Autoradiographic Distribution of 14C-labeled 3H-Imidazo[4,5-f]quinoline-2-aminues in Mice

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ABSTRACT

The highly mutagenic heterocyclic amines, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MelQ), are formed during heating of protein-rich foods. In order to gain information about the distribution and fate of IQ and MelQ in vivo, a whole-body autoradiographic study of i.v.-injected 14C-labeled IQ and MelQ has been performed in male NMRI, pregnant NMRI, and female C3H mice.

IQ and MelQ showed similar distribution patterns. At short survival times, the autoradiograms were characterized by an accumulation of radioactivity in metabolic and excretory organs (liver, kidney, bile, urine, gastric and intestinal contents, salivary glands, nasal mucosa, and Harder’s gland), as well as in lymphomyeloid tissues (bone marrow, thymus, spleen and lymph nodes) and in endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla, and prostate). The liver and kidney cortex were identified as sites of retention of nonextractable radioactivity, and in endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla, and prostate). The liver and kidney cortex were identified as sites of retention of nonextractable radioactivity. IQ and MelQ showed a strong affinity for melanin. IQ and MelQ showed similar distribution patterns. At short survival times, the autoradiograms were characterized by an accumulation of radioactivity in metabolic and excretory organs (liver, kidney, bile, urine, gastric and intestinal contents, salivary glands, nasal mucosa, and Harder’s gland), as well as in lymphomyeloid tissues (bone marrow, thymus, spleen and lymph nodes) and in endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla, and prostate). The liver and kidney cortex were identified as sites of retention of nonextractable radioactivity. IQ and MelQ showed a strong affinity for melanin. IQ and MelQ showed similar distribution patterns. At short survival times, the autoradiograms were characterized by an accumulation of radioactivity in metabolic and excretory organs (liver, kidney, bile, urine, gastric and intestinal contents, salivary glands, nasal mucosa, and Harder’s gland), as well as in lymphomyeloid tissues (bone marrow, thymus, spleen and lymph nodes) and in endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla, and prostate).

INTRODUCTION

The formation of highly mutagenic compounds during frying and broiling of protein-rich foods has recently attracted much interest (21). The heterocyclic amines IQ\(^1\) and MelQ were first isolated from broiled sardines (11, 12) and later also from fried beef and commerical beef extract (10, 20, 22). IQ and MelQ were concomitantly identified as extremely potent bacterial mutagens after metabolic activation (11, 12). The heterocyclic amines IQ\(^1\) and MelQ showed a strong affinity for melanin. IQ and MelQ showed similar distribution patterns. At short survival times, the autoradiograms were characterized by an accumulation of radioactivity in metabolic and excretory organs (liver, kidney, bile, urine, gastric and intestinal contents, salivary glands, nasal mucosa, and Harder’s gland), as well as in lymphomyeloid tissues (bone marrow, thymus, spleen and lymph nodes) and in endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla, and prostate).

In order to assess the relevance of these findings to human health, more information is needed about the fate of IQ and MelQ in vivo. In the present study, the results of a whole-body autoradiographic distribution study of 14C-labeled IQ and MelQ are reported.

MATERIALS AND METHODS

Chemicals. [2-14C]IQ (specific activity, 18.8 Ci/mol) and [2-14C]MelQ (specific activity, 24.0 Ci/mol) were synthesized by Dr. K. Olsson, Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden. The radiochemical purity was at least 97% as determined by high-pressure liquid chromatography, modified after the method of Turesky et al. (22), on a Hypersil column eluted with 15% (v/v) acetonitrile in 0.1 M ammonium formate, pH 4.3. The nature of the impurities is unknown. The compounds were dissolved in methanol:chloroform (1:1, v/v). For autoradiography, they were evaporated to dryness in a stream of nitrogen and dissolved in analytical grade dimethyl sulfoxide to a concentration of 1.25 μCi/μl.

Animals. Male and pregnant female NMRI mice were obtained from ALAB, Stockholm, Sweden. Female mice of the pigmented strain C3H were obtained from an inbred stock at the Department of Medical Radiobiology, Karolinska Institute, Stockholm, Sweden. The pregnant mice were used on the 17th day of pregnancy (day of vaginal plug is Day 1). The animals were fed a standard pellet diet for rodents (Ewos AB, Södertälje, Sweden) and were given tap water ad libitum.

