Drugs of the Future 2002, 27(4): 350-357

CCC: 0377-8282/2002

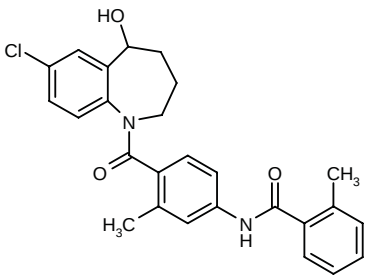
Tolvaptan

Prop INN

Treatment of Heart Failure
Vasopressin V_2 Antagonist

OPC-41061

(±)-*N*-[4-(7-Chloro-5-hydroxy-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-ylcarbonyl)-3-methylphenyl]-2-methylbenzamide



$C_{26}H_{25}ClN_2O_3$
Mol wt: 448.9475
CAS: 150683-30-0
EN: 255241

Abstract

Congestive heart failure is a condition in which impaired cardiac function causes circulatory congestion. Although there is no cure, several classes of agents are available or under development to improve cardiac function, relieve symptoms and improve quality of life of patients. Arginine vasopressin (AVP) is a potent antidiuretic hormone that plays a crucial role in the regulation of free water absorption, body fluid osmolality, blood volume, cell contraction and blood pressure and its diverse actions are mediated by the G-protein-coupled receptor subtypes V_1, V_2 and V_3. The systemic vasoconstriction and dilutional hyponatremia seen in patients with congestive heart failure are due, in part, to abnormally high levels of circulating AVP. AVP antagonism is therefore a feasible strategy to prevent disease progression in heart failure. The orally active benzazepine tolvaptan is a V_2 receptor antagonist that has demonstrated excellent preclinical and clinical aquaretic effects indicating its potential efficacy in the treatment of hyponatremia seen in congestive heart failure as well as other conditions such as liver cirrhosis and renal pathologies.

Synthesis

Treatment of 5-chloro-2-nitrobenzoic acid (I) with dimethyl sulfate and K_2CO_3 in acetone yields the methyl ester (II), which is reduced at the nitro group with $SnCl_2$ in HCl/EtOH to afford aniline (III). Protection of aniline (III) with tosyl chloride in pyridine gives the *p*-toluenesulfonamide (IV), which is alkylated with 4-bromobutyric acid ethyl ester (V) by means of K_2CO_3 in DMF to afford the diester (VI). Dieckmann cyclization of (VI) with potassium *tert*-butoxide in refluxing toluene provides benzazepinone (VIIa-b) as a mixture of ethyl and methyl esters, which is decarboxylated to (VIII) by heating with HCl in AcOH. Deprotection of the tosyl group of (VIII) with hot polyphosphoric acid furnishes benzazepinone (IX), which is condensed with 2-methyl-4-nitrobenzoyl chloride (X) by means of Et_3N in dichloromethane to give amide (XI). Reduction of the nitro group of (XI) with $SnCl_2$ in HCl/EtOH provides the corresponding aniline (XII), which is condensed with 2-methylbenzoyl chloride (XIII) by means of Et_3N in dichloromethane to provide diamide (XIV). Finally, tolvaptan is obtained by reduction of the ketone group of (XIV) by means of $NaBH_4$ in MeOH (1, 2). Scheme 1.

Introduction

Congestive heart failure is a progressive disorder involving left ventricular myocardial remodeling. It is caused by impaired cardiac function associated with circulatory congestion and is characterized as autonomic dysfunction, neurohormonal activation and overproduction of cytokines, which all contribute to progressive circulatory failure. According to the American Heart Association, approximately 4.7 million individuals in the U.S. have congestive heart failure and as many as 550,000 new cases are diagnosed annually. Heart failure occurs when the heart is damaged or overworked and is thus unable to effectively pump out all the blood that returns to it from the systemic circulation. The result is a

L.A. Sorbera, J. Castañer, M. Bayés, J. Silvestre. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Tolvaptan

