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エンドセリン受容体拮抗薬の末梢性浮腫評価を目的としたラット利尿モデルの検討
Evaluation of Peripheral Edema by Endothelin Receptor Antagonists Using a Rat Diuresis Model

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Introduction

Endothelin receptor antagonists are essential therapeutic options for pulmonary arterial hypertension (PAH); however, peripheral edema as an adverse effect remains a significant clinical challenge. In the present study, a rat diuresis model was employed to establish an experimental system for evaluating edema formation associated with endothelin receptor antagonists. By continuously monitoring urine output in a time-course manner in rats, antidiuretic effect of bosentan - a non-selective ETA/ETB receptor antagonist - and ambrisentan - a selective ETA receptor antagonist - were assessed.

Materials and Methods

This study was conducted as approved by the International Animal Care and Use Committee of NISSEI BILIS Co., Ltd., Shiga Laboratory.

Animals, Reagents and Drugs

- Animals
  Male SD rats, 12 to 14 weeks old (Japan SLC, Inc.)
- Reagents
  - Thiobutabarbital sodium (Inactin, Sigma)
  - Bovine serum albumin (BSA, Sigma)
  - γ-globulins from bovine blood (γ-globulin, Sigma)
  - 0.5% methylcellulose (MC) solution (FUJIFILM Wako Pure Chemical Corporation)
- Drugs
  - Ambrisentan (Tokyo Chemical Industry Co., Ltd.)
  - Bosentan (Bosentan hydrate, Bosentan Tablets 62.5mg MOCHIDA, Mochida Pharmaceutical Co., Ltd.)

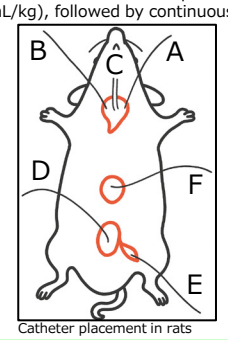
  Ambrisentan and bosentan were suspended in 0.5% MC solution.

Group design

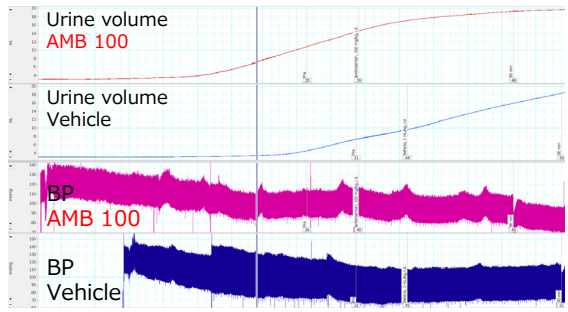
Groups	Drugs (intra-duodenal administration, 2 mL/kg)	No. of animals
Vehicle	0.5% MC solution	6
AMB 35	Ambrisentan, 17.5 mg/mL	5
AMB 100	Ambrisentan, 50 mg/mL	5
BOS 10	Bosentan, 5 mg/mL	6
BOS 30	Bosentan, 15 mg/mL	6
BOS 100	Bosentan, 50 mg/mL	6

Study procedures

- Anesthesia: Rats were fasted overnight and anesthetized via intraperitoneal injection of thiobutabarbital sodium (100 mg/kg).
- Catheter Placement for Continuous Infusion of Artificial Plasma and Diuretic Solution (A, B): Catheters were inserted into both jugular veins for continuous infusion of artificial plasma (25 mg/mL γ-globulin and BSA) and diuretic solution (10 mg/mL BSA) at designated flow rates using syringe pumps (HARVARD Apparatus).
- Tracheal Intubation (C): The trachea was surgically exposed, and a tracheal tube was inserted and secured.
- Bladder Catheterization and Urine Collection (D): A lower abdominal incision was made to expose the bladder, through which a urinary catheter was inserted and fixed. Urine output was continuously recorded using LabChart Pro (AD Instruments).
- Blood Pressure Monitoring (E): A femoral artery catheter was inserted to monitor arterial blood pressure (BP) and heart rate. These data were not included in the evaluation.
- Duodenal Catheter Placement and Drug Administration (F): A catheter was placed in the duodenum for drug administration. Once urine output reached at the baseline (0.10 mL/min), urine volume was measured for 30 minutes to obtain the pre-administration (Pre) value. Drugs were then administered intra-duodenally (2 mL/kg), followed by continuous measurement for 90 minutes.