 Autoradiography. Male NMRI mice and female C3H mice (body weight, 30 g) were given injections i.v. in a tail vein with 25 μCi of [14C]-IQ or [14C]MelQ (8.8 or 6.6 mg/kg, respectively). Pregnant NMRI mice (body weight, 36 to 46 g) were also given injections of 25 μCi of [14C]IQ or [14C]MelQ. The animals were killed by CO\(_2\) anoxia at 5 min, 20 min, 1 hr, 4 hr, 24 hr, and 4 days (male NMRI mice); at 20 min, 4 hr, 4 days (female C3H mice); and at 20 min, 4 hr, 24 hr (pregnant NMRI mice) after the injection. The mice were embedded and frozen in n-hexane cooled by solid carbon dioxide for whole-body autoradiography according to the method of Ullberg (23). Ten 20-μm sections and six 60-μm sections were taken from each animal on tape No. 810 (3M Co., St. Paul, MN), freeze-dried, and pressed against X-ray film (X-OMat MA or XAR-5, Kodak). In addition, 4 pairs of adjacent 20-μm sections were taken from each animal on tape No. 688 (3M Co.) at different sagittal levels. The sections were freeze-dried, and one section from each pair was extracted stepwise with water, 10% trichloroacetic acid, 50% methanol, butanol, and heptane for the evaluation of covalently bound radioactivity as described earlier (3). The sections were pressed against X-Omat MA film and exposed and developed in pairs.

RESULTS

The tissue distribution patterns of IQ and MelQ were very similar. Unless otherwise stated, the distribution patterns will be described together. Both IQ and MelQ were rapidly absorbed from the blood. Already at 5 min after i.v. injection, the radioactivity in the blood was very low. IQ and MelQ were widely distributed throughout the body, with the exception of the central nervous system, which indicates poor penetration of the blood-brain barrier by IQ and MelQ. A number of tissue localizations reflecting the metastatic and excretory pathways of IQ and MelQ were observed in all mice used in the study. In addition, radioactivity was registered in tissues belonging to the lymphomyeloid and endocrine-reproductive systems, pigmented tissues, and fetal organs.

Metabolic and Excretory Pathways of IQ and MelQ. The concentration of radioactivity in the liver reached a very high level immediately after the injection of IQ or MelQ (Fig. 1A). The liver radioactivity remained high at all survival times and could still be

\(^1\) The abbreviations used are: IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; MelQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline.

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registered at 4 days after the injection (Fig. 1B). At 1 hr and at all subsequent survival times, the liver radioactivity appeared in a mottled pattern, probably reflecting a centrilobular distribution (Figs. 1B and 3A). This pattern was particularly obvious after the extraction of whole-body sections to determine the presence of covalently bound radioactivity (Fig. 3B). Already at very short postinjection times (5 and 20 min), radioactivity appeared in the bile ducts, intestinal contents, kidney medulla, and urinary tract, indicating that IQ and MelQ are excreted via both bile and urine. A strong concentration of radioactivity in the kidney medulla and urinary tract was registered up to 4 hr after the injection, whereas the radioactivity in the intestinal contents was still fairly high at 24 hr with traces of radioactivity remaining at 4 days. In the kidney, radioactivity remained in the cortex (Figs. 1B and 3A) also after extraction of the sections (Fig. 3B), at all survival times.

The stomach contents showed a very high level of radioactivity from 20 min up to 4 hr after i.v. injection of IQ and MelQ. Other tissue localizations, which probably represent the excretory and/or metabolic fate of IQ and MelQ in the mouse, were the nasal mucosa (Figs. 1A and 3A), salivary glands (Fig. 1A), and Harder’s gland.

**Lymphomyeloid System.** The bone marrow accumulated a relatively high amount of radioactivity at short survival times (5 min to 1 hr) (Fig. 1A). The spleen, thymus, and lymph nodes were also identified as organs with a fairly high uptake of radioactivity at short survival times (lymph nodes in pigmented animals, see below). Fig. 2, A and B, shows the preferential accumulation of radioactivity in the cortical area of the thymus and in the germinal centers of the lymph nodes.

**Endocrine-reproductive System.** The most accentuated uptake of radioactivity in endocrine tissues was observed in the adrenal medulla, pancreatic islets, and hypophysis (Fig. 1A). The radioactivity in the adrenal medulla was evenly distributed up to 1 hr following the injection, at which time a strong concentration of radioactivity to the outer layer of the medulla occurred. This radioactivity was still visible at 24 hr. Radioactivity could also be registered in the pancreatic islets for as long as 24 hr. The hypophysial radioactivity showed a pronounced accumulation in the adenohypophysis at 20 min (Fig. 2C), but had almost disappeared at 1 hr.

The thyroid showed a low but persistent level of radioactivity throughout the study. At long survival times, an uneven distribution was seen within the thyroid, probably representing the accumulation of radioactivity in parafollicular cells (Fig. 2D).

