Characterization of the arteritis induced by infusion of rats with UK-61,260, an inodilator, for 24 h.
A comparison with the arteritis induced by fenoldopam mesylate

Received: 21 October 1994 / Accepted: 13 March 1995

Abstract  Administration of fenoldopam mesylate (FM), a dopaminergic agonist, or of cyclic cAMP phosphodiesterase inhibitors (PDE III), for example theophylline and caffeine, induces arteritis in the rat. In this study we characterized the arteritis induced by UK-61,260, an investigational inotropic agent with vasodilatory properties which displays an inhibitory action on cyclic AMP phosphodiesterase, in comparison with lesions induced by FM. The compounds were administered to Sprague-Dawley rats by intravenous infusion over 24 h (FM and UK-61,260), orally or subcutaneously (UK-61,260); the rats were killed and necropsied for pathological examination at various times between 0 h and 28 days post-infusion. Infusion of UK-61,260 at doses of 100, 300 or 400 mg/kg produced arteritis mainly in the mesenteric arteries and occasionally in the renal, pancreatic, gastric and coronary arteries. There were no arterial lesions after infusion of 30 mg/kg, or after administration of 30, 100 or 200 mg/kg per day subcutaneously for 7 days, or after acute administration of 100, 300, 400 or 600 mg/kg orally. Infusion of rats with 72 or 144 mg/kg FM produced arteritis over a wider range of tissues than did UK-61,260. However, the arterial lesions produced by infusion of either drug have the same initial aspect and a similar evolution with time. Immediately after the end of the infusion, minimal necrosis and haemorrhage occurred in the media only, without involvement of the endothelium or the perivascular space. This indicates that the media of the artery is the primary site of injury. The lesions seen 1 and 3 days post-infusion were characterized by severe medial necrosis and haemorrhage with perivascular acute inflammation and appeared macroscopically as haemorrhagic spots on the vessels. On days 7, 14 and 28 post-infusion, no medial necrosis or haemorrhage were present, while perivascular chronic inflammation and moderate smooth muscle hyperplasia were seen. It appeared, therefore, that the lesions underwent repair in 28 days, but footprints of the damage were still present 28 days post-infusion. The similarity between arteritis induced in rats by fenoldopam or by UK-61,260, at doses inducing PDE III inhibition, is consistent with the view that they have a similar pathogenesis. In our view it is probable that these pharmacologically and chemically distinct drugs trigger an increase in intracellular levels of cAMP which in turn triggers vascular damage. The arterial changes observed in the current study after acute administration may explain the increased incidence of polyarteritis nodosa occurring in long term toxicity studies with FM or PDE III inhibitors.

Key words  Phosphodiesterase inhibitor · Dopaminergic agonist · Arteritis · Medial necrosis · Vascular haemorrhage · Fenoldopam · UK-61,260

Introduction

A number of vasodilatory drugs are known to induce arteritis in muscular arteries of rats (Kerns et al. 1989a). Fenoldopam mesylate (FM), a dopaminergic (DA1) agonist, produced an increased incidence of polyarteritis nodosa after chronic oral administration or an acute arteritis after intravenous infusion for 24 h (Yuhas et al. 1985). Characterization of this latter lesion has helped in understanding the pathogenesis of polyarteritis nodosa (Kerns et al. 1989a, b).

Polyarteritis nodosa has also been described in rats, after repeated administration of cyclic AMP phosphodiesterase (PDE III) inhibitors, for example theophylline (Collins et al. 1988), caffeine (Johansson 1981), LY-195,115 and isomazol (Sandusky and Means 1987; Means et al. 1988; Sandusky et al. 1989, 1991), ICI-153,110 (Westwood et al. 1990), SKF-95,654 and isobutyl methylxantine (Kerns et al. 1991). To the best of our knowledge, acute arteritis has not been described after administration of PDE III inhibitors and the pathogenesis of polyarteritis nodosa produced by these drugs is unclear.
An increased incidence of polyarteritis nodosa occurred in a 2-year toxicity study in rats with UK-61,260 an investigational cardiac stimulant (Pfizer Central Research, unpublished results). The compound has inotropic properties with force-rate selectivity which were demonstrated in dogs treated over an oral dose range of 0.06–0.25 mg/kg (Alabaster and Rance 1987; Alabaster et al. 1987; Ellis et al. 1987; Collier et al. 1988; Bell et al. 1989).

In addition, UK-61,260 displays a vasodilator action on resistance and capacitance vessels (Ellis et al. 1988; Alabaster et al. 1989).

This compound is an inhibitor of canine myocardial PDE III (IC50 1.5 μM). However, at pharmacological doses, the PDE inhibitory activity of the compound appears too low to explain the inotropic effects. PDE inhibition only becomes significant at doses used for toxicological investigation (Pfizer Central Research, unpublished results).

