Chapter 12. Model of arterial hypertension induced by Angiotensin II

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Rationale for model development

Arterial hypertension is a problem of public health, due to end-organ damage and the direct role it has in cardiovascular morbidity and mortality. Arterial hypertension still remains the most important modifiable risk factor for a high range of cardiovascular diseases, including coronary artery disease, stroke, congestive heart failure, peripheral vascular disease and chronic kidney disease. The prevalence of arterial hypertension is very high and continues to increase with age, reaching over 70% in men and women 75 or older. Control rate of arterial pressure is just 18% of treated patients with hypertension. Arterial hypertension continues to be under diagnosed and untreated. The awareness, treatment and control of arterial pressure are known as being inappropriate. Due to these reasons, over the time, a series of experimental models had been developed. These experimental models, which simulate human cardiovascular diseases, provide useful information for understanding the root-cause and progression of disease, as well for discovering of possible therapeutic targets.

The renin-angiotensin system (RAS) is a complex system that plays an important role in maintaining the hemodynamic
stability, by regulation of arterial pressure and water-electrolyte balance. Increased activity of angiotensin II contributes to target organ damage and increases the risk of cardiovascular events by increasing both blood pressure, as well as direct effect, that angiotensin II (Ang II) has on vascular endothelial, cardiac and renal tissue by stimulating inflammatory phenomena and the development of atherosclerosis. Ang II reduces the ability of renal sodium excretion and initiates a series of events that lead to increased blood pressure. It is necessary to increase the blood pressure, using the phenomenon of pressure-natriuresis, to return at a normal excretion of sodium. Hypertension affects target organs (kidneys) and leads to the initiation of a vicious circle that contributes to maintaining high blood pressure. RAS may act on blood pressure via its effects on the kidney (control of salt and water), brain (thirst) and the sympathetic nervous system.

Ang II-induced hypertension is one of the more widely used pharmacological models of murine hypertension. Ang II is a peptide, which in vivo has a very short half-life. After a single administration, its plasma concentration has a peak, and then drops rapidly until it is removed. Duration of action of a single administration is limited to a few minutes and so its effect cannot be highlighted. Animal exposure to multiple daily doses leads to large fluctuations in plasma concentrations of Ang II. In particular, repeated injection of Ang II was stressful for the animal and required difficult periodicity. As fluid infusion, isotonic saline solutions are used. To eliminate these drawbacks, administration of Ang II is performed using Alzet osmotic minipumps. Angiotensin II can be delivered to mice or rats subcutaneously via Alzet osmotic mini pumps, for a period of up to 2-4 weeks. Typically, doses of 0.70-1.0 mg/kg per day
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are used for mice. Infusion rates of Ang II that have generated hypertension in rats have ranged from 50 to 500 ng/kg/min. The Ang II model can be used as a fast (7-10 days) or slowly-developing hypertension (4-8 week) to study Ang II-induced hypertension and end-organ damage. After 2 weeks of infusion, animals develop aortic and cardiac hypertrophy, as well as cardiac and renal fibrosis.

In our laboratory we used the 2001 model, which delivers fluids at a rate of approximately $1\mu l/h$ for 14 days. Administration of Ang II does not cause immediate increases in systemic blood pressure, but it rather leads to a slowly developing hypertension over a period of 6–10 days. In addition to the direct renal effects, promoting salt and water retention, Ang II induced hypertension produces a presumably baroreflex mediated sympathoinhibition, corresponding to the increased blood pressure. However, with a concomitant increase of dietary salt, the sympathetic nervous system does not respond to the increase in pressure with the appropriate inhibition.

Alzet osmotic mini-pumps – pump design and available models

Pump design

Alzet pumps use the osmotic pressure difference between a compartment within the pump, called the salt sleeve, and the tissue environment in which the pump is implanted. The high osmolality of the salt sleeve causes water to flux into the pump through a semipermeable membrane, which forms the outer surface of the pump (Figure 1). As the water enters the salt sleeve, it compresses the flexible reservoir, displacing the test
solution from the pump at a controlled, predetermined rate. Because the compressed reservoir cannot be refilled, the pumps are designed for single-use only.

