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Distribution and tumor penetration properties of a radiosensitizer 2-[^{14}C] misonidazole (Ro 07-0582), in mice and rats as studied by whole-body autoradiography*

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Summary. The hypoxic cell radiosensitizer 2-[^{14}C] misonidazole: 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (Ro 07-0582) MISO was administered to mice, control rats, and rats bearing chemically induced rhadbdomyosarcoma. The dose injected was 250 mg/kg and the delivered activity was 100 μCi/kg. Whole-body autoradiography was performed in all animals. We noted the highest uptake of radioactivity in the liver and the kidney. In the liver there was an accumulation of [^{14}C] from 5 min to the 2 hour after treatment, followed by a decrease; this observation is probably related to the metabolic pathway of the drug. The radioactivity was also concentrated in the renal medulla (30 min after injection); this organ is the excretion route for most of the misonidazole or its metabolites. Fecal excretion is also important following biliary elimination. Radioactivity is present in the central nervous system in the first hours after dosage. [^{14}C] Tumor activity was lowest 5 min after IP treatment. By contrast, 12 h after administration of labeled compound the highest activity was detected in this tissue.

Introduction

It is generally believed that hypoxic cells within both animal and human tumors contribute to the relative resistance to radiation therapy; hypoxic cells can be up to three times more resistant than well-oxygenated cells [10]. Accordingly, methods which overcame the contribution of hypoxia to radiation resistance could be expected to improve the effectiveness of radiotherapy. One of the more promising approaches to this problem involves preirradiation administration of drugs, such as 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol: misonidazole (Ro 07-0582), which mimics the effects of oxygen on the sensitivity of hypoxic cells. This compound has been shown to be an active hypoxic cell radiosensitizer both in vivo and in vitro [6, 19, 20]. Preliminary clinical trials have demonstrated that the effectiveness of misonidazole is closely related to the concentration of drug achieved in the tumor and the timing of radiation after drug administration [12, 17].

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Misonidazole is not routinely used in clinical practice, because of toxicological considerations [26]. Several other structurally related compounds which are more efficient radiosensitizers with a lesser neurotoxicity have been proposed [22]. However, misonidazole was the first molecule to be studied. As we plan to study several related compounds we fell it would be useful to know more about the reference compound.

The aim of this paper is to present results obtained by whole-body autoradiography in mice and tumor-bearing rats dosed with 2-[^{14}C] misonidazole. The results permit the localization of radioactive misonidazole within the organs, visualization of misonidazole loss, determination of the route of elimination, and its uptake in tumors, as described for chemotherapeutic agents [3].

Materials and methods

Drug. [^{14}C] Misonidazole (Fig. 1) was obtained as a crystalline powder with a specific activity of 239 μCi/mg from Roche Laboratories (USA). Labeled and unlabeled drugs were dissolved in NaCl 0.9% solution to give a volume activity of 4 μCi/ml and a concentration of 10 mg/ml.

Before use the radiochemical purity of the labeled compound was determined by thin-layer chromatography in a chloroform/methanol/acetic acid mixture (85 : 15 : 10, v/v) [8]. Radioactive chromatograms were scanned with a Berthold II radiochromatogram scanner. The radiochemical purity was found to be >98%.

Animals. All animals were caged separately and fed ad libitum. They were given [^{14}C]misonidazole 250 mg/kg IP, the activity thus delivered being 100 μCi/kg.

Mice. The study was performed with nine C3H mice weighing 20–22 g. They were sacrificed 5, 15, and 30 min and 2, 4, 8, 12, 24, and 48 h after treatment.

Rats. Twelve rats (6 control and 6 rhabdomyosarcoma-bearing rats) were dosed in the same conditions. These animals were sacrificed 5 and 30 min and 2, 12, 24 and 48 h after injection.

Solid tumors were produced by SC injection of a cell suspension (10^9/ml) obtained from a rhabdomyosarcoma tumor induced by Ni_3S_2. Three weeks after injection, a solid tumor was present in the flank region of the animal. This tumor was confirmed by necropsy and pathological studies [23].

