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**Review Article**

# Interaction of aldose reductase and superoxide dismutase activity in liver; influence of vasopressin hormone and its modulators

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## Abstract

The Liver is an important organ of the body for carbohydrate metabolism which includes ureogenesis, glycogenolysis, gluconeogenesis. The pituitary hormone vasopressin mediates its actions on the carbohydrate metabolism through the $V_1$ receptors expressed in the Liver. The vasopressin $V_1$ receptors are coupled to the phospholipase C mediated signaling pathway. In this article, the focus is on the effects of vasopressin on superoxide dismutase (SOD) and Aldose reductase (ALD) enzyme activity in the Liver. It also stresses on the role of calcineurin phosphatase which may be involved in ROS generation. The possible homeostatic balance between superoxide generation and polyol pathway during liver injury and the role of vasopressin hormone is also discussed.

**Keywords:** Vasopressin, superoxide, Aldose reductase, Aquaporin, TonEBP, Liver

## 1. Introduction

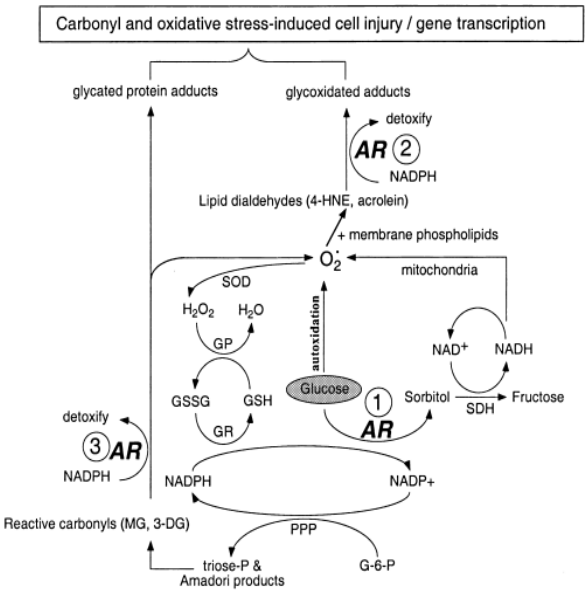
Vasopressin receptors V1a are expressed in the liver and arginine vasopressin (AVP), in addition to its well-known antidiuretic effects, is involved in the regulation of many other functions in liver processes such as ureogenesis, glycogenolysis, and gluconeogenesis [1-3]. Vasopressin receptors have been shown to be altered by some immunosuppressant agents. An experimental study has shown that 1 µM Cyclosporine (CsA) generated ROS in the cardiomyocytes. Small amounts of ROS are indispensable for many biochemical processes [4]. On the other hand in recent years, considerable evidence has accumulated supporting a role for ROS as activators of signaling pathways and transcription factors [5-6]. When the balance between pro- and antioxidants is disturbed in favor of the former, a situation of oxidative stress ensues [7]. The Aldose reductase (ALD) is an important enzyme in the polyol pathway. It was first described by Hers in 1956. Using NADPH as a cofactor, it reduces glucose to sorbitol in addition to reducing other sugars to their respective polyols [8].

The activation of the sorbitol pathway under hyperglycemic conditions is thought to be the cause of diabetic lesions in tissues where the import of glucose is independent of insulin, such as the lens, vascular cells, and nervous tissues [9-11]. Although ALD has been thoroughly studied for its role in the etiology of diabetic complications, its physiological functions are still not well understood. ALD is present in most tissues surveyed and has been implicated in a wide variety of physiological functions [12]. Reduction of glucose by the enzyme aldose reductase leads to the formation of sorbitol, which, in some tissues, is further oxidized to fructose upon catalytic oxidation by the enzyme sorbitol dehydrogenase [13].

Aldose reductase of the polyol metabolic pathway, apart from its role as a sorbitol producer and detoxifier of toxic aldehydes, is an osmoregulator in the lens and kidney, and regulator of sperm maturation, has been implicated in the etiology of long-term diabetic complications [14-15]. This enzyme is involved in many pathological processes that have become major threats to human health. Such pathologies include a number of cardiac disorders, inflammation, mood disorders, renal insufficiency, and liver abnormalities [16]. The injury to the Liver and recovery presents serious challenges to the clinical fraternity and it appears that the

ALD/SOD pathway act in some synergy to the defense of the hepatocyte cell functions. Endogenous vasopressin hormone and its receptors in the liver may possibly play an important role in the actions of ALD/SOD systems. Oxidative stress and the polyol pathway have recently been found to be linked in pathological states [13]. This review focusses on the role of the oxidative stress and polyol pathway during liver injury and possible importance of the vasopressin hormone and its receptor antagonists.

