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Effects of V_2-receptor antagonist tolvaptan and the loop diuretic furosemide in rats with heart failure

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ABSTRACT

Diuretics are frequently required to treat fluid retention in patients with chronic heart failure (CHF). Unfortunately, they can lead to a decline in renal function, electrolyte depletion, and neurohormonal activation. Arginine vasopressin (AVP) promotes renal water reabsorption via the V_2 receptor (V_2R) and its levels are increased in CHF. This study was conducted to characterize the diuretic effect of tolvaptan, a non-peptide AVP V_2R antagonist, and furosemide, a loop diuretic in a rat model of CHF after experimental autoimmune myocarditis. CHF was elicited in Lewis rats by immunization with porcine cardiac myosin, and 28 days after immunization rats were treated for 28 days with oral tolvaptan, and furosemide. CHF was characterized by left ventricular remodeling and impaired systolic and diastolic function. Tolvaptan produces a diuresis comparable to furosemide. Unlike tolvaptan, furosemide significantly increased urinary sodium and potassium excretion. Tolvaptan markedly elevated electrolyte-free water clearance (E-CH_2O) or aquaresis to a positive value and increased urinary AVP excretion. In contrast to tolvaptan, furosemide elevated only electrolyte clearance (E-Cosm) but not E-CH_2O. The differences in diuretic profile reflected the changes in plasma sodium and hormone levels. Tolvaptan dose dependently elevated plasma sodium concentration, but furosemide tended to decrease it. Furosemide significantly elevated plasma renin activity and aldosterone concentration. On the other hand, tolvaptan did not affect these parameters. Our results suggest that, tolvaptan have a potential medical benefit for the treatment of edematous conditions in CHF by removing excess water from the body without activating the RAAS or causing serum electrolyte imbalances.

1. Introduction

Chronic heart failure (CHF) is a clinical syndrome caused by heart disease that is characterized by abnormal sodium and water retention resulting in edema [1]. In CHF patients, diminished cardiac output activates the sympathetic nervous system and renin–angiotensin–aldosterone system (RAAS) and the non-osmotic release of arginine vasopressin (AVP).

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This causes a decrease in renal blood flow and an increase in the filtration fraction resulting in an increase in water and sodium retention and consequently edema [2]. Loop diuretics are frequently required to treat fluid retention in patients with CHF. Unfortunately, they can lead to a decline in renal function, electrolyte depletion (loss of sodium and other essential electrolytes, which may exacerbate the hyponatremia), and neurohormonal activation [3]. Furthermore, despite the widespread and long history of use of diuretics in CHF, some detrimental actions of diuretics continue to emerge. Importantly, by increasing distal tubular delivery of sodium, loop diuretics activate the tubuloglomerular feedback mechanism, which causes vasoconstriction of the afferent arteriole and reduction in renal blood flow [4–6]. Thus loop diuretics may compromise renal function, which has been identified in retrospective analyses as a powerful predictor for CHF mortality [7]. Potassium wasting diuretics, which have been associated with increased mortality, can also lead to serum potassium depletion, which in turn, can promote arrhythmias [8,9]. Furthermore, serum sodium concentration is one of the best predictors of cardiovascular mortality, with hyponatremia patients showing substantially shorter survival than patients with a normal sodium concentration [10]. It is not clear whether neurohormonal activation and decreased serum sodium level are the result of more advanced heart failure or whether they contribute to the progression of mortality. This has prompted efforts to develop more physiological strategies to treat volume overload. Thus, solute-free water diuretics (aquaretics) are preferable for the treatment of edematous state associated with CHF.

