THE DISTRIBUTION OF RADIOACTIVE CARBON FROM [1-¹⁴C]-GALACTOSAMINE IN MICE

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Tissue and organ distribution of radioactive carbon from ¹⁴C-labeled galactosamine in the mouse was investigated by whole-body autoradiography and biochemical analysis. At 5 min after intraperitoneal injection of ¹⁴C-galactosamine, high radioactivity was found in the abdominal cavity, liver and urine. The radioactivities of these organs were reduced by perchloric acid treatment of sections with the exception of urine. At 30 min after injection, the radioactivity of abdominal cavity became insubstantial, but the activity in the liver, small intestine and renal cortex was maintained at a high level which still remained 1 hr after injection, and was retained considerably after the acid treatment of sections. At 6 hr, the level of radioactivity became lower in many organs except for the liver, blood and renal cortex, at which time the activity of these organs was almost completely retained even after acid treatment of the sections.

The comparative radioactive values in organs as estimated by a liquid scintillation counter were consistent with the values estimated by densitometry of autoradiographs. In animals at 5 and 30 min after injection, paper chromatography of the acid-soluble fractions of liver and kidney had a radioactive spot for galactosamine-1-phosphate but no spot for galactosamine could be detected.

Amino sugars are important components of glycoconjugates, which are widely distributed throughout the body (4, 8). The carbohydrate moiety of glycoconjugates play an important role in cell recognition. In order to investigate the metabolic fate of amino sugars, many investigators have utilized ¹⁴C-glucosamine (1, 5, 9). On the other hand, White *et al.* reported the incorporation of D-[1-¹⁴C]galactosamine into serum proteins and tissues of the rat (15). Maley *et al.* have reported the metabolism of ¹⁴C-galactosamine in the rat liver after perfusion with ¹⁴C-galactosamine (7). MacNicoll *et al.* reported the metabolic fate of N-acetyl-galactosamine, a metabolite of galactosamine, in the perfused rat liver (6). Recently, the pathway of galactosamine metabolism in the liver has been established (14).

Little, however, is known about the distribution of ¹⁴C-galactosamine in the whole-body, although whole-body distribution of neutral sugars (10, 12) and glucosamine (2) have been examined. The present paper reports the distribution and metabolic fate of injected ¹⁴C-galactosamine into mice examined by whole-body autoradiography and biochemical analysis.

MATERIALS AND METHODS

Animals. Adult male albino mice of ICR strain, weighing approximately 22 g, were used. The animals were fed *ad libitum*.

Materials. D-[1-¹⁴C]galactosamine hydrochloride (specific activity: 45 mCi/mmol) was purchased from Amersham International Limited, England. The radiochemical purity was 99% as determined by paper chromatography. The substance was dissolved in physiological saline (50 μ Ci/ml).

Whole-body autoradiography. Animals were injected intraperitoneally with 7.5 μ Ci of radioactive galactosamine and sacrificed at 5 and 30 min, 1, 3, 6 and 24 hr after injection. The animals were anaesthetized with ether and frozen in a mixture of dry-ice and acetone. Whole-body sagittal sections (20 μ m) of the frozen animals were cut with a cryomicrotome (LKB-2250, Sweden).

Autoradiography of sections was performed before and after ice-cold 6% perchloric acid treatment in the manner previously reported (13). The autoradiographs of sections before acid treatment were designated "untreated autoradiographs" and those after acid treatment "treated autoradiographs". Some H-E stained whole-body sections were also prepared for tissue identification of autoradiographs (11).

Biochemical analysis. The animals injected intraperitoneally with 5 μ Ci of radioactive galactosamine were sacrificed by cervical dislocation at 5 and 30 min after injection and the blood, liver, kidney, small intestine, large intestine, Harderian gland, salivary gland, lung, brain, heart and testis were immediately removed. The acid-soluble, acid-insoluble and lipid fractions of each organ were obtained by the methods described in a previous report (2). The radioactivity of each fraction was determined by using a Beckman scintillation counter and paper chromatography was carried out for each fraction (2).