In the current paper, we report the conditions of appearance, the histological aspect and the evolution of the arteritis induced by acute administration of UK-61,260 in rats. The lesions were compared to those induced by FM, under the same experimental conditions. We speculate that both drugs induced arterial damage by a similar mechanism which may explain the increased incidence of polyarteritis nodosa in chronic studies with these compounds.

### Materials and methods

#### Compounds

UK-61,260 [6-(2,4-dimethyl-1H-imidazol-1-yl)-8 methyl-2-(1H)-quinolinone methanesulphonate] and fenoldopam mesylate [6-chloro-7,8 dihydroxy-1-(4’-hydroxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine] were supplied by Pfizer Central Research, Sandwich, UK. For intravenous and subcutaneous administration UK-61,260 and FM were dissolved in saline [0.9% (w/v) NaCl]. For oral administration, UK-61,260 was dissolved in demineralized water.

#### Animals and housing conditions

We used male Sprague-Dawley rats [Crl: COBS-VAF-CD(1/1)] (France) obtained from Charles River, France. They were housed under standard conditions for toxicology studies at Pfizer, Amboise (temperature 20 ± 3 °C, relative humidity 60 ± 20%, 12 h lighting/day) and had free access to a standard laboratory diet (UAR A04C pellets, Usine d’Alimentation Rationelle, France) and tap water. The rats were acclimatised for a period of about 1 week prior to use. The animals were about 46 days old and had a mean body weight in the range 190–240 g.

#### Oral and subcutaneous administration of UK-61,260

Three groups of four rats received a single oral dose, by oesophageal intubation, of 30, 100 or 300 mg/kg UK-61,260, while two groups of six rats received two oral administrations of 200 or 300 mg/kg of the drug at 6-h intervals. Simultaneously, five rats were treated with demineralized water.

Three groups of five rats were injected subcutaneously with 30, 100 or 200 mg/kg per day UK-61,260 for 7 days, while five rats were injected with the vehicle. All animals which survived the treatment were killed on the day following the last administration.

#### Continuous intravenous infusion

UK-61,260, FM or vehicle were administered at a rate of 5.4 μl/min into a tail vein through a polyethylene catheter which was connected to a plastic syringe mounted in a Razel model A infusion pump (Razel Scientific Instruments, Stamford, Conn., USA). The total volume infused was approximately 7.8 ml/animal.

#### Characterization of the lesion immediately following the end of infusion

Rats were infused for 24 h with a dose of 300 mg/kg UK-61,260 (13 animals) or 72 mg/kg FM (3 animals). A control group of eight animals was infused with the vehicle. Immediately at the end of treatment the rats were killed.

#### Characterization of the lesion 24 h after infusion and dose-effect relationship

UK-61,260 was infused for 24 h at doses of 30, 100, 200, 300 or 400 mg/kg to groups of 5, 5, 10, 14 and 5 animals, respectively. FM was administered at doses of 72 or 144 mg/kg to groups of 16 and 12 animals, respectively. A total of 23 rats were infused with the vehicle. The rats were killed 24 h after completion of the infusion.

#### Study of the evolution of the lesion

The rats were infused for 24 h with a dose of either 300 mg/kg UK-61,260 or 72 mg/kg FM. For each compound, a total of 20 treated animals were allocated to one of five groups of four animals. Rats which survived the infusion were killed 24 h, 3, 7, 14 or 28 days post-infusion. A group of eight control rats were infused with saline and were killed 24 h post-infusion.

#### Necropsy and histopathological examination

After completion of each study, the rats were killed by exposure to carbon dioxide and necropsied. Sections were taken from the following tissues from all animals: lung, heart, liver, kidneys, thyroid, stomach, pancreas, cranial mesenteric arteries, testes, epididymides, ductus deferens. All tissues were fixed for a minimum of 48 h in 10% formalin and embedded in paraffin wax. Sections 4–5 microns thick were cut and stained with haematoxylin (Mayer) and eosin (HE). Martius Scarlet Biebrich (MSB) staining for fibrin was performed on selected tissues.

### Results

#### Induction, dose-response and distribution of the arteritis

No arteritis occurred after oral or subcutaneous treatment with UK-61,260. Infusion of rats with either UK-61,260 or FM for 24 h did induce arteritis and was thus used in all subsequent experiments. There were no vascular changes in rats infused with vehicle.

In animals killed 24 h post-infusion, arteritis produced by UK-61,260 was visible macroscopically as haemorrhagic spots on blood vessels (Fig. 1) and microscopically as medial necrosis and haemorrhage. The incidence of arteritis was dose-related in the range 100–300 mg/kg but did not increase between 300 and 400 mg/kg (Table 1). There were no vascular lesions after 30 mg/kg. A number of animals