Distribution of [*nitrile*-^{14}C]rorifone in mice by whole body autoradiography

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ABSTRACT 10-methyl-sulphonyl-decyl[^{14}C]-nitrile([*nitrile*-^{14}C]rorifone) was given to ♂ mice by intragastric gavage about 152 kBq and the mice were frozen by immersion in dry ice/hexane at 3, 9, 24 h and 3 d. At 3 h, high concentrations of radioactivity were observed in salivary gland, bile, kidney, urine, and gastrointestinal contents. A moderate level of radioactivity was seen in the blood, lung, spleen, esophagus, liver, oral cavity and aortic wall. In 9-24 h, remarkable accumulations of radioactivity were found in salivary glands and gastric mucosa and contents. The intense localization of ^{14}C in salivary gland and gastric mucosa and less retention in gastric contents persisted throughout the entire period of 3 d.

KEY WORDS 10-methyl-sulphonyl-decyl[^{14}C] nitrile ([*nitrile*-^{14}C]rorifone); whole body autoradiography; tissue distribution, salivary glands

Roripa montana (Wall) Small is distributed widely in China. It has been used as a folk medicine to relieve cough and asthma, and as a mucolytic agent. Clinical trials showed some efficacy in chronic bronchitis and as an expectorant[1].

Rorifone (10-methyl-sulphonyl-decylnitrile) is a white crystalline substance which was isolated from the whole herb. The pharmacological studies revealed that it was one of the active principles[1,2]. Clinical trials with rorifone revealed a marked improvement of chronic bronchitis.

This report is a study of the localization of [*nitrile*-^{14}C] rorifone by whole body autoradiography; the work was done to learn the pharmacodynamics and possible mechanism of the compound.

MATERIALS AND METHODS

Adult, ♂ Swiss-Webster mice weighing 30-35 g were fasted about 19 h prior to treatment. [*nitrile*-^{14}C] rorifone was synthesized by Shanghai Institute of Materia Medica; the label was in the CN group. This compound had a specific activity of 133.2 kBq/mg. Mice were given by gavage 0.5 ml of a suspension containing [*nitrile*-^{14}C] rorifone in 1% carboxylmethylcellulose (CMC). Each mouse received 107.3 to 166.5 kBq of [*nitrile*-^{14}C] rorifone; this corresponded to a dose of rorifone 27-36 mg/kg. After treatment, the mice were placed in a cage with a raised wire-mesh bottom fed with standard chow and water *ad libitum*.

$CH_3SO_2CH_2(CH_2)_7CH_2{}^{14}CN$

[*nitrile*-^{14}C] rorifone

10-methyl-sulphonyl-decyl [^{14}C]nitrile

At 3, 9 and 24 h and 3 d following the ig [*nitrile*-^{14}C]rorifone, each mouse was anesthetized with ether and immersed in a dry ice/hexane bath. The frozen mice was attached to a microtome stage by embedding in 1.5% CMC and quickly frozen in the dry ice/hexane bath.

Sagittal sections, 20 and 40 μm thick,

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of the frozen mice were taken onto #800 Scotch tape using a Slee cryostat at -20℃. The freeze-dried sections were mounted to Kodak AA X-ray film at -14℃: exposure times were 16 wk (3 h mouse): 36 wk (9 h mouse) and 55 wk (24 h and 3 d mice). After the appropriate exposures, the Scotch tape with the section attached was removed from the film: the film was developed and fixed. The technique of whole body autoradiography was described previously[3].

RESULTS

In mice given a single ig [*nitrile*-^{14}C] rorifone, radioactivity was present in highest concentrations in salivary gland, bile, kidney, urine and gastrointestinal contents at 3 h. Moderate concentrations were seen in blood, lung, spleen, esophagus, liver, oral cavity and aorta. There were minimal radioactivities in bone, testis, muscle and myocardium (Fig 1. See Plate 2).

By 9 h, remarkable accumulations of ^{14}C were found in salivary glands, gastric mucosa and contents and urine (Fig 1). Blood, lung, oral cavity, esophagus, gall bladder and aorta still retained low levels of radioactivity.

Pronounced accumulations of ^{14}C were seen in salivary glands and gastric mucosa and lower levels in the stomach contents at 24 h and 3 d: intense localization occurred in urine at 24 h (Fig 1). Moderate amounts of radioactivity were noted on the surfaces of oral cavity and esophagus. Minimal uptakes were seen in aortic wall, buccal cavity and lungs.

In general, Figures A to D relate the change of concentration in various tissues over the 3 d study period. Little radioactivities were found in spleen or skeletal muscles and no uptake in brain or spinal cord. The increasing levels in the salivary glands with persistence in gastric mucosa and contents, and the decreasing levels in the circulating blood, lung and kidney were noted. The radioactivity in the salivary gland was not homogeneous: it was higher in the parenchyma in discrete areas.

DISCUSSION

The fate of rorifone was previously studied in mice and rabbits, and showed that thiocyanate is one of the metabolites excreted in urine. The report revealed that rorifone is cleaved *in vivo* and the cyanate oxidized to the less toxic thiocyanate which is excreted in urine.

Radioactivity seen in the autoradiographs probably represents both the metabolites and unchanged rorifone. The intense localization of radioactivity in salivary glands, urinary bladder, gall bladder and stomach seems to be related to the routes of elimination of the rorifone or its metabolites.