Catheter placement in rats

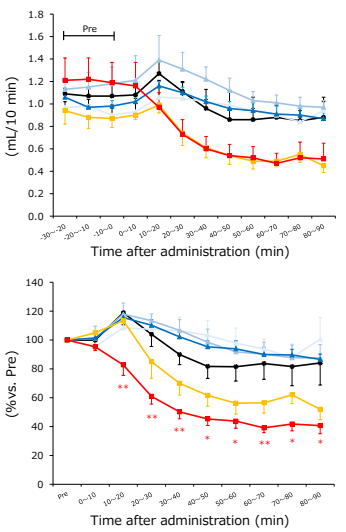


Measured Parameter

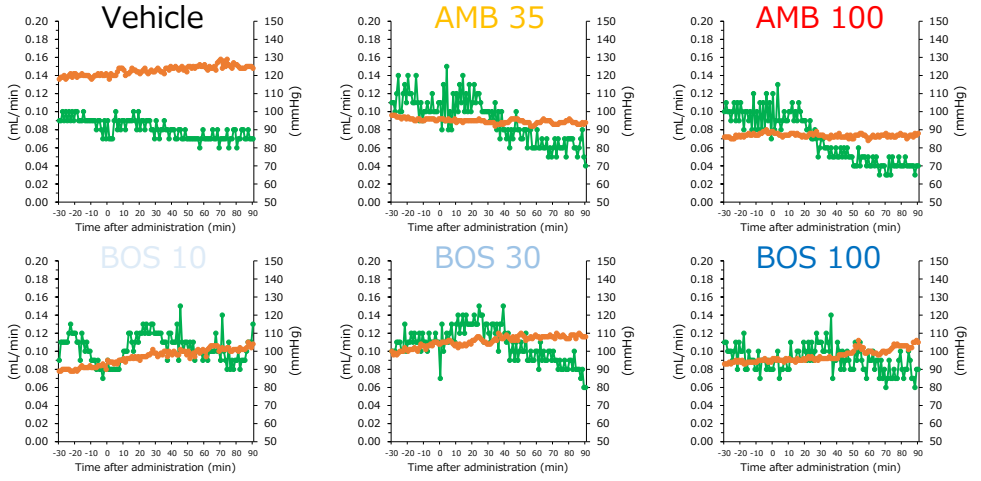
- Urine volume
  Urine volume was calculated every 10 minutes during the 30 minutes before and 90 minutes after duodenal administration of the drugs. The average volume during the pre-administration 30 minutes was set as 100%.

Results

Urine volume



Temporal changes in individual urine volume and MBP



Vehicle (n=6) AMB 35 (n=5) AMB 100 (n=5) BOS 10 (n=6) BOS 30 (n=6) BOS 100 (n=6) Urine volume (left axis) MBP (rignt axis)

Each value represents the mean ± SEM.
* and ** : $p<0.05$ and $p<0.01$ vs. Vehicle by Dunnett's test

Conclusion

Ambrisentan administration at 35 and 100 mg/kg demonstrated dose-dependent antidiuretic effects, with the 100 mg/kg group showing significantly decreased urine output compared to the vehicle group. In contrast, bosentan at doses ranging from 10 to 100 mg/kg did not induce notable changes in urine volume. These findings align with clinical observations and likely reflect the mechanism by which ambrisentan induces edema more frequently than bosentan. This model proves valuable for evaluating peripheral edema caused by endothelin receptor antagonists. It may also serve as a predictive tool for the assessment of adverse effects associated with therapeutics for diseases involving endothelin, such as pulmonary arterial hypertension (PAH).

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☑ The author has no conflict of interest to disclose with respect to this presentation.