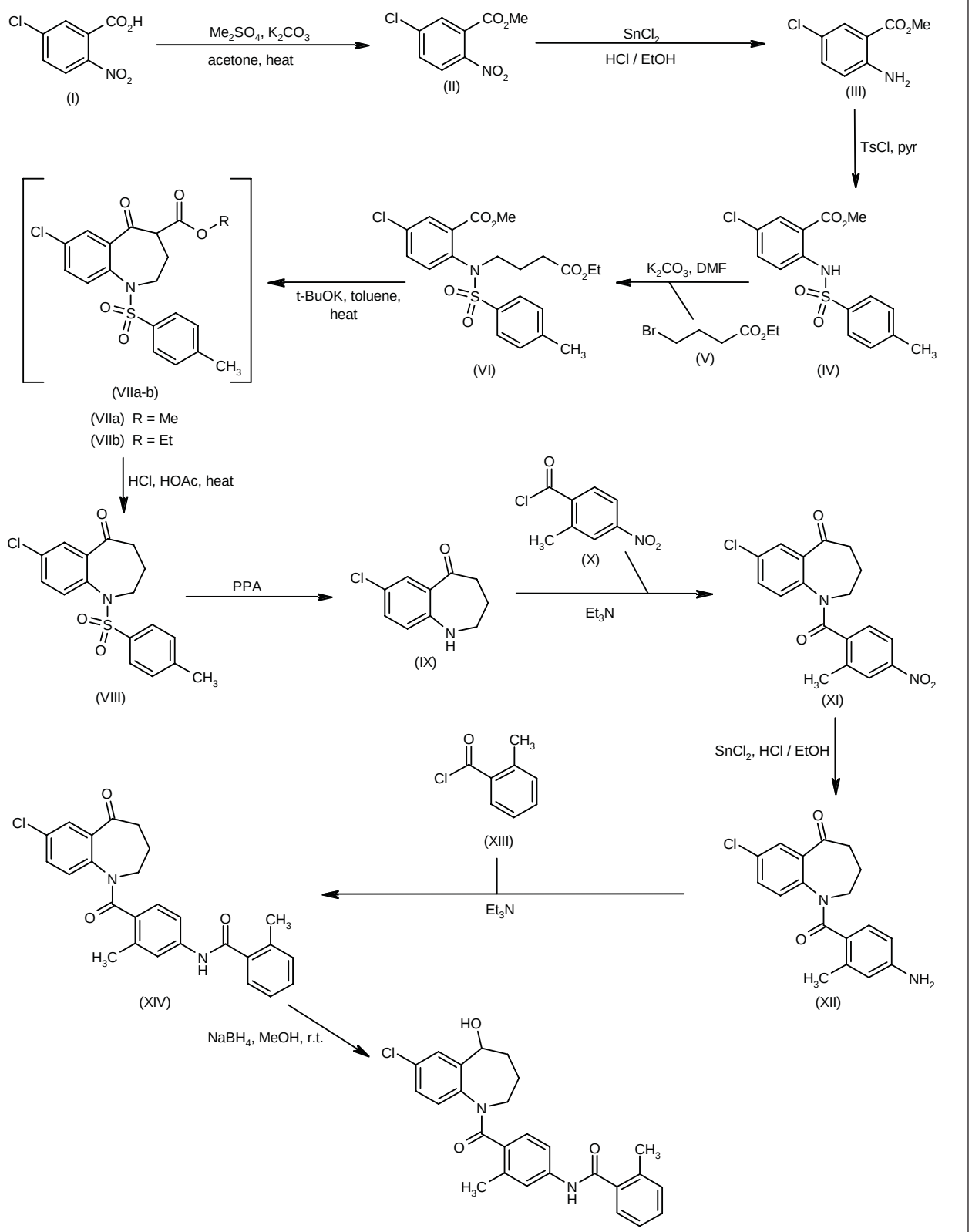
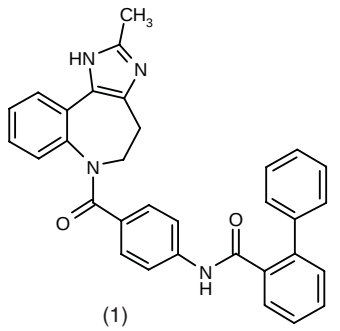


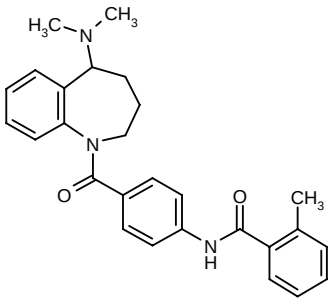
Table I: Vasopressin antagonists under development (Prous Science Integrity®).

