THE DUTCH RABBIT IN TOXICITY TESTING
CHEMICAL - INDUCED CREATINE KINASE RELEASE;
A SPECIES- AND STRAIN-SPECIFIC RESPONSE?

Organon Int. B.V., Drug Safety Rand D Labs.,
P.O. Box 20, 5340 BH Oss, The Netherlands

INTRODUCTION

Within the framework of preclinical safety testing a compound having cardiovascular activity (CC), was studied in rabbits. For the study of its teratogenic potential, CC was administered orally to Dutch rabbits from day 6 to 18 of pregnancy at dose levels of 25, 50 and 100 mg/kg body weight. Food consumption decreased in a dose-related manner. At 100 mg/kg no live foetuses could be recovered on day 29 of pregnancy; at 50 mg/kg only half of the number of dams had live young, whereas at 25 mg/kg no embryotoxicity was observed. Inhibition of food consumption was found in association with overfilling of the stomach, contraction of its pyloric part, empty intestines and high plasma levels of creatine kinase (CK). These effects were not present in oral toxicity studies in Wistar rats and Beagle dogs. New studies were designed to investigate species- and strain-specificity and the underlying mechanism of toxicity.

MATERIALS AND METHODS

Rabbit strains

The following strains were used:
- Dutch rabbits (spf) supplied by the Broekman Inst., Someren, The Netherlands
- New Zealand White (NZW) rabbits from the same breeder
- Chinchilla rabbits from HSD/Cpb, Zeist, The Netherlands
- Himalayan rabbits (HY/CR) from Charles River, France

If not stated otherwise adult male animals were used.

Husbandry

Animals were housed individually in galvanized iron cages measuring 50x50x40 cm (lxbxh) under standard conditions of temperature (21 +/- 3 C), humidity (60 +/- 20%) and artificial illumination (12 hrs off, 12 hrs on). They had free access to tap water from bottles and to pelleted food (LKK 20, Hope Farms, Woerden, The Netherlands). The study with Himalayan rabbits was carried out at RL-CERM, Riom, France.

Dosing

For oral and intraperitoneal administration CC was suspended in an aqueous medium of 0.5% gelatin containing 5% mannitol. The suspension was administered intragastrically through a polyethylene catheter (Pharmaplas Disposable Catheters).

For intravenous administration CC was dissolved in 30 % polyethylene-glycol (PEG300) in saline to the limit of solubility of 1.7 mg/ml. The drug solution was infused into the marginal ear vein at a pump rate of 0.5 ml/min.

Clinical observations, ECG recordings and autopsy

Animals were daily observed for behavioural changes and clinical signs.
Electrocardiographic recordings were obtained by bipolar leads I, II, III and unipolar leads aVR, aVL and aVF (Cardiscript IV, Schwartzer, W-Germany). For autopsies animals were killed by destruction of the brain stem (Supercash pistol, Accles and Shelvoke Ltd., No. 78130) followed by rupture of the diaphragm.

Laboratory investigations
Plasma from heparinized blood, collected by puncture of the marginal ear vein, was assayed for creatine kinase (CK) at 30°C according to the recommendations of the GSCC (1) and in some studies for lactate dehydrogenase (LDH) also at 30°C (2).

Analysis of results
Pre-dosing values of food intake and plasma CK showed large inter-individual variation. To reduce variation each animal served as its own control by expressing the post-dosing values as a percentage of the pre-dosing value. A decrease in food intake was considered significant for values lower than 75% and an increase of CK for values higher than 200%.

RESULTS
Intragastric route of administration
Following a single i.g. dose of 100 mg/kg food intake decreased abruptly. The number of faecal droppings was sharply decreased during a subsequent period of 7 hrs. Food intake was restored to the pre-dosing value within 2 or 3 days.

Plasma CK levels in samples collected 24 hrs post-dosing were increased as compared with the pre-dosing value. From a study with i.g. dose levels of 10, 20 and 30 mg/kg it appeared that the minimum dose level giving a 2-fold increase in CK activity was 30 mg/kg. Food intake at that dose level was depressed to about 40% of the pre-dosing value.

The time course of the CK rise was studied in a female rabbit using an i.g. dose level of 100 mg/kg. Plasma CK activity increased sharply between 16 and 24 hrs post-dosing. The values before dosing and at 16, 20 and 24 hrs after dosing were 277, 646, 30000 and 57400 U/L, respectively.

Following multiple dosing the suppression of food intake lasted the entire treatment period and returned to normal thereafter. The CK rise was first observed 24 hrs after the 1st dose, whereas subsequent doses caused a low or undetectable CK rise.

Intravenous route of administration
Following intravenous administration of CC at dose levels of 6.9 or 10.7 mg/kg a slight reduction in food intake was observed. In contrast to the oral route the peak level of CK activity was observed 7 hrs post-dosing.

The values were 19300 and 13700 U/L at 7 and 24 hrs post-dosing, respectively. A significant rise occurred already at 2 hrs post-dosing (1896 U/L).

In the rabbit which received 10,7 mg/kg plasma CK and LDH levels were determined. The peak level for LDH was also found at 7 hrs post-dosing.

Intraperitoneal route
A high plasma CK level was also observed 24 hrs following an intraperitoneal injection of CC (100 mg/kg), despite the fact that at autopsy a significant amount of test compound was found as a precipitate in the abdominal cavity. At 24 hrs post-dosing the plasma CK level was 31600 U/L (more than 80-fold the pre-dosing value).

Other rabbit strains
Intragastric administration of CC (100 mg/kg) to NZW, Chinchilla