RESULTS

Whole-body autoradiography. At 5 min after intraperitoneal injection of radioactive galactosamine, high radioactivity was observed in the abdominal cavity, liver, kidney and content of urinary bladder (Fig. 1a). The treated whole-body autoradiograph, however, did not show substantial radioactivity in any of organs except for the kidney and urinary bladder (Fig. 1b). This indicates that a large part of galactosamine metabolite in major organs is incorporated into acid-soluble substances. At 30 min after injection, high radioactivity was found in the liver, small intestine and renal cortex (Fig. 2). The radioactivity in the abdominal cavity was negligible at this time, indicating that the injected galactosamine was nearly completely absorbed from the cavity. At 1 hr after injection, high radioactivity was detected in the liver and small intestine, and relative high radioactivity was observed in the blood, lung, renal cortex and hair follicle glands (Fig. 3a). At this time the activity of these organs was considerably retained after acid-treatment of sections (Fig. 3b). This indicates that a large part of radioactive galactosamine metabolites in these organs is incorporated into their acid-insoluble fractions. At 3 hr after injection, high radioactivity was detected in the liver, kidney, small intestine, blood and urine (Fig. 4). The radioactivity in the region corresponding to the renal

FIGS. 1a, b. Whole-body autoradiographs at 5 min after intraperitoneal injection of D-[1- ^{14}C]-galactosamine. a) untreated. b) treated with 6% perchloric acid. a, b $\times 1.1$

FIG. 2. Whole-body autoradiographs (untreated) at 30 min after intraperitoneal injection of D-[1- ^{14}C]galactosamine. $\times 1.1$

medulla and urine became negligible after acid treatment of the sections. At 6 hr, the level of radioactivity became lowered in many organs, but a relatively high level was retained in the liver, blood, renal cortex and urine (Fig. 5a). At this time the activity of the organs was almost completely retained even after acid-treatment of sections except for the urine. This indicates that the radioactive galactosamine metabolites are almost completely incorporated into their acid-insoluble fractions. At 24 hr after injection, most organs showed a decrease in radioactivity (Fig. 6). As a whole, the distribution of ^{14}C -galactosamine appears as a different pattern than

Figs. 3a, b. Whole-body autoradiographs at 1 hr after intraperitoneal injection of D-[1- ^{14}C]-galactosamine. a) untreated. b) treated with 6% perchloric acid. a, b $\times 1.1$

Fig. 4. Whole-body autoradiograph (untreated) at 3 hr after intraperitoneal injection of D-[1- ^{14}C]galactosamine. $\times 1.1$

that of ^{14}C -glucosamine (2).

Biochemical analysis. Organ samples obtained from the mice injected with ^{14}C -galactosamine were separated into acid-soluble, acid-insoluble and lipid fractions. Table 1 shows the total radioactivity of the organs at 5 min after injection. High radioactivity was seen in the liver and kidney. Relatively high radioactivity could be observed in the lung, small intestine and Harderian gland. The activity in the

FIGS. 5a, b. Whole-body autoradiographs at 6 hr after intraperitoneal injection of D-[1- ^{14}C]-galactosamine. a) untreated. b) treated with 6% perchloric acid. a, b $\times 1.1$

FIG. 6. Whole-body autoradiograph (untreated) at 24 hr after intraperitoneal injection of D-[1- ^{14}C]galactosamine. $\times 1.1$

acid-insoluble fractions of organs were ranged from 4% to 18% of the total radioactivity. As shown in Table 2, at 30 min after injection the highest radioactivity of organs was in the liver and a relatively high level radioactivity was observed in the kidney and small intestine. At this time about 44% of blood radioactivity was in its acid-insoluble substances. The relative radioactivity of organs to blood showed an increase at 30 min in the liver, kidney and small intestine.

The radioactivity of lipid fractions of organs did not exceed 5% of the total radioactivity at 5 and 30 min after injection.

Figure 7 shows the paper chromatograms of acid-soluble components of organs (glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, galactosamine, UDP-N-acetylglucosamine, galactosamine-1-phosphate and glucosamine-6-phosphate were used as markers). As shown in Fig. 7a, at 5 min after injection the liver had two radioactive spots for galactosamine-1-phosphate and an unidentified substance. A spot for galactosamine-1-phosphate was also detected at 30 min. However, no spot for galactosamine could be detected at any of these times. Figure 7b shows the kidney 5 and 30 min after injection. Radioactive spots detected were for galactosamine-1-phosphate and glucose, while no spot for galactosamine was detected.