Drug	Source	Mechanism of Action	Phase
1. Conivaptan Hydrochloride	Yamanouchi	Vasopressin V_2 and V_{1A} antagonist	II
2. OPC-31260	Otsuka	Vasopressin V_2 antagonist	II
3. Tolvaptan	Otsuka	Vasopressin V_2 antagonist	II
4. SR-121463A	Sanofi-Synthélabo	Vasopressin V_{1A} antagonist	I

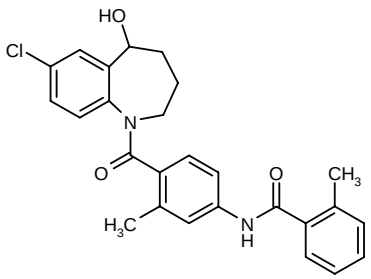


(1)

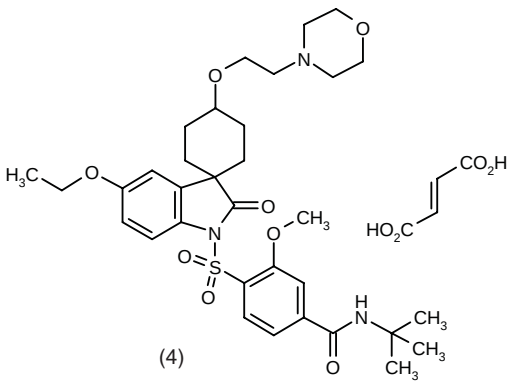
.HCl



(2)



(3)



(4)

back up of the blood returning to the heart and consequent fluid retention in other parts of the body. Kidney dysfunction can also be evident so that sodium and water excretion is impaired, thus further increasing fluid retention. Although there is no cure for congestive heart failure, several classes of agents incorporating various therapeutic strategies are available and under development to improve cardiac function, relieve symptoms, prolong life and improve quality of life of patients (3).

Arginine vasopressin (AVP) is a potent antidiuretic, vasoconstricting, neurohypophysial hormone that plays an essential role in the regulation of free water reabsorption, body fluid osmolality, blood volume, blood pressure, cell contraction, cell proliferation and adrenocorticotropin (ACTH) secretion. AVP action is mediated by 3 specific G-protein-coupled receptor subtypes: V_1-vascular (V_1) types a and b, V_2-renal (V_2) and V_3-pituitary (V_3). Activation of each receptor type results in distinct pharmacological effects through activation of intracellular second messengers. Patients with symptomatic heart failure are known to have AVP levels double those observed in normal subjects which are responsible in part for the systemic vasoconstriction and dilutional hyponatremia observed in these patients. Moreover, dilutional hyponatremia is the most common electrolyte disorder seen in hospitalized patients suffering from congestive heart failure as well as other conditions such as liver cirrhosis and renal pathologies, and the condition increases mortality rates by 60-fold (4, 5). Thus, there is considerable therapeutic interest in developing AVP receptor antagonists which could alleviate hyponatremia. Blockade of the V_1 receptor could be potentially useful as a treatment for congestive heart failure, arterial hypertension and peripheral vascular disease. V_2 receptor blockade would also be effective against congestive heart failure progression as well as liver cirrhosis, nephrotic syndrome and any condition involving excessive fluid retention and subsequent dilutional hyponatremia. On the other hand, blockade of the V_3 receptors could be effective in treating ACTH-secreting tumors (5-9).

AVP antagonism has become an attractive strategy to prevent disease progression in heart failure and several agents including both V_2 receptor antagonists and dual V_2/V_{1a} receptor antagonists are currently under development, as seen in Table I. The benzazepine, tolvaptan (OPC-41061) is an orally active, specific and selective nonpeptide V_2 receptor antagonist that has exhibited potent aquaretic effects and has been chosen for further

Table II: Binding affinity for human recombinant receptors and V_2 selectivity index of V_2 receptor antagonists (Prous Science Integrity®).

Compound	hV_{1a}	hV_{1b}	hV_2	SI[a]
Arginine-Vasopressin (10, 20-22)	0.56-1.51	0.30-0.59	0.78-3.27	0.44
Conivaptan HCl (20, 21)	4.30-6.30	>10,000->60,000	1.10-1.92	3.51
OPC-31260 (21)	77.6	>10,000	24.7	3.14
SR-121463A (21)	304	52,100	2.75	111
Tolvaptan (10)	12.3	>100,000	0.43	28.6

Binding affinity assessed by displacement of [^{3}H]-vasopressin (K_i, nM). [a]SI: V_2 selectivity index calculated as $V_{1a}[K_i]/V_2$ [K_i]. Arginine-vasopressin included for comparison.