**Figure 1: Liver injury and ALD/SOD pathway**




## 2. Aldose reductase and oxidative stress pathway

Aldose reductase enzyme belongs to the aldo-keto reductase enzyme superfamily and is an important enzyme in the polyol pathway. Crystallized complexes of ALD with ligands and site directed mutagenesis allowed the enzyme structure to be identified. The enzyme is a single polypeptide domain composed of 315 amino acid residues [17]. The peptide chain at the amino terminus folds into a β /α-barrel structural motif containing atleast eight parallel β strands which are connected to each other by eight peripheral α helical segments running anti-parallel to the β sheet. The active site is located in a large and deep crevice in the C-terminal end of the β barrel, and the NADPH cofactor binds in an extended conformation at the bottom of the active site.

Aldose reductase thus is an extremely sensitive enzyme and susceptible to oxidative modification in the presence of free metal ions/thiols and glutathione. Oxidant-induced changes include alterations in kinetic parameters, decreases sensitivity to inhibitors, and increased proteolytic susceptibility. This modification has been demonstrated to involve oxidation of a critical active site cysteine thiol (Cys2') which leads to glutathione/thiol adduct formation. Induction of aldose reductase under oxidizing conditions may therefore represent a mechanism whereby the cell strives to maintain critical levels by compensating for oxidative inactivation of pre-existing enzyme [18]. The immunosuppressant drug CsA increases the cell surface expression of the type 1A vasopressin receptor ($V_{1A}$ receptor) at the protein and mRNA levels, probably via a posttranscriptional effect on mRNA stabilization.

Thus increasing endogenous $O_2^{-\cdot}$ production by 50% and can have a biological effect, and cause an increase in $V_{1A}$ receptor expression [4]. The consequence of this is a higher responsiveness of the tissue to AVP, resulting in increased vasoconstriction in blood vessels. Recently, accumulating evidences have indicated the detrimental role of polyol pathway in the ishcemia reperfusion (I/R) events, which could be reversed by ALD inhibitors [19]. Until now, the potential mechanisms underlying this beneficial effect have not been completed elucidated.

## 3. Liver injury and role of vasopressin

The vasopressin receptors were found to be expressed in the Liver apart from Kidney and brain by Ostrwoski *et al* in 1992 [20]. In liver failure, vasopressin analogues for e.g terlipressin or ornipressin are able to reverse hepatorenal syndrome and restore renal function similar to the effect of vasopressin in septic shock [21-22]. In a previous study it was demonstrated that vasopressin directly decreases portal vein flow and pressure in patients undergoing liver transplantation, a consequence of splanchnic vasoconstriction. In that study there was a statistically significant increase of blood pressure after vasopressin but the patients did not receive a bolus injection of vasopressin but only a continuous infusion. This may not be sufficient dose in cirrhotic patients with an altered volume of distribution to achieve adequate plasma levels of vasopressin and increase blood pressure, higher doses may be required to cause sustained increases of blood pressure similar to what has been observed in another study after a bolus injection.

Vasopressin may be an interesting option for managing uncontrolled hemorrhage in the extremities and below the diaphragm. Vasopressin leads to peripheral vasoconstriction via $V_1$-receptors in the vasculature and shifts blood primarily from the skeletal muscle, cutaneous, and splanchnic bed to the heart and brain [23-24]. This indicates that vasopressin may reflect two advantages in uncontrolled hemorrhagic shock in the abdomen i.e it

may decrease bleeding first by shifting blood away from the injury and by improving vital organ blood flow. The vasopressin receptor antagonist have potential uses in many disorders and including liver disorders and have been nicely reviewed by pioneering scientists in the field [25-26] (Table 1).

Ascites is a serious hepatic complication with large amount of fluid in peritoneal cavity and abdominal distension. This condition could be due to cirrhosis of liver or due to metastatic cancer and complications like the Budd Chiari syndrome. The formation of ascites involves an increase in the hepatic portal vein pressure, which may cause systemic arterial vasodilation, resulting in a decrease in the effective blood volume. This decrease in blood volume triggers the activation of the sympathetic nervous system and the renin-angiotensin system; the resultant cascade of events causes the release of arginine vasopressin AVP, which is also known as the antidiuretic hormone. AVP plays an important role in water reabsorption in the renal collecting ducts. However, as mentioned previously, the treatments for refractory ascites are limited. A diet of controlled salt intake, adequate diuretics treatment, large volume paracentesis, transjugular intrahepatic portosystemic shunting and liver transplantation are the current clinical recommendations. However, targeting the AVP mechanism presents a potential novel treatment approach for refractory ascites.

