

September 14th, 2016

**Name of Journal:** World Journal of Gastroenterology  
**Manuscript Type:** Basic Study

**Title of manuscript:** Effects of asymmetric dimethylarginine on renal arteries in portal hypertension and cirrhosis

**Authors:** Gloria Segarra, Belén Cortina, María Dolores Mauricio, Susana Novella, Paloma Lluch, Javier Navarrete-Navarro, Inmaculada Noguera, Salvador Lluch, Pascual Medina

#### **Animal care and use statement**

The animal protocols were designed to minimize pain or discomfort to the animals. Male Sprague-Dawley rats (200–250 g) were acquired from Charles River, and housed according to institutional guidelines (constant room temperature 22°C, 12 h light/dark cycle, 60% humidity, standard rat chow and water *ad libitum*). All protocols were approved by the Institutional Ethics Committee at the University of Valencia (No. UV20121124), and conformed to the Guide for the Care and Use of Laboratory Animals published in Directive 2010/63/EU of the European Parliament.

Rats were assigned to a sham-operated group (Sham; n = 15), partial portal vein ligation group (PPVL; n = 15) or bile duct excision group (BDE; n = 15) in a random way. After induction of anesthesia by isoflurane (5%, by induction chamber), rats received isoflurane 2–3% by mask. To assess the adequacy of anesthesia during the surgery, parameters such as responsiveness (e.g. no response to toe pinching), respiratory rate, and heart rate were monitored. Analgesia with Butorphanol was used pre-operatively for preemptive analgesia and post-operatively every 4-12 h during the day of the surgery.



*Surgical procedures*

Pre-hepatic portal hypertension induced by partial portal vein ligation (PPVL) was performed by placing a 20-gauge needle on the portal vein. A non-absorbable surgical thread ligature was placed around the needle and portal vein, and the needle was then withdrawn. The studies were performed 14-16 days after PPVL, when the hyperdynamic circulation accompanying portal hypertension was fully established. Secondary biliary cirrhosis was induced by bile duct excision (BDE). The bile duct was cut between a ligature close to the hilum of the liver and another one close to the duodenum. The studies were performed 28 days after BDE when secondary biliary cirrhosis had developed. For the sham operation (Sham), the duodenum, portal vein, and bile duct were exposed during laparotomy, and the abdomen was closed 15 min later.

Pascual Medina, Ph.D.  
Department of Physiology  
School of Medicine  
University of Valencia  
Avenida Blasco Ibáñez 15  
46010 Valencia