Table III: Binding affinity for rat receptors, V_2 selectivity index and in vivo *diuretic activity of V_2 receptor antagonists (Prous Science Integrity®).*

Compound	V_{1a} K_i (nM)	V_2 K_i (nM)	SI V_{1a} [K_i]/V_2 [K_i]	Diuretic Activity [a]ED_{300}, mg/kg po (iv)
Arginine-Vasopressin (10, 23, 24)	1.0-1.45	0.95-3.23	0.60	–
Furosemide	–	–	–	11.7[9]
Conivaptan HCl (23, 25)	0.48	3.04	0.14	0.23[9]
OPC-31260 (23, 25, 26)	193-748	21.7-42.3	14.7	3.20[9]-3.80[c][11]
SR-121463A (23, 26)	5,480-10,600	1.42-2.82	3,775	NR
Tolvaptan (10)	325	1.33	244	0.54[c][11]
VP-343 (27, 28)	110*	0.77*	143	0.22[9,10]
VP-365 (28)	127	1.18*	108	0.31[10]

Binding affinity assessed by displacement of [^{3}H]-vasopressin in rat liver and rat kidney for V_{1a} and V_2 receptors, respectively. *IC_{50} (nM); [a]ED_{300} (mg/kg) as the effective dose able to triplicate the urine volume compared to vehicle-treated rats. Arginine-vasopressin and furosemide included for comparison.

development as a treatment for disease progression in congestive heart failure (1, 3).

Pharmacological Actions

Investigation of a series of orally active, novel benzazepine V_2 receptor antagonists revealed the superior activity of tolvaptan. Radioligand binding studies using rat liver and kidney plasma membrane preparations in vitro and examining displacement of [^{3}H]-AVP reported high affinity binding of tolvaptan to the kidney V_2 receptor (IC_{50} = 0.003 μM) as compared to the liver V_{1A} receptor (IC_{50} = 0.58 μM) (8). Further characterization of tolvaptan binding to AVP receptors was performed using HeLa cells expressing cloned human or rat AVP receptors. Results showed that tolvaptan antagonized [^{3}H]-AVP binding to human V_2 receptors more potently than AVP (K_i = 0.43 ± 0.06 *vs.* 0.78 ± 0.08 nM) and was 29 times more effective in antagonizing human V_2 receptors as compared to human V_{1a} receptors (K_i = 12.3 ± 0.8 nM); tolvaptan was ineffective against [^{3}H]-AVP binding to the human V_{1b} receptor (K_i = > 100,000 nM). Similarly, tolvaptan was 244 times more selective in antagonizing rat V_2 receptors (K_i = 1.33 ± 0.30 nM) over rat V_{1a} receptors (K_i = 325 ± 41 nM). Moreover, tolvaptan was 3 and 26 times more potent in antagonizing V_2 and V_{1a} receptors, respectively, from humans as compared to rats. Experiments also showed that tolvaptan dose-dependently inhibited AVP (1 nM)-induced increases in cAMP production with no evidence of agonist activity noted (10).

The binding affinity and selectivity index for tolvaptan and reference compounds are reported in Tables II and III.

Tolvaptan was shown to have potent diuretic efficacy *in vivo*. Treatment of rats with a single oral dose (0.3, 1, 3, 10 or 30 mg/kg) resulted in a 12-fold higher mean volume of spontaneously voided urine collected at 2 h postdosing and a reduction in urine osmolality (175 ± 15 *vs.* 714 ± 136 mOsm/kg); no significant differences in urine output were observed at 4 h postdosing. Multiple dosing with the agent (1 and 10 mg/kg once daily for 28 days) also significantly increased urine volume and decreased urine osmolality during 0-4 h postdosing. The calculated ED_3 value (*i.e.*, the dose necessary to cause a 3-fold increase in 2-h urine volume as compared to controls) was 0.54 mg/kg p.o. A significant increase in urinary excretion of sodium and urea nitrogen was observed in the tolvaptan-treated animals at 0-4 h postdosing; however, there were no differences in net urinary creatinine excretion indicating that the agent did not affect the glomerular filtration rate. Urinary AVP excretion was also significantly increased at 0-24 h postdosing. No differences in serum osmolality, sodium, creatinine and urea nitrogen concentrations were seen at 24 h postdosing nor were changes in pituitary or serum AVP content or the number and affinity of AVP receptors in the kidney and liver observed throughout the treatment period (1, 10).

A study comparing the diuretic effects of tolvaptan (1 and 10 mg/kg) with those of the loop diuretic furosemide (10 and 10 mg/kg) in conscious rats showed that both agents dose-dependently and comparably increased urine volume. However, the higher tolvaptan dose significantly increased electrolyte-free water clearance to a positive value while furosemide only increased electrolyte clearance. In this regard, tolvaptan dose-dependently increased serum sodium concentrations while furosemide tended to decrease them. Unlike furosemide (100 mg/kg) which significantly increased serum renin and aldosterone, tolvaptan had no effect on the renin-angiotensin-aldosterone system. Additive diuretic effects were observed when tolvaptan was administered concomitantly with furosemide and dose-dependent increases in serum osmolality and sodium concentration were seen when tolvaptan was combined with the high dose of furosemide (11).