Similar to other neurohormones that are activated in CHF, circulating AVP is elevated in patients with CHF [11]. The precise role of AVP in the pathophysiology of cardiovascular disease is controversial. AVP acts via three receptor types: V_{1a}, V_{1b} (V_3), and V_2. AVP regulates various physiological processes including vascular tone regulation, cardiovascular contractility and body fluid regulation through activation of V_{1a} and V_2 receptors, respectively [11]. The recent development of non-peptide orally active AVP-receptor antagonists has allowed reevaluation of the precise role of AVP in experimental animal models of hypertension [12,13] and heart failure [14,15]. Tolvaptan is a modified benzazepine derivative that was selected as a potent human V_2-receptor (V_2R) antagonist through a series of structural conversions of mozavaptan. The potent aquaretic properties of tolvaptan in rats and its pharmacological profile were reported by Yamamura et al. [16]. Tolvaptan exerts an aquaretic effect by blocking the V_2 receptors at the renal collecting ducts and thereby inhibiting water reabsorption. AVP binding studies of this agent reported a 29:1 (V_2:V_{1a}) receptor selectivity in cloned human AVP receptors and 250 times more potent to rat V_2R than to rat V_1R and produced aquaresis after single and multiple dosing in rats [16]. The first human study using this V_2R antagonist (tolvaptan) in patients with CHF was performed by Gheorghiade et al. [17]. They found that, patients with blockade of V_2 receptors had an increase in urine volume and a decrease in body weight that were maintained throughout the study. Recently several investigators have demonstrated that addition of tolvaptan to the standard therapy in patients with heart failure decreased body weight and edema, corrected hyponatremia and appeared to be well-tolerated with no adverse effects on heart rate (HR), blood pressure, electrolytes, neurohormonal activation or renal function, despite its potent aquaretic effect [18–20].

Though, the differences between aquaresis and natriuresis have been investigated acutely in normal rats and human CHF [3,21], but not been explored chronically with the same experimental procedures. Therefore, we compared the diuretic effects of tolvaptan, a non-peptide AVP V_2R antagonist and furosemide, a most commonly used loop diuretic, in rat model of myosin-induced CHF after experimental autoimmune myocarditis (EAM).

2. Materials and methods

2.1. Materials

Tolvaptan (7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1-benzazepine) was a gift from Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan), and furosemide was purchased from Wako (Osaka, Japan). Lewis rats (male, 8 weeks old) were purchased from Charles River Japan Inc., Kanagawa, Japan.

2.2. Experimental design

All experiments were carried out using 8-week-old male Lewis rats and were performed in accordance with the guidelines of our institute [22]. Lewis rats were injected in the footpads with antigen-adjuvant emulsion according to the procedure described previously [22,23]. In brief, porcine cardiac myosin was dissolved in phosphate-buffered saline at 5 mg/ml and emulsified with an equal volume of complete freund's adjuvant with 11 mg/ml *Mycobacterium tuberculosis* H37RA (Difco Lab., Detroit, MI, USA). CHF in rats was induced by immunization with 0.1 ml of emulsion once by subcutaneous injection into their rear footpads (0.1 ml to each footpad). The morbidity of EAM was 100% in rats immunized by this procedure [22,23]. Rats immunized with myosin became ill and immobile on day 14, and their activity gradually recovered beginning at the fourth week. Nine (23%) out of the 39 rats died between days 14 and 28 after immunization. All hearts from the dead rats showed extensive myocardial necrosis and pericardial effusion. Twenty-eight days after immunization, the postmyocarditis dilated cardiomyopathy (DCM) develops in the rats. The surviving 30 rats were divided into five groups and received oral administration (p.o.) of tolvaptan (3 mg/(kg day), group T3, $n = 6$; 10 mg/(kg day), group T10, $n = 6$), furosemide (30 mg/(kg day), group F30, $n = 6$; 100 mg/(kg day), group F100, $n = 6$) or vehicle (1% hydroxypropyl methyl cellulose, group V, $n = 6$) for 28 days. Age matched Lewis rats without immunization was used as normal controls. Doses of tolvaptan (3 and 10 mg/kg) and furosemide (30 and 100 mg/kg) were selected on the basis of aquaretic properties demonstrated in an earlier reports [15,21,24]. Hirano et al. [21] reported that aquaretic properties of tolvaptan at 1 and 10 mg/kg are equal to furosemide at 10 and 100 mg/kg, respectively. Moreover, very recently we have reported aquaretic properties of tolvaptan at a dose of 3 and 10 mg/kg [15]. Hence we selected