Potential role of P2X7 receptor in regulating kidney stem cells in the course of acute kidney injury
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Abstract

The role of the P2X7R in developing acute kidney injury is unknown. In this study, we developed acute kidney injury mouse model system using 0.75% adenine and examined for renal damage using histology on day 2 and day 4. P2X7 antagonist (A438079) was used to study the recovery process after initial damage and it shows positive histological data. The P2X7R expression on the day 2 and day 4 of acute kidney injury was studied using immunohistochemistry and Western blotting. Result shows elevated expression as acute kidney injury progress. Later the P2X7R expression was compared with apoptotic signal and stem cell specific marker (CD133). The results conclude that the apoptotic signals are mainly associated with advanced stage of acute kidney injury but not much in day 2. Similarly, CD133 expression was masked in latter stages of injury following elevated expression in the initial stages.

Introduction

Acute kidney injury is the form of kidney injury that develops in an immediate manner (Mehta et al., 2007) after a particular causes like low blood pressure, on exposure to destructive substances, following inflammation in kidney or may be due to improper flow of urine. The patient with acute kidney injury faces 30-40% mortality (Lameire et al., 2006) even it has sub lethal damage of kidney cells and their mortality rates increase further in chronic kidney damage as it is suffering with lethal damages (Coca et al., 2009).

The assessing the kidney damage together with their functional aspect are poorly understood (Murugan and Kellum, 2011). Even after a lot of research there is no specific improvement in this field with suitable therapy to treat acute kidney injury other than some development in supportive care (Murugan and Kellum, 2011). The problem with acute kidney injury is associated with cell death that are mediated by either apoptosis or through necrosis (Bonventre and Yang, 2011).

P2X7 is a membrane receptors that are mainly associated with the large pore formation on activation by ATP that results to form membrane blabbing to initiate cell death (Birch et al., 2013). Studies with P2X7R expression demonstrate that its expression in normal kidney is under, low level (Turner et al., 2002) but in different pathological condition their expression elevated to the marked level (Harada et al., 2000). Still the basic functions of P2X7 in different pathological condition of acute kidney injury are largely unexplored. In this paper, we are assessing the role of the P2X7R in different condition of acute kidney injury together to find out their correlation they have with kidney stem cells in their recovery process.

Materials and Methods

Experimental animal

The healthy male mice of strain (C57BL/6) were used (age: 8 weeks; weight: 15-20 g). All the mice were kept...
in a well ventilated room. The mice were developed with acute kidney injury by ingestion of 0.75% adenine (Morishita et al., 2011). The life of the mice ingested with 0.75% adenine cast can tolerate up to a maximum of 6 days. Following ingestion, the mice were analyzed for kidney damage on day 2 and day 4. One group of mice was injected with P2X7 antagonist (A438079) as previously described (Taylor et al., 2009) on day 2 of post-ingestion of 0.75% adenine and their recovery from developing acute kidney injury was studied on day 4.

**Immunohistochemistry**

For performing immunohistochemistry, the tissues were initially fixed with formalin and embedded in paraffin (Ascon et al., 2006). They were then subjected to thin sectioning of size 6 µm. The sections were then de-paraffinized using two times of xylene wash and dehydrated with isopropyl alcohol. The steps were followed with antigen retrieval using Tris EDTA (pH 9.0) and after washing they were immersed in 10% H2O2 solution for 30 min to mask the endogenous peroxidase. The sections were then incubated with 4% BSA solution for 30 min to minimize non-specific binding and latter the sections incubate with suitable antigen specific primary antibody, Anti-P2RX7 antibody (ab77413, abcam) or with Anti-CD133 antibody (ab19898, abcam) for 8 hours at 4°C. After washing the sections thoroughly with 1x PBS, they were incubated with suitable secondary antibody for 1 hour at room temperature. After washing for two times the sections were specifically bound to suitable secondary antibody. After throughout washing the specifically bind antibody are further stained using diaminobenzidine kit.

**TUNEL assay**

Apoptotic cells show a characteristic DNA fragment which were identified using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (Fulda and Pervaiz, 2010). End labelled of DNA fragments was carried out by incorporating Brdu, a thymidine analogue to the 3' ends of DNA strands. The incorporated Brdu in the apoptotic cell were then distinguished using anti-BrdU antibody (EMD Millipore, ab27413) following staining with diaminobenzidine kit.