**Table 1: Potential uses of Vasopressin Receptor Antagonists**

| Receptor | Compound (Non-peptide antagonist) | Potential uses |
|---|---|---|
| **$V_1a$** | Relcovaptan (OPC – 21268, Sanofi Synthelabo, France) | Raynaud's syndrome, dysmenorrhoea, preterm labor, ACTH –independent macronodular adrenal hyperplasia |
| **$V_1b$** | Nelivaptan (SSR – 149415, Sanofi Aventis, France) | Depressive disorders |
| **$V_2$** | Lixivaptan (VPA-985, Cardiokine, USA) | Hyponatremia (SIADH, cirrhosis, CHF) |
| | Tolvaptan (OPC – 41061, Otsuka, Japan) | Hyponatremia, CHF, Cardiovascular mortality, kidney function impairment |
| | Mozavaptan (OPC-31260, Otsuka, Japan) | Hyponetremia (only SIADH) |
| | Satavaptan (SR-121463, Sanofi Aventis, France) | Hyponatremia, CHF, Cirrhosis |
| **$V_1a$ + $V_2$** | Conivaptan (Astellas, Japan) | Hyponatremia, CHF, Euvolemic hyponatremia, Hypotension, Bleeding complications |

ACTH: Adrenocorticotropic hormone; CHF: Congestive heart failure; SIADH: Syndrome of inappropriate antidiuretic hormone secretion

## 4. Aldose reductase/Superoxide generation and intervention with vasopressin analogues

Hepatic ischaemia reperfusion injury (IRI) contributes to primary liver nonfunction or late dysfunction and leads to a higher incidence of acute and chronic rejection. It also contributes to late-phase tumor recurrence and metastasis. The outcome is particularly not good in diseased livers such as fatty liver, which is more prone to hepatic IRI. Hence, elucidating the mechanism and minimizing the adverse consequences of hepatic IRI would be important for improving liver surgery outcomes and the long-term survival. Liver IRI is a typical inflammatory response involving a complex web of interactions between various cellular and humoral contributors [27]. Acute ischemia leads to the activation of the endothelium with an increase in permeability, expression of many adhesion

molecules, and production of oxidative stress. The transcription factor, Nuclear factor kappa beta (NF-κB) and macrophages are induced and activated, leading to the production of cytotoxic free radicals and inflammatory cytokines. Hepatocyte necrosis and apoptosis represent two major direct causes of liver dysfunction following severe I/R impairment and represent different extremes on a continuum of cell death [28-29]. They are the common triggers of mitochondrial impairment and resultant energy failure. During this cascade, excessive ROS and lipid peroxidation, coupled with an imbalance in pro- and antiapoptosis proteins (e.g., Bax and Bcl-2, respectively), promote the mitochondrial permeability transition [30]. These changes may, in turn, initiate and interact with the following mitochondrial events: respiratory chain uncoupling, the release of cytochrome, the downstream activation of caspase 3, and so forth [31].

In this cited study, the increased Bax/Bcl-2 ratio which resulted from AR overexpression clearly lowered the mitochondrial membrane potential and contributed to the overactivation of the caspase pathway. However, treatment with ALD inhibitors significantly alleviated the I/R-induced increase of ROS, Bax/ Bcl-2 ratio, and activation of caspase 3, which could eventually improve the liver function. Thus, aldose reductase enzyme may modify the formation of reactive dicarbonyl compounds and lipid aldehydes and subsequently protein-bound adducts associated with oxidative stress and tissue damage [32].

Vasopressin receptor antagonists like the Nelivaptan, Satavaptan and Tolvaptan can block the $V_1$ and $V_2$ receptors and produce beneficial effects by antagonizing the aldose reductase enzyme activity, sorbitol formation and reducing the ALD mRNA expression and potentially treat liver IRI.

## 5. Estimation of Superoxide dismutase activity in Liver

All enzyme activities are measured simultaneously in triplicate for each sample using a Shimadzu UV-160 spectrophotometer and a temperature controlled cuvette holder. The activity of Total SOD is assayed by the adrenaline method [33] based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome. One unit of SOD activity is defined as the amount of protein causing 50% inhibition of the autoxidation of adrenaline at 26°C. The activity of Mn-SOD was obtained after the inhibition of Cu/Zn-SOD with KCN. Cu/Zn-SOD activity was calculated as a difference between Tot-SOD and Mn-SOD activities. Total protein concentration is determined according to the method of Lowry et al. (1951) using bovine serum albumin as a reference and expressed in mg/mL protein. Protein electrophoretic profiles were examined by the standard method of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), [34].