TABLE 1. *Radioactivity in various organs of the mouse at 5 min after injection of ^{14}C -galactosamine*

organ	total radioactivity per wet weight of organs [$\times 10$ cpm/g]	radioactivity ratio of organs to the blood
blood	148	1.0
liver	495	3.34
kidney	434	2.93
small intestine	217	1.47
large intestine	167	1.13
Harderian gland	317	2.14
salivary gland	80	0.54
lung	247	1.67
brain	10	0.07
heart	97	0.66
testis	160	1.08

TABLE 2. *Radioactivity in various organs of the mouse at 30 min after injection of ^{14}C -galactosamine*

organ	total radioactivity per wet weight of organs [$\times 10$ cpm/g]	radioactivity ratio of organs to the blood
blood	40	1.0
liver	1025	25.6
kidney	273	6.83
small intestine	339	8.48
large intestine	33	0.83
Harderian gland	82	2.05
salivary gland	23	0.58
lung	35	0.88
brain	14	0.35
heart	27	0.68
testis	20	0.50

FIG. 7. Paper chromatograms of the acid-soluble fractions of the liver (a) and kidney (b). The radioactivities of the fractions were 12000 cpm and 6000 cpm for the liver and kidney respectively. (—) at 5 min after injection, (---) at 30 min after injection. Arrows indicate the migration of standards: 1, glucosamine-6-phosphate; 2, UDP-N-acetylglucosamine; 3, galactosamine-1-phosphate; 4, galactosamine; 5, galactose; 6, glucose; 7, N-acetylglactosamine; 8, N-acetylglucosamine.

DISCUSSION

The distribution of radioactive carbon from ^{14}C -galactosamine was demonstrated by whole-body autoradiography and biochemical experiments. In the present report, labeled galactosamine, injected into the abdominal cavity of the mouse, was completely absorbed from the cavity within 30 min after injection of ^{14}C -galactosamine. High radioactivity was found in the urine at 5 min after injection. This indicates that a considerable part of the administered radioactive galactosamine is excreted through the urinary system within a short time after injection.

Whole-body sections were treated with 6% perchloric acid for the treated autoradiograph. This procedure enabled us to estimate the incorporation rate of radioactive galactosamine carbon into the acid-insoluble substances of the tissues. Results obtained from untreated and treated whole-body autoradiographs (Figs. 1, 3, 5) showed that free galactosamine appeared first as acid-soluble components in

various organs and then was gradually incorporated into acid-insoluble substances which are assumed to be glycoproteins and mucopolysaccharides. As shown in Fig. 1b, high radioactivity was retained in the kidney and urinary bladder even after acid treatment of the sections. It seems that the radioactivity is too high to be completely washed out. The liver had the highest radioactivity during the period examined, and radioactivity ratio of blood to liver increased with time. These findings agree with those obtained for ^{14}C -glucosamine (2) and support the report by Robinson *et al.* (9) that the liver is the major site of biosynthesis of plasma glycoprotein.

In order to compare the results of biochemical analysis with those obtained by densitometry of whole-body autoradiographs, the radioactivities of organs estimated by a liquid scintillation counter are represented in Tables 1 and 2 as the total counts/min per wet weight of organ. These tables also show the radioactivity ratios of organs to blood at 5 and 30 min after injection. The ratios were consistent with those obtained by whole-body autoradiography stated above.

Paper chromatographic examination of acid-soluble substances demonstrated that the distribution of galactosamine carbon in acid-soluble substances is different from organ to organ (Fig. 7). The galactosamine in the acid-soluble fraction was not radioactive even at 5 min after injection in any of the organs examined. This indicates that ^{14}C -galactosamine may immediately be metabolized after injection. Maley *et al.* (7) reported that after liver perfusion with $[1-^{14}\text{C}]$ galactosamine several labeled acid-soluble metabolites could be identified including galactosamine-1-phosphate, UDP-galactosamine, UDP-glucosamine, UDP-N-acetylgalactosamine and UDP-N-acetylglucosamine. Recently the pathway of galactosamine metabolism has been established in the liver (14). In the present study, labeled galactosamine-1-phosphate was detected in the liver and kidney of mouse at 5 min after injection of $[1-^{14}\text{C}]$ galactosamine, according to Keppler and Decker (3) who reported that galactosamine-1-phosphate is a predominant early metabolite of galactosamine in rat liver. At 30 min after injection, we also found ^{14}C -labeled galactosamine-1-phosphate in the liver, but the labeled UDP-N-acetylglucosamine could not be found. These findings indicate that in the liver the labeled galactosamine-1-phosphate is accumulated at least 30 min after injection, and this is supported by some other reports (3, 14). In the kidney ^{14}C -labeled acid-soluble compounds separated by paper chromatography were galactosamine-1-phosphate and glucose. Transfer of radioactive carbon from ^{14}C -labeled galactosamine into glucose has not been reported so far. It may be supposed that galactosamine might be metabolized through another pathway in the kidney. At the same time the present investigation also revealed some unidentified acid-soluble substances into which the radioactive carbon of $[1-^{14}\text{C}]$ galactosamine is incorporated. This requires further investigation to ascertain the metabolic pathways of galactosamine.

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