**Western blotting**

The protein lysate was prepared from normal mice kidney and from the mice developed with acute kidney injury. For acute kidney injury developing mice, the samples were collected after day 2 and day 4 after 0.75% adenine injection. The protein samples were initially resolved in 12% SDS-PAGE gel and the further protocol was followed according with the previously described procedure (Hamidouche et al., 2008). For performing Western blotting, the membranes were initially primed with either anti-P2RX7 antibody (ab77413, abcam), anti-CAD antibody (C7852, Sigma-Aldrich) or with Anti-CD133 antibody (ab19898, abcam) and their signals were visualized with the help of suitable secondary antibody together with signalling reagents.

## Results

**Assessing the damage following adenine ingestion**

Following 0.75% adenine we observed significant histological changes (Figure 1). The control kidney shows normal structures with compact tissue pattern. But after intake of mice with 0.75% adenine the kidney shows pathological changes and in particularly the compact nature of tissue pattern was disturbed. On the 2nd day of post-exposure with 0.75% adenine, the dense packing of solid tissue shows initial damages with widen renal tissue, together with enlarged nucleus. The condition proves worsen on day 4 with unpacked loose tissue structure of the affected kidney.

**Rescue with P2X7 antagonist**

A438079 a chemical that helps to specifically target P2X7R and aids in potentially block them. The P2X7R is thought to be involved in initial pathogenesis of acute kidney injury. The mice developed with initial form of AKI were treated with P2X7 antagonist (100 mg/kg, ip) on the day 2. Once the P2X7 antagonist absorbed in the body system, it exerted a protective role. On examining the histological kidney tissue on the day 4, we observed significant restoration of abnormal tissue pattern as that of normal tissue (Figure 1D). The affected tissue pattern reverted and it was observed with a progressive histological pattern that is similar to that of control.

**Correlation of P2X7R expression with apoptotic and CD133 expression**

In the present study, the obtained histological data were further analyzed in depth using specific molecular markers that helps to understand whether P2X7R has a role of apoptosis or growth stimulating role. The normal kidney tissue had limited expression of P2X7R (Figure 2A) but by inducing acute kidney injury their expression got up-regulated (Figure 2B, C). The more specific observation was P2X7R expression enhanced with severe damage to kidney tissue as observed on day 4 of acute kidney injury (Figure 2C).

The role of P2X7R expression in the developing pattern of AKI was further studied using an apoptotic assay (Figure 2D-F). Surprisingly, we observed the apoptotic signal that shows on day 2 of acute kidney injury (Figure 2E) was magnified as the acute kidney injury progress that is on the day 4 (Figure 2F). The results concluded the acute kidney injury developed on day 4 showed severe cell death and at this stage the acute kidney injury could not be reverted. Similarly the corre-
The specific signals obtained through immunohistochemistry were further analysed using Western blotting technique. The results confirms that P2X7R over express as acute kidney injury progress (Figure 3, Lane 1–3) and similarly apoptotic activity shows elevated expression on day 4 (Figure 3, Lane 6) of acute kidney injury. The specific pattern of expression gets diluted on day 4 of acute kidney injury (Figure 2I).
Acute kidney injury when compared to day 2 (Figure 3, Lane 5). But the expression of CD133 was rapidly down-regulated on day 4 (Figure 3, Lane 9) following over expression as observed on day 2 (Figure 3, Lane 8). For all the experiments, tubulin was used.

Discussion

Normally the kidney express a very low level of P2X7R (Turner et al., 2002) as our results suggest but its elevated expression are observed in many abnormal conditions like chronic kidney disease (Ji et al., 2012). Recently the therapy based on P2X7R in cirrhosis condition are revealed out (Adebayo et al., 2013). Adenine which stimulates acute kidney injury formation was validated with histological examination. Our histological studies confirm as the acute kidney injury progress the condition of renal damage significantly worsen and with P2X7 antagonist (A438079) the damage can be reverted to certain extend. The results suggest the P2X7 antagonist (A438079) interact in an effective manner and further studies in this direction give more defined understanding in this area of research.