## 6. Assay of Aldose reductase enzyme activity

Aldose Reductase Enzyme Activity can be measured with minor modifications based on previous method shown by Aniket Kumar *et al* [35]. In tubes containing the liver homogenate were subjected to centrifugation. It was further washed with saline thrice and stored for further analysis. By adding 50 mM sodium phosphate buffer at pH 7.4 and 150 mM NaCl, a 10% suspension was made and is lysed by repeated freezing and thawing. Insoluble debris was then removed by centrifugation. ALD activity was measured spectrophotometrically using a properly diluted hemolysate utilizing a T60 LABINDIA UV-VIS spectrophotometer

## 7. Conclusion

The imbalance of vasoregulatory genes and early overexpression of several adhesion molecules play important roles in the disturbance of hepatic microcirculation at the early phase after liver transplantation using small-for-size grafts as well possibly in other liver injuries. Recent studies demonstrate that besides reducing glucose to sorbitol, Aldose reductase efficiently reduces lipid aldehydes and their conjugates with GSH. This has opened new dimensions in understanding the detoxification of reactive aldehydes generated during lipid peroxidation. Using kinetic, structural, and physiological studies, researchers have investigated the mechanisms by which aldose reductase selectively recognizes and catalyzes the reduction of Lipid peroxidation and their reduced glutathione conjugates. On the other hand vasopressin may modulate the effects of these enzymes in the liver and Vasopressin receptor antagonists like Tolvaptan, Lixivaptan are effective to treat refractory ascites and/or edema in decompensated cirrhotic patients and is, therefore, a promising aquaretic agent.

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## References


[1] Valtin H. Genetic models of diabetes insipidus. In: Windhager E E, editor. Handbook of physiology. Section 8: renal physiology. II. Bethesda, Md: *American Physiology Society*. 1992; 1281–1315.

[2] Drew, P.J.T., Monson, J. P., Metcalfe, H. K., Evans, S.J.W., Iles, R. A., Cohen, R. D. The effect of arginine vasopressin on ureagenesis in isolated rat hepatocytes. *Clin. Sci*. 1985 ; 69 : 231–233.

[3] Kraus-Friedmann, N., Feng, L. The role of intracellular $Ca^{2+}$ in the regulation of gluconeogenesis. *Metabolism*.1996; 45: 389–403.

[4] Buetler T M, Krauskopf A, Ruegg UT. Role of Superoxide as a Signaling Molecule. *News Physiol. Sci*. 2004; 19 (3) :120-123.

[5] Ryter S W,Hong PK , Hoetzel A, Wong X, Choi AMK.Mechanisms of cell death in oxidative stress. *In Antioxidants and Redox Signaling*. 2007; 9(1): 49–89.

[6] Allen, R G., and Tresini, M. Oxidative stress and gene regulation. *Free Radic. Biol. Med*. 2000; 28: 463–499.

[7] Ullrich V, Zou M H, and Bachschmid M. New physiological and pathophysiological aspects on the thromboxane A (2)-prostacyclin regulatory system. *Biochim. Biophys. Acta*.2001; 532: 1–14.

[8] Hers H G. The mechanism of transformation of glucose to fructose by the seminal vesicles. *Biochim Biophys Acta*. 1956; 22:202–203.

[9] Pugliese G, Tilton R G, Williamson J R. Glucose-induced metabolic imbalances in the pathogenesis of diabetic vascular disease. *Diabetes Metab Rev*. 1991; 7:35–59.

[10] Kinoshita J H, Nishimura C. The involvement of aldose reductase in diabetic complications. *Diabetes Metab Rev*. 1988;4:323–337.

[11] Del Corso A, Camici M, Mura U. *In vitro* modification of bovine lens aldose reductase activity. *Biochem Biophys Res Commun*. 1987; 148(1): 369-75.

[12] Schade SZ, Early SL, Williams TR, Kézdy FJ, Heinrikson RL, Grimshaw CE, Doughty CC. Sequence Analysis of Bovine Lens Aldose Reductase. *Journal of Biological Chemistry*. 1990; 265(7): 3628-3635.

[13] Suzen S, Das-Evcimen N, Varol P, Sarıkaya M.Preliminary evaluation of rat kidney aldose reductase inhibitory activity of 2-phenylindole derivatives: affiliation to antioxidant activity. *Medicinal Chemistry Research*. 2007; 16 (3): 112-118.

