[\textsuperscript{14}C]cadralazine: absorption, distribution and excretion in rat and dog

G. BONARDI, E. ROSSI, and M. PELLEGATTI
ISF Laboratories for Biomedical Research, Milan, Italy

Received for publication: August 5, 1981

Key words: Absorption, distribution, excretion \([\textsuperscript{14}C]\)cadralazine, rat, dog

SUMMARY

Absorption, distribution and excretion of \([\textsuperscript{14}C]\)cadralazine in rat after oral and i.v. administration of 3 mg/kg were studied. Plasma levels after oral administration of 10 and 45 mg/kg were also evaluated. A direct relation between dose and plasma levels was demonstrated. The drug was well absorbed, disappeared very rapidly from plasma, and was distributed in all the organs examined, with the highest concentration in liver, kidney, and gastrointestinal tract. For more than 4 days excretion was essentially through the urine (75.6\% after i.v. and 80\% after oral administration), whereas faecal and biliary excretions were quite low. The total recovery was respectively 77.6\% and 83.2\% after i.v. and oral administration of 3 mg/kg, with the greatest amount (65-70\% of the administered dose) appearing in the first 4 to 7 hours. Placental transfer and excretion of radioactivity with milk were demonstrated. Drug-protein binding was 25.9\%. Elimination of \(\text{\textsuperscript{14}C}O_2\) was not observed. Plasma levels in dog, after oral and i.v. administration of 1 mg/kg of labelled compound, showed similar behaviour to that observed in the rat. Binding of the radioactivity to erythrocytes was found; the radioactivity values observed up to 24 hours were constant with time and not dependent on the decreasing plasma levels. The total recovery (urine and faeces) in the dog over 4 days was 71.1\% and 82.1\% after oral and i.v. dose, respectively. Preliminary metabolic approaches in rats showed that cadralazine was essentially excreted as unchanged drug in the presence of minor metabolites.

INTRODUCTION

Cadralazine, ISF 2469, ethyl-2-(6-{(2-hydroxypropyl)methylamino}-3-pyridazinyl)hydrazinecarboxylate, is a new vasodilator compound endowed with a slow-in-onset and long-lasting antihypertensive activity in the experimental animal and man. It was selected from a class of hydrazinopyridazine compounds synthesized in our laboratories (1, 2), and previous papers have reported its pharmacological properties (3-6) and clinical effectiveness (7, 8).

The present work is devoted to the study of the pharmacokinetic profile of cadralazine in rat and dog by using the labelled compound with \(\text{\textsuperscript{14}C}\).

MATERIALS AND METHODS

Labelled cadralazine (3,6-\textsuperscript{14}C in the pyridazine ring - Fig. 1) was synthesized at the Radiochemical Centre (Amersham, U.K.) according to the method of Pifferi et al. (1). [3,6\textsuperscript{14}C]-Dihydroxypyrirdazine, prepared from [1,4\textsuperscript{14}C] maleic anhydride and hydrazine, was treated with POCl\textsubscript{3} to give [3,6-\textsuperscript{14}C]

\[
\text{H}_3\text{C}-\text{CH}-\text{CH}_2-N-\text{N}^\bullet \text{N}^\bullet \text{N} \\
\text{O} \quad \text{C}_2\text{H}_5
\]

Fig. 1: Chemical structure of cadralazine; \(\bullet\), labelled carbon.
dichloropyridazine. Reaction of the latter with \(N-2\)-hydroxypropyl ethylamine and \(N\)-ethylpiperidine gave \([3,6-^{14}C]\)-3-chloro-6-\([N-(2-hydroxypropyl)ethylamino]\) pyridazine, which was refluxed with carbethoxyhydrazine to obtain the final product. The specific activity of the product was 5.0 mCi/mmol, with a radiochemical purity higher than 98% as evaluated by thin-layer chromatography (TLC) on silica gel impregnated with octadecylsilane. The following solvent systems were used: (a) chloroform:methanol (9:1); (b) ethyl acetate:methanol (85:15); (c) methanol:water (80:20).

**Animal experiments**

Male and female Charles River rats of the Sprague Dawley strain, fasted for 18 h and with an average body weight of 150 to 180 g (except where otherwise specified), were used. Male beagle dogs, weighing 9 to 11 kg and fasted for 16 h, were also used.

Labelled cadralazine, solubilized as hydrochloride in distilled water, was used as such or appropriately diluted with the cold compound for administration to the animals.

Rats received \([^{14}C]\)cadralazine as a single intravenous dose (3 mg/kg) or as increasing oral doses (3, 10, 45 mg/kg), and blood samples were collected to determine plasma levels of radioactivity at different times after administration. Urinary and faecal excretion and organ distribution were studied in rats treated i.v. and orally with 3 mg/kg. The organs and tissues considered were brain, heart, aorta, lungs, kidneys, liver, adrenals, spleen, stomach, small and large intestine. Biliary excretion was studied in rats under urethane anaesthesia that weighed about 300 g, had a cannulated bile duct, and had been treated with 3 mg/kg i.v. and orally of labelled compound. Bile samples were collected at different hours after administration. Placental transfer was studied at the 17th day of pregnancy in two female rats weighing 320 and 330 g, fasted for 16 h, and treated i.v. with 3 mg/kg of \([^{14}C]\)cadralazine. Maternal and foetal blood were withdrawn 1 h after administration. Milk excretion was studied at the 10th day after parturition in one rat weighing 255 g, fasted for 16 h, and treated orally with 3 mg/kg of labelled compound. Evaluation of \([^{14}C]\)CO\(_2\) was performed on rats orally treated with 3 mg/kg by collecting expired CO\(_2\) for 24 h.

Dogs received i.v. and orally 1 mg/kg of \([^{14}C]\) cadralazine; samples of blood, urine and faeces were collected at various times after administration. Protein binding was studied in vitro by the equilibrium dialysis technique.

**Biological sampling methods**

At prefixed times after dosing, blood of rats was withdrawn from the abdominal aorta, mixed with heparin, and the plasma separated; organs were removed and weighed. For elimination studies, rats were individually housed in glass metabolic cages. Urine, faeces and expired CO\(_2\) (absorbed on Carbosorb) were collected. For the study on excretion with milk, aliquots of milk obtained by suction at definite times were absorbed on filter paper (1.5 X 1.5 cm), which was then weighed and treated with Lipoluma (Packard).

Dogs were housed in metabolic cages and urine and faeces were collected at fixed times after administration. Plasma was separated from heparinized blood by centrifugation, and erythrocytes were washed with saline until there was no detectable level of radioactivity in the supernatant. All the samples were frozen for storage until the analysis.

**Radioactivity measurements**

Radioactivity measurements were performed after chemical dissolution or after direct oxidation. In the first case, aliquots of biological samples (plasma, urine, tissues etc.) were treated with Soluene 350 (Packard), isopropyl alcohol and hydrogen peroxide, then Dimilume (Packard) was added, and counting was performed. In the second case, aliquots of biological samples were burned with a Packard Model B 30b oxidizer, and the combustion products were collected in Carbosorb:Permafluor (6:12). Radioactivity measurements were performed by using a Packard model 3255 scintillation counter, and quenching corrections were obtained by using the external standard method.

**TLC studies**

Chromatograms were run on Silica gel 60F\(_{254}\) (Merck), 0.25 mm, 5 X 20 cm) using chloroform: methanol:water (70:25:5) or chloroform:methanol (9:1) as eluting solvent systems. Radioactive compounds were then visualized with a Packard model 7201 radiochromatogram scanner.