The rate of delivery by an Alzet pump is controlled by the water permeability of the pump’s outer membrane. Thus, the delivery profile of the pump is independent of the drug formulation dispensed. Drugs of various molecular configurations, including ionized drugs and macromolecules, can be dispensed continuously in a variety of compatible vehicles at controlled rates. The molecular weight of a compound, or its physical and chemical properties, has no bearing on its rate of delivery by Alzet pumps.

Models available

The volume delivery rate of Alzet pumps is fixed at manufacture. They are available with a variety of delivery rates between 0.11 and 10 µL/hr and delivery durations between 1 day and 6 weeks (Figure 2). While the volume delivery rate of the pump is fixed, different dosing rates can be achieved by
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varying the concentration of agent in the solution or suspension used to fill the pump reservoir.

Fig. 2. Models available (Copyright Alza Corporation)

Filling the pump:

The first step in preparing the pump is the dissolution of Ang II. Ang II is commonly supplied as a lyophilized powder in glass bottles. Ang II in solution has a high affinity for glass, thus solutions must be prepared in plastic recipients. It is important to use sterile technique. Gloves are used always, as natural oils from hands will damage the exterior of the pump casings.
Pump filling procedure (Figure 3) starts with the empty pump (body and flow regulator) being weighed and recorded. A supplied needle is used with pumps to draw infuse into syringe and remove air bubble from it. The filling should be slow and will stop when infuse gets out of the pump. The needle is withdrawn and flow regulator is inserted into pump. The pump is reweight and recorded. The increase in weight should equal the filling volume. The pump is kept upright until it is implanted.

![Fig 3. Body and flow regulator of the pump](image)

**Implantation**

The site for subcutaneous implantation of Alzet pumps in mice and rats is on the back, slightly posterior to the scapulae. The contents of the pump (Ang II) will be delivered into the local subcutaneous space. Absorption of the compound by local capillaries results in systemic administration.

**Steps:**
1. The animal is anesthetized.
2. The skin is shaved and washed over implantation site.
3. A suitable incision, adjacent to the back of the animal (a mid-scapular), is made.

4. A hemostat is inserted through the incision, by opening and closing the jaws of the hemostat, and subcutaneous tissue is spread, to create a pocket for the pump. The pocket should be large enough to allow some free movement of the pump (e.g. 1 cm longer than the pump). Avoid making the pocket too large, as this will allow the pump to turn around or slip down on the flank of the animal. The pump should not rest immediately beneath the incision, which could interfere with the healing of the incision.

5. A filled pump is inserted into pocket with the delivery port first. This minimizes interaction between the compound delivered and the healing of the incision.

6. The wound is closed with clips or sutures. Two metal clips will normally suffice.

Angiotensin II hypertension in the rat

In our laboratory we use this Ang II experimental model to induce hypertension. The flow chart presented in Figure 4 depicts the timing for the essential steps.
Arterial blood pressure measurements

Systolic blood pressure was determined twice a week in conscious rats using tail-cuff plethysmography method (Figure 5). Measurements were done at the same hour of the day (10.30 a.m.). Before the beginning of the measurements, the rats were conditioned six to seven times to the procedures.
A gradual increase in systolic blood pressure occurs as early as three days after insertion of the Ang II pump. After 2 weeks of infusion, we observed a group average increase in systolic blood pressure of approximately 40-50 mmHg, as compared to control rats, which received implants with saline only (Figure 6).
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Fig. 6. Blood pressure recording using the tail-cuff method in a control rat (saline, upper panel, systolic pressure = 118 mmHg) and a rat treated with Ang II (lower panel, systolic pressure = 188 mmHg).
We consider the method very efficient in producing a live model for arterial hypertension, a model that we use for a wide range of experiments regarding both pathophysiological events in hypertension, but also in testing antihypertensive drugs.

References:

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