The disposition of 3-O-methyl-(+)
-catechin in the rat and the marmoset following oral administration

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SUMMARY

The tissue distribution of an alkyl substituted flavanol, 3-O-methyl-(+)
-catechin, has been investigated. Following oral administration, 3-O-methyl[14C](+)
catechin was well absorbed, peak levels of serum radioactivity for three dose levels being
recorded within one hour of administration. Despite extensive absorption, it has been demonstrated that over the period 0-24 h
after administration much of the radioactivity in the carcasses of rats was associated with the contents of the alimentary canal.
This appears to be largely due to the enterohepatic circulation of the major metabolite 3,3'-O-dimethylcatechin glucuronide,
since the present study indicates that some 60% of biliary excreted metabolites are reabsorbed in the first enterohepatic
circulation. Metabolism of 3-O-methyl-(+)
-catechin was rapid since the unchanged compound was detected in serum only at
high dose levels and trace amounts only of 3-O-methyl-(+)
catechin were detected in intestinal contents 3 h after dosing. Radiometric examination of tissue samples following 3-O-[14C]methyl-(+)
catechin administration indicated that maximal tissue levels were observed at 30 min. After 6 h only trace amounts were detectable.

INTRODUCTION

The major pathway of metabolism of 3-O-methyl- (+)-catechin (3-methoxy-5,7,3',4',-tetrahydroxyflavan) in the mouse, rat and marmoset is methylation followed by conjugation with glucuronic acid, although the extent of glucuronyla
tion varied between the three tested species (1). 3-O-Methyl-(+)
catechin was shown to be resistant to ring fission by the intestinal microflora of each species investigated. This is in contrast to the metabolism of the closely related flavanol (+)-catechin, which although in part
methylated (2), undergoes catabolism to CO2, phenylvalerolactones and phenolic acids in the rat, guinea pig, monkeys and man (3-7, 13).

No information has previously been available on the tissue distribution of 3-O-methyl-(+)
catechin. In the present study the factors determining the levels of this compound and its metabolites in the gut contents and tissues of the rat and the serum of the marmoset are explored.

MATERIALS AND METHODS

Materials

Two batches of 3-O-[14C]methyl-(+)
catechin had specific activities of 0.154 µCi/mg (46.8 µCi/mmol) and 2.000 µCi/mg (607.8 µCi/mmol) respectively. Each preparation was shown by radioscanning, liquid scintillation counting and autoradiography of paper and thin layer chromatograms to be radiochemically pure.
Labelled and non-labelled 3-0-methyl-(+)-catechins were donated by Zyma S.A., Nyon, Switzerland.

Animals and animal experiments

Rats (Birmingham Wistar Strain, specific pathogen-free; c300 g body weight) and marmosets were maintained under previously described conditions (1,8). Doses of 3-0-methyl-(+)-catechin were dissolved in water and administered intragastrically to rats by stomach intubation. Oral doses to marmosets were administered dissolved in Cytacon Syrup (Glaxo Laboratories, Greenford, Middlesex). Male animals of each species and a dose level of 25-30 mg/kg were used throughout.

Blood samples from rats were obtained by atrial puncture post mortem. Blood samples were obtained from marmosets by withdrawal from a femoral vein.

The ligation of rat bile ducts and the investigations of enterohepatic circulation were carried out by previously described methods (9,10).

Preparation and autoradiography of rat whole body sections

Rats were sacrificed at various times after dosing by CO₂ anoxia and the carcasses rapidly frozen by immersion in a mixture of acetone and dry ice. Frozen carcasses were mounted on the table of a microtome-cryostat and sections 15 μ thick were cut and dried at -20°C (11). Dried sections were mounted in apposition to X-ray film for a suitable time and developed.

Measurement of radioactivity

Radioactivity in urine, bile and serum was measured by liquid scintillation counting and that in tissue samples and alimentary canal contents by combustion techniques (1,12).

Measurement of 3-0-methyl-(+)-catechin in serum and gut contents

Aliquots of serum (1 or 2 ml) were diluted to 20 ml with distilled water and continuously extracted with diethyl ether for 2 hr. Extracts were evaporated to dryness and redissolved in 100 μl of methanol and filtered through a 1.0 μm pore PTFE filter in a BAS Microfilter System (Anachem Ltd., Luton, Beds.). High performance liquid chromatography of the filtrate was performed on a Waters HPLC system incorporating a μ-Bondapak C₁₈ column eluted with 30% (v/v) methanol in 2% (v/v) acetic acid (flow rate 1 ml/min) (2). Detection was at 280 nm in a CE 212 variable wavelength UV monitor (Cecil Instruments Ltd., Cambridge).

Under these conditions the retention volume of 3-0-methyl-(+)-catechin was 12.2 ml recovery from serum was 92.2 ± 3.2 (S.D.)% and the limit of accurate measurement was 50 ng of 3-0-methyl-(+)-catechin/ml serum.

The contents of the stomach and small intestine of sacrificed rats were separately freeze dried and ground to a fine powder. This was extracted with methanol in a Soxhlet system for 2 h. The extract was evaporated to dryness and redissolved in an appropriate volume of methanol for filtration and HPLC. Recovery of 3-0-methyl-(+)-catechin and its metabolites (measured as recovery of ¹⁴C from gut contents) was 89.0 ± 7.5% and the limit of detection was 0.5% of the dose in each individual stomach or intestine sample assayed.

Paper chromatography

Separation of metabolites of 3-0-methyl-(+)-catechin in bile, urine, methanolic extracts of alimentary canal contents and acetone-deproteinised serum samples was carried out on Whatman 3MM paper developed in butan-2-ol: acetic acid:water 5:1:2 (v/v/v). Metabolites were located by autoradiography, radioscaning (Packard 7200 radiochromatogram scanner) or by liquid scintillation counting of chromatogram segments.

RESULTS

Distribution of radioactivity in tissues

Autoradiography of tissue sections: Autoradiography of tissue slices from rats which had received intragastric doses (25-30 mg/kg body weight) of 3-0-[¹⁴C]methyl-(+)-catechin clearly showed the radioactivity to be concentrated in the contents of the alimentary canal over the period 30 min-24 h after administration. Sections from the animal sacrificed 30 min after dosing showed the contents of the stomach and intestines to be heavily labelled (Fig. 1). Radioactivity was however also detectable in the blood in the heart chambers, liver, kidney and skin.