Pharmacokinetics

An open-label study involving 12 healthy male volunteers examined the absorption, distribution, metabolism and excretion of a single oral dose (60 mg) of [^{14}C]-labeled (100 μCi) and unlabeled tolvaptan. Tolvaptan was extensively metabolized with a total recovery of 98.9 ± 8% of the radioactivity in urine and feces. The C_{max} for plasma radioactivity was 1063.9 ± 194.2 ngEq/ml reached at a t_{max} of 3 ± 1.9 h. The metabolites detected in plasma postdosing were (in rank order of mean C_{max}): DM-4103 > DM-4104 > DM-4107 > DM-4111 > DM-4110 > DM-4105 > DM-4119. DM-4103, unchanged tolvaptan and the other 6 metabolites accounted for 52.5, 2.7 and about 5%, respectively, of the plasma [^{14}C]-AUC value. DM-4107, DM-4111 and tolvaptan + other metabolites accounted for 24.5, 14.4 and 6.8%, respectively, of the total [^{14}C] detected in urine. Tolvaptan, DM-4107 and other metabolites accounted for 32.7, 21.7 and 21.5%, respectively, of the total [^{14}C] in feces. The major route of excretion for tolvaptan was feces, with < 1% excreted in urine (12).

A randomized, open-label, 3-period crossover study conducted in 12 healthy male volunteers determined that there was no clinically significant pharmacokinetic or pharmacodynamic interactions between tolvaptan (30 mg) and furosemide (80 mg) or hydrochlorothiazide (100 mg). Neither furosemide nor hydrochlorothiazide altered the C_{max} and AUC values of tolvaptan (0.28 μg/ml and 168 μg·h/ml) and the percent changes in urine volume, plasma AVP and plasma renin observed with tolvaptan were unaffected by the diuretics (13).

Clinical Studies

A randomized, double-blind, placebo-controlled study involving 250 patients with heart failure (NYHA class I-III) and signs of congestion (*e.g.*, edema or rales) and on standard therapy (without fluid restrictions) including stable furosemide (20-240 mg/day), showed the efficacy of tolvaptan (30, 45 or 60 mg once daily for 25 days) in significantly reducing body weight (–0.79 ± 0.99, –0.96 ± 0.93 and –0.84 ± 0.02 kg for the respective doses *vs.* +0.32 ± 46 kg for placebo) and markedly reducing leg edema without adversely affecting serum electrolytes. Tolvaptan was well tolerated with thirst and dry mouth the most common adverse events reported. Tolvaptan significantly and dose-dependently increased urine volume as compared to placebo (3.9 ± 0.6, 4.2 ± 0.9 and 4.6 ± 0.4 l/24 h, respectively, *vs.* 2.3 ± 0.2 l/24 h for placebo on day 1) and dose-dependently decreased mean urine osmolality. Treatment with tolvaptan resulted in a significant increase in serum sodium within the normal ranges (< 4 mEq/l) as compared to placebo; no changes in serum potassium, laboratory parameters or blood pressure were observed. Of the patients receiving 30, 45 and 60 mg tolvaptan and placebo, 34, 23, 23 and 32% had hyponatremia at baseline. On day 1 postdosing, both normonatremic and hyponatremic patients exhibited significant dose-dependent increases in urine volume and increases in serum sodium. However, by day 25 sodium levels returned to baseline in normonatremic patients and remained normalized in hyponatremic patients treated with the agent (14-16) (Box 1).

A multicenter, open-label study involving 28 patients hospitalized with hyponatremia (serum sodium < 135 mEq/l) due to heart failure (71%) or cirrhosis (29%) with signs of extracellular volume expansion, who were randomized after a 2-day run-in period to fluid restriction (1200 cc/24h) or tolvaptan (10 mg/day increased up to 60 mg/day as needed) without fluid restriction, showed that treatment with tolvaptan was effective in correcting hyponatremia without requiring fluid restrictions. Tolvaptan was well tolerated and resulted in a more rapid and marked normalization of serum sodium as compared to standard therapy. Similar efficacy of treatment was observed in patients with heart failure and cirrhosis. Serum sodium was increased 5.73 ± 3.25 mEq/l in the group receiving tolvaptan without fluid restrictions as compared to 1 ± 4.69 mEq/l in the fluid restriction group (17) (Box 2).