The uptake in the ovaries was even and relatively low in contrast to parts of the male reproductive tract, the testicular interstitium, caput and cauda epididymis (Fig. 2E), seminal vesicles (Fig. 1A), ampulla and prostate, which all showed a marked uptake of radioactivity at survival times from 5 min to 1 hr.

**Pigmented Animals.** The general distribution patterns of IQ and MelQ were the same for both pigmented and albino mice. However, the uptake in the retina and other pigmented tissues of C3H mice exceeded that of any other tissue at all survival times (Fig. 2F). No uptake was seen in the retina of albino mice. This finding demonstrates the melanin affinity of IQ and MelQ. The radioactivity in the pigmented tissues was not affected by the extraction procedure. In addition, a much more pronounced and persistent accumulation of radioactivity in lymph nodes and in Harder’s gland took place in pigmented than in albino mice (Fig. 2F).

**Pregnant Animals.** The general distribution patterns of IQ and MelQ were the same in pregnant albino mice as in male albino mice and nonpregnant pigmented mice. Both IQ and MelQ passed the placenta and reached the fetus. At 20 min after i.v. injection to the mother, the fetal distribution of radioactivity was even, but a distinct uptake of radioactivity in the fetal liver was observed. At 4 hr postinjection time, the fetal liver radioactivity had disappeared, but the amniotic fluid, the fetal urinary bladder, and the fetal intestinal contents were all heavily labeled. At 24 hr, some radioactivity still remained in the fetal intestine and in the amniotic fluid (Fig. 3A). The radioactivity in fetal tissues was completely extractable at all survival times. Fig. 3B shows the disappearance of all fetal radioactivity after extraction of a whole-body section taken 24 hr after i.v. administration of MelQ to the mother. However, the maternal liver and kidney cortex contain covalently bound radioactivity.

**Other Tissue Localizations of IQ and MelQ.** A weak but clearly discernible uptake of radioactivity in the bronchi of the lung was seen in all animals at all survival times after administration of IQ (Fig. 2A) but not after administration of MelQ. Radioactivity was also observed in the walls of the large arteries at short survival times (5 min to 1 hr) after i.v. injection of both IQ and MelQ (Fig. 1A).

**DISCUSSION**

The distribution patterns of IQ and MelQ were characterized by a very rapid uptake of radioactivity in metabolic (mainly the liver) and excretory organs. The autoradiographic evidence of a fast and efficient urinary and biliary excretion of labeled material is supported by similar findings in rats (17). However, it should be noted that radioactivity, which was nonextractable, remained in the liver for the full duration of the present study. In addition, the liver radioactivity appeared in a mottled pattern, suggesting a centrilobular distribution. IQ has been shown to be N-hydroxylated by a distinct form of cytochrome P-450 in liver microsomes (25), and the highest concentration of cytochrome P-450 has been demonstrated in centrilobular hepatocytes. The binding of radioactivity in the liver is interesting in view of the genotoxicity of IQ to hepatocytes (2) and the hepatocarcinogenicity of IQ in mice (15).

IQ and MelQ are basic compounds, which probably explains their excretion via the stomach. The gastric excretion of other i.v.-injected basic compounds has been described (1, 8). IQ and MelQ most likely pass the gastric mucosa by passive diffusion and become ionized in the acid gastric contents, i.e., they are ion-trapped in the stomach. This route of excretion would also exist after p.o. administration of IQ and MelQ. After absorption from the intestine, IQ and MelQ could be reexcreted into the gastric contents and thus made to “circulate” and come into repeated contact with the gastric mucosa. This finding may be related to the increased frequency of forestomach tumors observed after chronic feeding of mice with IQ (15). However, no covalent binding of radioactivity to the gastric mucosa was observed in the present study.

The autoradiographic distribution patterns of IQ and MelQ were further characterized by an accumulation of radioactivity in lymphomyeloid and endocrine tissues. At the present time, the toxicological significance of these tissue localizations is difficult to judge. It can be noted that the radioactivity was extractable.
and disappeared relatively rapidly. A similar distribution pattern has been reported for a number of other biologically active amines (7, 9, 13, 19). In these earlier studies, the possibilities of toxic effects, e.g., on the synthesis and release of polypeptide hormones, were discussed in relation to the specific uptake of amine compounds in endocrine and lymphomyeloid tissues. However, it may also be that these tissue localizations do not reflect a primary physiological action of IQ or MelQ (or other amines), but rather the fact that IQ and MelQ may benignly share the transport and/or storage mechanisms which exist for biogenic amines. It can be noted that a similar distribution pattern, especially as regards the endocrine and lymphomyeloid systems, was found in an autoradiographic study of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), another cyclic amine compound formed during heating of protein-rich foods (4).