This study reveals that most of [*nitrile* - ^{14}C]rorifone is rather rapidly eliminated by renal excretion. However, there is a remarkable retention in the salivary gland and mucosa of stomach. This strong accumulation of radioactivity in salivary glands and stomach may be due to an enterosalivary circulation. The possibility exists that there is an action of the drug in stimulating other secretory processes in addition to those in the salivary gland that, in part at least, may be responsible for its efficacy on the respiratory tract.

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[腈-^{14}C]蕺菜素在小鼠整体自显影分布

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提要　用整体放射自显影法研究[腈-^{14}C]蕺菜素（10-甲磺酰基癸[^{14}C]腈）在小鼠分布。每鼠约ig 152 kBq（^{14}C）蕺菜素后，在3，9，24 h和3 d冰冻杀死，切片。服药后3 h，^{14}C高度浓集在唾液腺、胆汁、肾、尿和胃肠内含物。血、肺、脾、食管、肝、口腔和主动脉壁含有中等量^{14}C。9-24 h，唾液腺、胃粘膜和胃肠内含物仍有明显^{14}C潴留，3 d内仍见到少量^{14}C。

关键词　[腈-^{14}C]蕺菜素（10-甲磺酰基癸[^{14}C]腈）；体放射自显影术；　组织分布；　唾液腺

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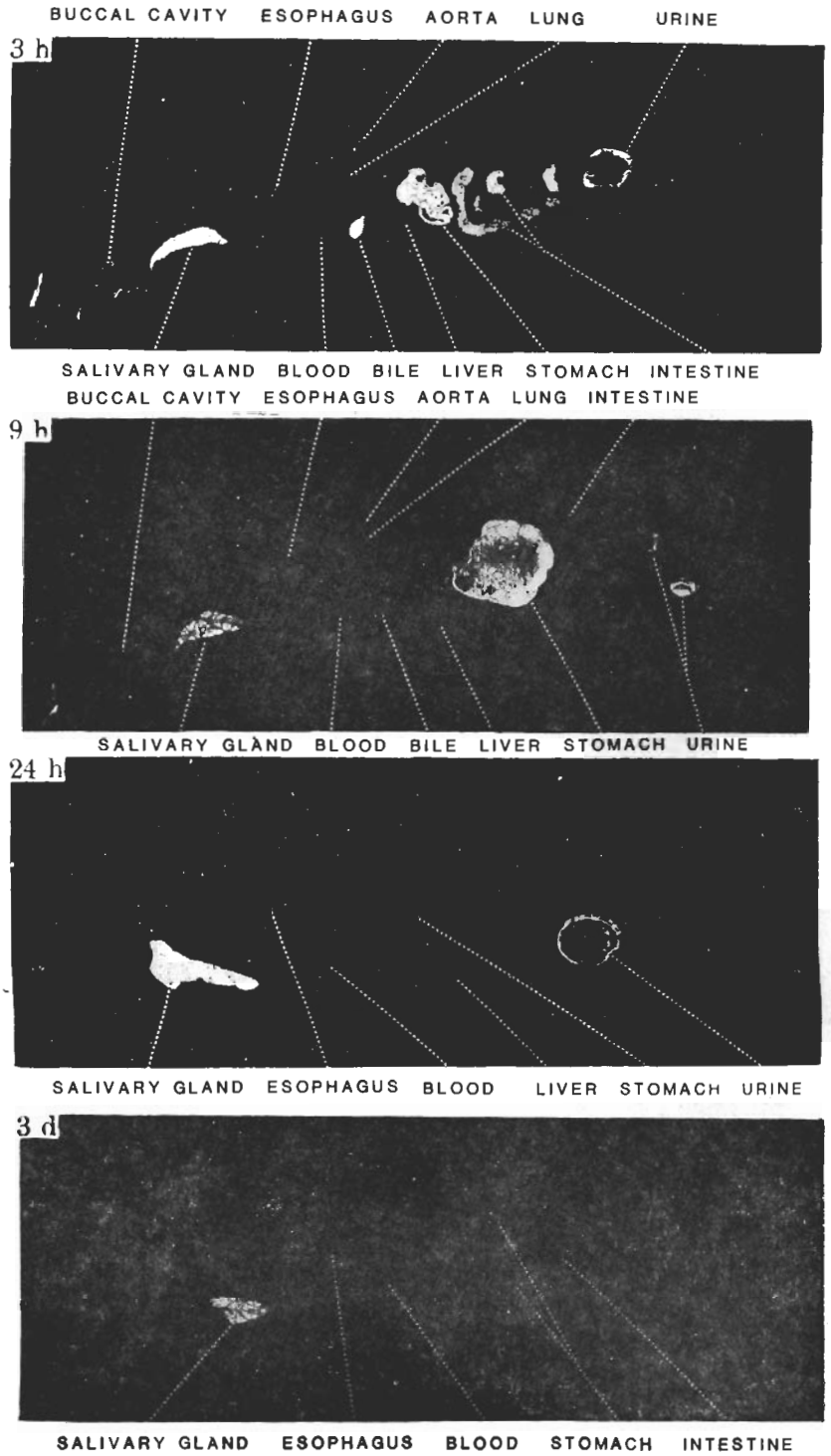

Fig 1. Autoradiographs of 20-μm-thick sections from ♂ mice given ig [*nitrile*-^{14}C]rorifone 107.3-166.5 kBq (27 - 36 mg/kg).

(See p 148)