A randomized, placebo-controlled study with a 2-day run-in period and involving 83 patients with heart failure (NYHA class II-III) and signs of congestion, compared the efficacy of tolvaptan (30 mg) alone, furosemide (80 mg) alone or a combination of the two agents administered for 7 days. Patients were maintained on their standard therapy without fluid restrictions but were removed from their baseline diuretic therapy and placed on a low-sodium diet (2 g/day). Tolvaptan was well tolerated. Treatment with tolvaptan resulted in a significant increase in serum sodium within the normal range as compared to placebo and furosemide alone. Treatment with tolvaptan also resulted in reductions in leg edema, dyspnea rales and hepatomegaly as compared to placebo. Significantly greater changes in body weight as compared to baseline were seen in patients treated with

Box 1: Effects of tolvaptan in the treatment of chronic heart failure (14-16) [Prous Science Integrity®].

Design	Randomized, placebo-controlled clinical study
Population	Patients with chronic heart failure (n=250)
Treatments	Tolvaptan, 30 mg o.d. p.o. + standard therapy and stable furosemide [20-240 mg/d] x 25 d (n=64) Tolvaptan, 45 mg o.d. p.o. + standard therapy and stable furosemide [20-240 mg/d] x 25 d (n=62) Tolvaptan, 60 mg o.d. p.o. + standard therapy and stable furosemide [20-240 mg/d] x 25 d (n=62) Placebo + standard therapy and stable furosemide [20-240 mg/d] x 25 d (n=62)
Results	Body weight (kg) change @ d 1: T45* (–0.96) ≥ T60* (–0.84) ≥ T30* (–0.79) > P (0.32) [*p <0.001 *vs.* P] Body weight (kg) change in patients with basal hyponatremia @ d 1: T45* (–1.2) ≥ T60* (–0.5) ≥ T30 (–0.2) ≥ P (0.3) [*p <0.05 *vs.* P]; @ d 25: T45* (–1.3) ≥ T60* (–0.7) ≥ T30 (–0.4) ≥ P (0.4) [*p <0.05 *vs.* P] Body weight (kg) change in patients with basal normonatremia @ d 1: T30* (1.0) ≥ T60* (-0.9) ≥ T45* (–0.9) > P (0.3) [*p <0.05 *vs.* P]; @ d 25: T30* (1.0) ≥ T45* (1.0) ≥ T60* (–0.4) > P (0.7) [*p <0.05 *vs.* P] Serum sodium (mEq/l) change in patients with basal hyponatremia @ d 1: T60* (5.2) ≥ T30* (3.6) ≥ T45* (3.3) > P (1.2) [*p <0.05 *vs.* P]; @ d 25: T60* (4.3) ≥ T30 (2.1) ≥ T45* (1.5) ≥ P (1.0) [*p <0.05 *vs.* P] Serum sodium (mEq/l) change in patients with normonatremia @ d 1: T45* (2.9) ≥ T60* (2.6) ≥ T30 (2.3) ≥ P (–1.8) [*p <0.05 *vs.* P]; change @ d 25: P (–0.6) ≥ T45 (–0.5) ≥ T60 (–0.4) ≥ T30 (0.2) Urine volume (l/24 h), change @ d 1: T60* (4.6) ≥ T45* (4.2) ≥ T30* (3.9) > P (2.3) [*p <0.001 *vs.* P] Mean urine osmolality: T dose-dependent decrease Net fluid loss (> 1000 ml): T not dose-dependent Edema reduction: T > P [p <0.05] Serum potassium, other laboratory values and blood pressure: no change
Conclusions	Tolvaptan reduced body weight and edema and normalized serum sodium in patients with heart failure and hyponatremia without adversely changing serum electrolytes

Box 2: Effects of tolvaptan in the treatment of hyponatremia (17) [Prous Science Integrity®].

Design	Randomized, open, multicenter clinical study
Population	Patients with heart failure or cirrhosis and hyponatremia (n=28)
Treatments	Tolvaptan, 10 —> 60 mg p.o. o.d. (n=17) Controls [fluid restriction (<1200 cc/24 h)] (n=11)
Results	Serum sodium (mEq/l), change @ 300 h: T (4.4%) > P (0.8%) [p = 0.0065] Response to tolvaptan was similar in patients with heart failure and cirrhosis
Conclusions	Tolvaptan was well tolerated, effective and rapid in reducing hyponatremia associated with heart failure or cirrhosis without the need for fluid restriction

Box 3: Effects of tolvaptan versus furosemide in congestive heart failure (18) [Prous Science Integrity®].