Several (poly)cyclic amines have been shown to accumulate in melanin-containing tissues (7, 9, 13, 19). The accumulation of foreign compounds in melanin may give toxic effects (13). Both IQ and MelQ showed a very strong affinity for melanin, since the radioactivity in pigmented tissues was unaffected by the extraction procedure, which may be worthy of further investigation. In pigmented animals, a more accentuated uptake of IQ and MelQ was seen in the lymph nodes and in Harder's gland than in albino animals. This is probably explained by the presence of melanin in lymph nodes and Harder's gland of pigmented animals (13).

The metabolic capacity of the fetal mouse liver is very low, even in late gestation (14, 16). Thus, it seems probable that the uptake of IQ- and MelQ-derived radioactivity in the fetal liver represents the ability of the mouse fetus to excrete preformed metabolites of IQ and MelQ in the bile, rather than a metabolic capacity. The accumulation of foreign compounds in the fetal liver of experimental animals in late gestation is a relatively frequent observation, and it is often associated with fetal excretion into the intestine (24). The lack of formation of reactive IQ or MelQ metabolites in the fetus is demonstrated by the complete extractability of the fetal radioactivity. The heavy labeling of the amniotic fluid is probably due to the fetal renal excretion of water-soluble metabolites of IQ and MelQ.

In summary, a large proportion of a dose of IQ or MelQ given i.v. seems to be efficiently excreted by the mouse in 24 hr. The present autoradiographic findings do, however, pinpoint the liver as a site of IQ- and MelQ-mediated toxicity. The presence of covalently bound radioactivity in the kidney cortex may also indicate a harmful effect of IQ and MelQ and the kidney.

In all other organs, with the exception of melanin-containing tissues, IQ and MelQ were only transiently present, and no covalent binding was observed. Although many of these tissue localizations are difficult to associate with any specific toxic effects of IQ and MelQ, it is hoped that the results of the autoradiographic study may usefully incite, direct, and perhaps aid in the interpretation of future studies of the toxicity of IQ, MelQ, and similar substances.

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REFERENCES

Fig. 1. Whole-body autoradiograms of male NMRI mice 5 min after i.v. injection of 14C-labeled IQ (A) and 4 days after i.v. injection of 14C-labeled MeIQ (B). At the short survival time, an uptake of radioactivity is seen in metabolic and excretory organs (liver, kidney, intestinal contents, nasal mucosa, salivary gland), endocrine and reproductive tissues (hypophysis, thyroid, adrenal medulla, pancreatic islets, seminal vesicles), and lymphomyeloid tissues (bone marrow, spleen). Radioactivity can also be seen in the wall of the aorta. At the long survival time, radioactivity remains only in the liver (in a mottled pattern, probably reflecting a centrilobular distribution) and in the kidney cortex.
Fig. 2. Details of whole-body autoradiograms illustrating the accumulation of IQ- and MelQ-derived radioactivity in specific parts of lymphatic, endocrine, reproductive, and pigmented tissues. A, accumulation in the cortex of the thymus (male NMRI mouse, 20 min after i.v. injection of IQ). B, uptake in the germinal centers of 2 cervical lymph nodes (female C3H mouse, 20 min after i.v. injection of MelQ). C, accumulation in the adenohypophysis (female C3H mouse, 20 min after i.v. injection of IQ). D, uptake in the thyroid. The uneven distribution of radioactivity probably reflects an affinity for parafollicular cells of the thyroid (male NMRI mouse, 4 days after i.v. injection of MelQ). E, accumulation in the testis and epididymis. The testicular radioactivity shows an interstitial localization (male NMRI mouse, 20 min after i.v. injection of IQ). F, retention in pigmented tissues (melanin of the eye). Radioactivity is also visible in Harder’s gland and in a lymph node, tissues which also contain melanin (female C3H mouse, 4 days after i.v. injection of MelQ).
Fig. 3. Whole-body autoradiograms of a pregnant NMRI mouse in late gestation 24 hr after i.v. injection of MelQ. B, made from an extracted section to register covalently bound radioactivity. The highest levels of radioactivity are seen in the maternal nasal mucosa, thyroid, liver and kidney, and in the amniotic fluid and fetal intestine. All fetal radioactivity is extractable indicating the absence of reactive metabolites of MelQ in the fetus. However, the maternal liver (note the mottled appearance of the radioactivity) and kidney cortex contain bound radioactivity.
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