidative stress (16,17). Induction of autophagy in certain situations has been shown to be protective. For example, autophagy induction after nutrient deprivation is essential for cell survival (14). Recent discoveries suggest that autophagy during ischemia may have protective function by providing cells with the energy derived from lysosomal degradation of cellular materials (18). Thus, it is plausible that prolonged activation of PPAR γ in young mice confers hepatoprotection by more effective recycling of cellular components for energy.

A previous study from our lab showed that another PPAR, PPAR α , was protective against hepatic I/R injury (19). However, that study did not assess the activation kinetics of PPAR α , or whether there was any difference in PPAR α activation relevant to age. Whether PPAR α has similar regulatory effects as PPAR γ in this response warrants further study.

In summary, we show that during hepatic ischemia, PPAR γ activation is significantly prolonged in young mice compared to older mice. This increased activity was associated with higher expression of PPAR γ 1 in the nucleus. PPAR γ activation was also associated with autophagy in the liver. Stimulation of PPAR γ in adult mice with rosiglitazone treatment resulted in increased autophagy, demonstrating a direct link between PPAR γ activation and induction of autophagy. These data indicate an age-dependent difference in PPAR γ activity and suggest that previously documented hepatoprotective effects of PPAR γ may be related to induction of autophagy.

Acknowledgments

This work was supported by National Institutes of Health grants AG025881 and DK56029 to A.B.L. Special thanks to Dr. Ronald Jandacek and Therese Rider at the Mouse Metabolic Phenotyping Center at the University of Cincinnati (supported by DK59630) for performing the fatty acid analysis.

References

1. Lentsch AB, Kato A, Yoshidome H, McMasters KM, Edwards MJ. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. *Hepatology* 2000;32:169–173. [PubMed: 10915720]
2. Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G15–26. [PubMed: 12488232]
3. Okaya T, Blanchard J, Schuster R, Kuboki S, Husted T, Caldwell CC, Zingarelli B, Wong H, Solomkin JS, Lentsch AB. Age-dependent responses to hepatic ischemia/reperfusion injury. *Shock* 2005;24:421–427. [PubMed: 16247327]

4. Calkins CM, Bensard DD, Moore EE, McIntyre RC, Silliman CC, Biffl W, Harken AH, Partrick DA, Offner PJ. The injured child is resistant to multiple organ failure: a different inflammatory response? *J Trauma* 2002;53:1058–1063. [PubMed: 12478028]
5. Clavien PA, Selzner M, Rudiger HA, Graf R, Kadry Z, Rousson V, Jochum W. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann Surg* 2003;238:843–850. [PubMed: 14631221]discussion 851–842
6. Markmann JF, Markmann JW, Markmann DA, Bacquerizo A, Singer J, Holt CD, Gornbein J, Yersiz H, Morrissey M, Lerner SM, McDiarmid SV, Busuttil RW. Preoperative factors associated with outcome and their impact on resource use in 1148 consecutive primary liver transplants. *Transplantation* 2001;72:1113–1122. [PubMed: 11579310]
7. Kuboki S, Shin T, Huber N, Eismann T, Galloway E, Schuster R, Blanchard J, Zingarelli B, Lentsch AB. Peroxisome proliferator-activated receptor-gamma protects against hepatic ischemia/reperfusion injury in mice. *Hepatology* 2007;47:215–224. [PubMed: 18085707]
8. Akahori T, Sho M, Hamada K, Suzuki Y, Kuzumoto Y, Nomi T, Nakamura S, Enomoto K, Kanehiro H, Nakajima Y. Importance of peroxisome proliferator-activated receptor-gamma in hepatic ischemia/reperfusion injury in mice. *J Hepatol* 2007;47:784–792. [PubMed: 17936399]
9. Lentsch AB, Yoshidome H, Cheadle WG, Miller FN, Edwards MJ. Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and Kupffer cells. *Hepatology* 1998;27:507–512. [PubMed: 9462650]
10. Deryckere F, Gannon F. A one-hour miniprep technique for extraction of DNA-binding proteins from animal tissues. *Biotechniques* 1994;16:405. [PubMed: 8185907]
11. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 1995;83:803–812. [PubMed: 8521497]
12. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A* 1997;94:4318–4323. [PubMed: 9113987]
13. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, Reddy JK. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc Natl Acad Sci U S A* 1995;92:7921–7925. [PubMed: 7644514]
14. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ* 2005;12 (Suppl 2):1542–1552. [PubMed: 16247502]
15. Rodway HA, Hunt AN, Kohler JA, Postle AD, Lillycrop KA. Lysophosphatidic acid attenuates the cytotoxic effects and degree of peroxisome proliferator-activated receptor gamma activation induced by 15-deoxyDelta12,14-prostaglandin J2 in neuroblastoma cells. *Biochem J* 2004;382:83–91. [PubMed: 15171682]
16. Takagi H, Matsui Y, Sadoshima J. The role of autophagy in mediating cell survival and death during ischemia and reperfusion in the heart. *Antioxid Redox Signal* 2007;9:1373–1381. [PubMed: 17627477]
17. Levine B, Yuan J. Autophagy in cell death: an innocent convict? *J Clin Invest* 2005;115:2679–2688. [PubMed: 16200202]
18. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circ Res* 2007;100:914–922. [PubMed: 17332429]
19. Okaya T, Lentsch AB. Peroxisome proliferator-activated receptor- α regulates postischemic liver injury. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G606–G612. [PubMed: 14615282]

Figure 1.

Hepatic activation of PPAR γ during ischemia in different age groups of mice. Liver nuclear extracts were analyzed by EMSA. Y, young mice (4–5 weeks of age); A, adult mice (10–12 weeks of age); O, old mice (10–12 months of age). Results are representative of 4 sets of experiments.

Figure 2.

Hepatic expression of endogenous ligands (left) and potential ligands (right) for PPAR γ . 15d-PGJ₂ was measured in liver extracts by ELISA. Fatty acids were determined by gas chromatography at the Mouse Metabolic Phenotyping Center at the University of Cincinnati. Data are means $\pm$ SEM with n=4 per group. * P <0.05 compared with sham-operated mice.

Figure 3.

Expression of PPAR γ isoforms in liver cytoplasmic and nuclear extracts during hepatic ischemia. PPAR γ 1 and PPAR γ 2 expression were determined by Western blot. Y, young mice (4–5 weeks of age); A, adult mice (10–12 weeks of age); O, old mice (10–12 months of age). Results are representative of 3 sets of experiments.

Figure 4.

Autophagy in the liver during hepatic ischemia. Liver lysates were analyzed for autophagy activity by Western blot for LC3B. Conversion of LC3B-I to LC3B-II is an indicator of autophagy. Y, young mice (4–5 weeks of age); A, adult mice (10–12 weeks of age); O, old mice (10–12 months of age). Results are representative of 4 sets of experiments.

Figure 5.

Effect of rosiglitazone on hepatic autophagy during ischemia in adult mice. Mice were treated orally with methylcellulose (veh) or 10 mg/kg rosiglitazone (rosi) dissolved in methylcellulose 30 minutes prior to the induction of ischemia. Liver lysates were analyzed for autophagy activity by Western blot for LC3B. Results are representative of 2 sets of experiments.