Design	Randomized, comparative, placebo-controlled clinical study
Population	Patients with heart failure in NYHA class II-III and signs of congestion (n=83)
Treatments	Tolvaptan, 30 mg o.d. p.o. x 7 d (n=20) Furosemide, 80 mg o.d. p.o. x 7 d (n=22) Tolvaptan, 30 mg o.d. p.o. + Furosemide, 80 mg o.d. p.o. x 7 d (n=20) Placebo (n=21)
Results	Body weight (kg), change @ 7 d: T* (–1.37) ≥ TF* (–1.13) ≥ F (–0.54) ≥ P (0.72) [*p <0.01 *vs.* baseline] Urine output (ml/d), change @ 7 d: T*/** (2646) ≥ TF*/** (2585) ≥ F* (894) ≥ P (423) [*p <0.01 *vs.* baseline; **p <0.001 *vs.* F] Serum sodium increase within the normal range: T > P [p <0.02 *vs.* P] Serum potassium or other laboratory values: no changes Reductions in leg edema, dyspnea, jugular venous pressure, rales and hepatomegaly: T > P
Conclusions	Tolvaptan alone was well tolerated and reduced body weight and edema without adversely affecting serum electrolytes in patients with heart failure and signs of volume overload

tolvaptan alone (–1.37 ± 1.61 *vs.* –0.54 ± 1.59 for furosemide alone and +0.72 ± 2.42 kg in placebo) or in combination with furosemide (–1.13 ± 1.49 kg). In addition, urine output was significantly higher in patients treated with tolvaptan alone (+2646 ± 1503 ml/24 h) or tolvaptan in combination with furosemide (+2585 ± 2119 ml/24 h) as compared to patients treated with furosemide alone (+894 ± 853 ml/24 h) or placebo (+423 ± 786 ml/24 h). Tolvaptan treatment was not associated with changes in serum potassium, laboratory parameters or blood pressure (18) (Box 3).

Tolvaptan is currently undergoing phase II trials as a treatment for congestive heart failure (19).

Source

Otsuka Pharmaceutical Co., Ltd. (JP).

References

1. Kondo, K., Ogawa, H., Yamashita, H. et al. *7-Chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoyl-amino)benzoyl]-2,3,4,5-tetrahydro-1H-1-benzazepine (OPC-41061): A potent, orally active nonpeptide arginine vasopressin V_2 receptor antagonist.* Bioorg Med Chem 1999, 7: 1743-54.

2. Ogawa, H., Miyamoto, H., Kondo, K., Yamashita, H., Nakaya, K., Komatsu, H., Tanaka, M., Kora, S., Tominaga, M., Yabuuchi, Y. (Otsuka Pharmaceutical Co., Ltd.). *Benzoheterocyclic cpds.* US 5985869.

3. Prous Science Drug R&D Backgrounders: *Heart failure (online publication).* Updated March 25, 2002.

4. Thibonnier, M., Conarty, D.M., Preston, J.A., Wilkins, P.L., Berti-Mattera, L.N., Mattera, R. *Molecular pharmacology of human vasopressin receptors.* Adv Exp Med Biol 1998, 449: 251-76.

5. Andreoli, T.E. *Edematous states: An overview.* Kidney Int Suppl 1997, 59: S2-10.

6. Johnston, C.I. *Vasopressin in circulatory control and hypertension.* J Hypertens 1985, 3: 557-69.

7. Goldsmith, S.R. *Vasopressin as vasopressor.* Am J Med 1987, 82: 1213-9.

8. Dahia, P.L., Ahmed-Shuaib, A., Jacobs, R.A., Chew, S.L., Honegger, J., Fahlbusch, R., Besser, G.M., Grossman, A.B. *Vasopressin receptor expression and mutation analysis in corticotropin-secreting tumors.* J Clin Endocrinol Metab 1996, 81: 1768-71.

9. de Keyzer, Y., Lenne, F., Auzan, C., Jégou, S., René, P., Vaudry, H., Kuhn, J. M., Luton, J.-P., Clauser, E., Bertagna, X. *The pituitary V_3 vasopressin receptor and the corticotroph phenotype in ectopic ACTH syndrome.* J Clin Invest 1996, 97: 1311-8.

10. Yamamura, Y., Nakamura, S., Itoh, S. et al. *OPC-41061, a highly potent human vasopressin V_2-receptor antagonist: Pharmacological profile and aquaretic effect by single and multiple oral dosing in rats.* J Pharmacol Exp Ther 1998, 287: 860-7.

11. Hirano, T., Yamamura, Y., Nakamura, S., Onogawa, T., Mori, T. *Effects of the V_2-receptor antagonist OPC-41061 and the loop diuretic furosemide alone and in combination in rats.* J Pharmacol Exp Ther 2000, 292: 288-94.

12. Tammara, B.K., Sekar, K., Bramer, S.L. *The disposition of a single dose of ^{14}C-OPC-41061 in healthy male volunteers.* Annu Meet Am Assoc Pharm Sci (AAPS) (Nov 14-18, New Orleans) 1999, Abst.

