

Treatment Protocol

This study used four groups of six-week old C57BL/6 mice: conventional (n=6) and GF (n=6) mice were infused with angiotensin-II (AngII) for 4 weeks ($400\text{ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Alzet 1004) and in parallel control groups, conventional (n=6) and GF (n=6) mice instead received saline (0.9%w/v, Nova Tech Inc.) via minipumps (saline). Each group had equal numbers of males and females. All mice were housed individually (to avoid 'cage' effects due to coprophagia) on a 14/10 light/dark cycle, were fed the same diet (Purina Conventional Mouse Diet, LabDiet® JL Rat/Mouse and Auto 6F #5K67) and had free access to water during the experimental period.

For both conventional and GF mice, minipumps (Alzet®) were implanted subcutaneously after intraperitoneal (I.P.) anesthesia (Ketamine 50mg/kg, Xylozine 10mg/kg). A 2-4 mm subcutaneous incision was made to insert the minipump on the dorsal flank. After surgery, mice received a single subcutaneous dose (4mg/kg) of ostilox (meloxicam, Norbrook®) to relieve post-operative pain and meloxicam in the drinking water (0.2 mg/Kg) for next 24 hours.