The role of the P2X7R in cell growth and apoptosis remains unclear. Here we pointed out that it has triggered apoptosis signal in advanced acute kidney injury when comparing with initial day 2 acute kidney injury. The results give a conclusion that P2X7R is important for regulation of advance stage of acute kidney injury. Other than this our results demonstrate the role of the P2X7R in triggering stem cells in initial damage but when the damage become severe the stem cell population gradually reduced which confirms the unable situation to revert the damage. All the results obtained through immunohistochemistry are further analysed and confirmed using western blotting. The comparative analysis of both the data shows almost similar results which helps to validate the obtained data.

Conclusion

P2X7 assists in forming acute kidney injury. The progress in acute kidney injury is associated with increasing in expression of P2X7R. Other than that the specific investigation with apoptotic and CD133 suggests the severe form of AKI supports elevated expression of apoptotic protein together with reduced expression of CD133. The comparative analysis reveals the regulative function of P2X7.

Ethical Issue

All the procedures to be followed for performing the experiments were approved by the institutional animal welfare and ethical committee.

Conflict of Interest

The authors declare no conflicts of interest.

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References

Adebayo D, Oria M, Davies N, Habestion A, Garcia-Martinez R, Unwin R, Jalan R. 571 P2X7 receptor in acute kidney injury of cirrhosis: A potential novel target of therapy. J Hepatol. 2013; 58: S234.

Ascon DB, Lopez-Briones S, Liu M, Ascon M, Savransky V, Colvin RB, Soloski MJ, Rabb H. Phenotypic and functional...
characterization of kidney-infiltrating lymphocytes in renal ischemia reperfusion injury. J Immunol. 2006; 177: 3380-87.

Birch RE, Schwiebert EM, Peppiatt-Wildman CM, Wildman SS. Emerging key roles for P2X receptors in the kidney. Front Physiol. 2013; 4: 262.

Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest. 2011; 121: 4210-21.

Coca SG, Yusuf B, Shlipak MG, Garg AX, Parikh CR. Long-term risk of mortality and other adverse outcomes after acute kidney injury: A systematic review and meta-analysis. Am J Kidney Dis. 2009; 53: 961-73.

Fulda S, Pervaiz S. Apoptosis signaling in cancer stem cells. Int J Biochem Cell Biol. 2010; 42: 31-38.

Hamidouche Z, Hay E, Vaudin P, Charbord P, Schüle R, Marie PJ, Fromigué O. FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/β-catenin signaling-dependent Runx2 expression. FASEB J. 2008; 22: 3813-22.

Harada H, Chan CM, Loesch A, Unwin R, Burnstock G. Induction of proliferation and apoptotic cell death via P2Y and P2X receptors, respectively, in rat glomerular mesangial cells. Kidney Int. 2000; 57: 949-58.

Ji X, Naito Y, Weng H, Endo K, Ma X, Iwai N. P2X7 deficiency attenuates hypertension and renal injury in deoxycorticosterone acetate-salt hypertension. Am J Physiol Renal Physiol. 2012; 303: F1207-15.

Lameire N, Van Biesen W, Vanholder R. The rise of prevalence and the fall of mortality of patients with acute renal failure: What the analysis of two databases does and does not tell us. J Am Soc Nephrol. 2006; 17: 923-25.

Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: Report of an initiative to improve outcomes in acute kidney injury. Critical Care. 2007; 11: R31.

Morishita Y, Ohnishi A, Watanabe M, Ishibashi K, Kusano E. Establishment of acute kidney injury mouse model by 0.75% adenine ingestion. Ren Fail. 2011; 33: 1013-18.

Murugan R, Kellum JA. Acute kidney injury: What's the prognosis? Nature Rev Nephrol. 2011; 7: 209-17.

Taylor SR, Turner CM, Elliott JJ, McDaid J, Hewitt R, Smith J, Pickering MC, Whitehouse DL, Cook HT, Burnstock G, Passey CD, Unwin RJ, Tam FW. P2X7 deficiency attenuates renal injury in experimental glomerulonephritis. J Am Soc Nephrol. 2009; 20: 1275-81.

Turner CM, Vonend O, Chan C, Burnstock G, Unwin RJ. The pattern of distribution of selected ATP-sensitive P2 receptor subtypes in normal rat kidney: An immunohistological study. Cells Tissues Organs. 2002; 175: 105-17.
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