13. Tammara, B., Bricmont, P., Bramer, S. *Pharmacokinetic (PK) and pharmacodynamic (PD) interaction between OPC-41061 (OPC), furosemide (F) and hydrochlorothiazide (H).* 100th Annu Meet Am Soc Clin Pharmacol Ther (March 18-20, San Antonio) 1999, Abst PIII-100.

14. Gheorghiade, M., Niazi, I., Ouyang, J., Czerwiec, F., Kambayashi, J., Orlandi, C. *Chronic effects of vasopressin receptor blockade with tolvaptan in congestive heart failure: A randomized, double-blind trial.* Circulation 2000, 102(18, Suppl.): Abst 2869.

15. Gheorghiade, M., Konstam, M.A., Udelson, J.E., Ouyang, J., Orlandi, C. *Vasopressin receptor blockade with tolvaptan in chronic heart failure: Differential effects in normonatremic and hyponatremic patients.* J Am Coll Cardiol 2002, 39(5, Suppl. A): 171A.

16. Orlandi, C., Ouyang, J., Kambayashi, J., Konstam, M.A., Gheorghiade, M. *Beneficial effects of chronic therapy with tolvaptan, a novel vasopressin receptor blocker, in patients with congestive heart failure.* Circ J 2002, 66(Suppl. 1): Abst OE-360.

17. Gheorghiade, M., Orlandi, C., Gottlieb, S., Corder, C., Udelson, J.E., Ouyang, J., Konstam, M.A. *Vasopressin V_2 receptor blockade versus fluid restriction in the treatment of hyponatremia: Results from a multicenter randomized controlled trial.* Circulation 2001, 104(17, Suppl. 2): Abst 2075.

18. Udelson, J.E., Orlandi, C., O'Brien, T., Sequeira, R., Ouyang, J., Konstam, M.A. *Vasopressin receptor blockade in patients with congestive heart failure: Results from a placebo controlled, randomized study comparing the effects of tolvaptan, furosemide, and their combination.* J Am Coll Cardiol 2002, 39(5, Suppl. A): 156A.

19. *Pipeline status of compounds with CV indications.* Otsuka Web Site April 4, 2002.

20. Tahara, A., Saito, M., Sugimoto, T. et al. *Pharmacological characterization of YM087, a potent, nonpeptide human vasopressin V_{1A} and V_2 receptor antagonist.* Naunyn-Schmied Arch Pharmacol 1998, 357: 63-9.

21. Tahara, A., Saito, M., Sugimoto, T. et al. *Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells.* Br J Pharmacol 1998, 125: 1463-70.

22. Nakamura, S., Yamamura, Y., Itoh, S. et al. *Characterization of a novel nonpeptide vasopressin V_2-agonist, OPC-51803, in cells transfected with human vasopressin receptor subtypes.* Br J Pharmacol 2000, 129: 1700-6.

23. Tahara, A., Tsukada, J., Ishii, N., Tomura, Y., Wada, K., Kusayama, T., Yatsu, T., Uchida, W., Tanaka, A. *Characterization of rodent liver and kidney AVP receptors: Pharmacologic evidence for species differences.* Regul Pept 1999, 84: 13-9.

24. Nakamura, S., Hirano, T., Tsujimae, K., Aoyama, M., Kondo, K., Yamamura, Y., Mori, T., Tominaga, M. *Antidiuretic effects of a nonpeptide vasopressin V_2-receptor agonist, OPC-51803, administered orally to rats.* J Pharmacol Exp Ther 2000, 295: 1005-11.

25. Tahara, A., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Takanashi, M., Yatsu, T., Uchida, W., Tanaka, A. *Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V_{1A} and V_2 receptor antagonist, in vitro and in vivo.* J Pharmacol Exp Ther 1997, 282: 301-8.

26. Serradeil-Le Gal, C., Lacour, C., Valette, G. et al. *Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V_2 receptor antagonist.* J Clin Invest. 1996, 98: 2729-38.

27. Naito, A., Ohtake, Y., Hasegawa, H. et al. *Pharmacological profile of VP-343, a novel selective vasopressin V_2 receptor antagonist, in rats.* Biol Pharm Bull 2000, 23: 182-9.

28. Ohtake, Y., Naito, A., Hasegawa, H. et al. *Novel vasopressin V_2 receptor-selective antagonists, pyrrolo[2,1-a]quinoxaline and pyrrolo[2,1-c][1,4]benzodiazepine derivatives.* Bioorg Med Chem 1999, 7: 1247-54.