[14] Gabbay K H. Hyperglycemia, polyol metabolism, and complications of diabetes mellitus. *Annu Rev Med*. 1975; 26:521-36.

[15] Gui T, Tanimoto T, Kokai Y, Nishimura C. Presence of a closely related subgroup in the aldo-ketoreductase family of the mouse. *Eur J Biochem*. 1995; 227(1-2):448-53.

[16] Alexiou P, Pegklidou K, Chatzopoulou M, Nicolaou I, Demopoulos VJ. Aldose reductase enzyme and its implication to major health problems of the 21 (st) centuries. *Curr Med Chem*. 2009; 16(6):734-52.

[17] Rondeau JM, Tete-Favier F, Podjarny A, Reymann JM, Barth P, Biellmann JF, Moras D. Novel Nadph-Binding Domain Revealed by the Crystal Structure of Aldose Reductase. Nature. 1992; 355(6359) 469-472.

[18] Landis GN and Tower J. Superoxide dismutase evolution and life span regulation. *In Mechanisms of Ageing and Development*. 2005; 126 (3): 365–379.

[19] Springate J E, Feld L G, Van Liew J B, Fildes R D, Acara M A. Diuretic and natriuretic effects of sorbinil, an aldose reductase inhibitor. *Pharmacol Res*. 1991; 23:279–283.

[20] N L Ostrowski, S J Lolait, D J Bradley, A M O'Carroll, M J Brownstein, and W S Young, 3rd. Distribution of $V_{1a}$ and $V_2$ vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology*. 1992 Jul; 131(1):533-5.

[21] Martin-Llahi M, Pepin MN, Guevara M, Diaz F, Torre A, Monescillo A, Soriano G, et al. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology*. 2008; 134:1352–1359.

[22] Fabrizi F, Dixit V, Messa P, Martin P. Terlipressin for hepatorenal syndrome: A meta-analysis of randomized trials. *Int J Artif Organs*. 2009; 32:133–140.

[23] Wagener G, Gubitosa G, Renz J, Kinkhabwala M, Brentjens T, Guarrera JV, Emond J, *et al*. Vasopressin decreases portal vein pressure and flow in the native liver during liver transplantation. *Liver Transpl*. 2008;14:1664–1670.

[24] Holmes CL, Walley KR, Chittock DR, Lehman T, Russell JA. The effects of vasopressin on hemodynamics and renal function in severe septic shock: a case series. *Intensive Care Med*. 2001; 27:1416–1421.

[25] Greenberg A, Verbalis JG: Vasopressin receptor antagonists. *Kidney Int* 2006; 69:2124-2130.

[26] Ali F, Guglin M, Vaitkevicius P, Ghali JK: Therapeutic potential of vasopressin receptor antagonists. *Drugs* 2007; 67: 847-858.

[27] Jaeschke H. Reactive oxygen and mechanisms of inflammatory liver injury: present concepts. *Journal of Gastroenterology and Hepatology*, 2011; 26 (supplements 1): 173–179.

[28] Zhai Y, Busuttil RW, Kupiec-Weglinski JW. Liver ischemia and reperfusion injury: new insights into mechanisms of innate-adaptive immune-mediated tissue inflammation. *Am J Transplant*. 2011; 11:1563–1569.

[29] Fondevila C, Busuttil RW, Kupiec-Weglinski JW. Hepatic ischemia/ reperfusion injury- a fresh look. *Exp Mol Pathol*. 2003; 74:86–93.

[30] Boros P, Bromberg JS. New cellular and molecular immune pathways in ischemia/reperfusion injury. *Am J Transplant*. 2006; 6:652–658.

[31] Selzner M, Clavien PA. Fatty liver in liver transplantation and surgery. *Semin Liver Dis*. 2001; 21:105–113.

[32] Vanderjagt DL, Hunsaker LA, Vanderjagt TJ, Gomes MS, Gonzales DM, Duk LM & Royer ME. Inactivation of glutathione reductase by 4-hydroxynonenal and other endogenous aldehydes. *Biochem Pharmacol*. 1997; 53: 1133–1140.

[33] Misra, H.P and I.Fridovich. The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem*. 1972; 247: 3170-3175.

[34] Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature*. 1970; 227: 680-685.

[35] Kumar A, Margaret Shanthi F. X., Mahalaxmi C., Ramaswamy A, Bina Isaac and Tyagi MG. Rottlerin Mediated Modulation of Tonicity-Responsive Enhancer Binding Protein (TonEBP) In Wistar Rats. *Biomedical & Pharmacology Journal*. 2014; 7(2): 665-673.