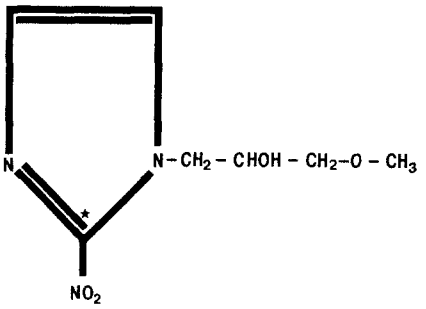

Fig. 1. Structural formulae of misonidazole. The position of the carbon label in the drug is shown by (*)

Technique. All the animals were prepared for whole-body autoradiography [24, 25]. First, they were frozen by rapid immersion in a freezing medium composed of methanol and dry ice. They were then embedded in a 10% aqueous solution of carboxymethylcellulose and frozen to form a firm support all around the carcass. The frozen animals were cut sagittally until an appropriate section appeared. Whole-body sagittal sections were then obtained either with a Leitz 1300 k cryomicrotome or with a PMV 450 semiautomatic microtome. Before each section was collected, adhesive tape was fixed on the surface of the animal to be sectioned. Then the microtome stage was moved and a 20-μm-thick section was obtained on the adhesive tape. After the sections had been collected they were dried by storage overnight at −20°C in a cryostat. The dried sections were then pressed directly against a single-coated X-ray film (Kodak Kodirex) in a light-tight bag and stored at −30°C. At the end of a 1-month exposure period the section and the film were separated. The former was stained when necessary with Mayer's hematoxylin for histological examination; the latter was developed, fixed, and rinsed. All these operations were performed in well-defined, identical conditions to produce autoradiograms that could be compared.

Moreover, sections were obtained from animals injected with unlabeled misonidazole. These control sections were prepared by the same procedure and pressed against Kodak Kodirex X-ray film to verify that no positive or negative chemography occurred with this film under the conditions of our experiment.

Results

Mice. Results are given in Table 1 and Fig. 2.

Blood. Radioactivity in the bloodstream was highest in animals sacrificed 5 min after injection: then it decreased quickly to trace amounts, which were still present in the blood stream 48 h after injection.

Liver. The incorporation or radioactivity in this organ can be described as follows: first, there is an accumulation of [^{14}C] from 5 min to the 2nd hour after the injection of the labeled compound. Then we observed a decrease till the 24th h, at which time the hepatic radioactivity was very low.

Gallbladder. Radioactivity was found in bile within 5 min of the injection. The maximum biliary activity seemed to occur between 2 h and 4 h. At that time, the gallbladder was one of the most intensely labeled organs in the body. After 4 h, the biliary radioactivity decreased rather quickly, so that in an animal killed 24 h later no radioactivity was detectable in bile.

Kidney. The radioactive content was comparable to that described for the liver. The kidney activity was in the same order of magnitude as the liver. From 5 min to 30 min after IP treatment the distribution was rather homogeneous, but in animals sacrificed after this time the distribution of radioactivity was heterogeneous. There was an accumulation of radioactivity in the medulla and a decrease of activity in the renal cortex.

Table 1. Distribution of radioactivity in the body of mice following injection of IP labeled misonidazole, as studied by whole-body autoradiography

	5 min	15 min	30 min	2 h	4 h	8 h	12 h	24 h	48 h
Brain	+	+(+)	+(+)	+(+)	+	(+)	((+))	((+))	0
Blood	++	++	++	+(+)	+	(+)	((+))	((+))	0
Lung	++	++	++	++	+	(+)	((+))	((+))	0
Stomach	+(+)	(+)	(+)	+++	+++(+)	++	++	(+)	0
Intestine	+(+)	++	+++	+++++	+++++	++++	++++	++	0
Liver	+++	+++	+++	+++(+)	++	+	+	(+)	((+))
Brown fat	++(+)	+++	++(+)	+++	++(+)	+	(+)	((+))	((+))
Muscle	++	++(+)	++	++	+	(+)	((+))	0	0
Kidney	++(+)	+++	+++	+++(+)	++	(+)	(+)	((+))	((+))
Bladder	-	+++	+++++	+++++	+++++	++++++	++	0	0
Spleen	++	++	++	++(+)	++	(+)	((+))	((+))	0
Adrenal gland	++	++	++	++	+(+)	(+)	((+))	((+))	0
Thymus gland	+(+)	+(+)	+(+)	+(+)	(+)	((+))	((+))	((+))	0
Thyroid gland	++	++	+(+)	+(+)	(+)	((+))	((+))	((+))	0
Hypophysis	++	++(+)	++	+(+)	(+)	((+))	((+))	((+))	0
Pancreas	+(+)	+(+)	+(+)	++	+	(+)	((+))	((+))	0
Gallbladder	+	++	++(+)	+++++	+++++	++++	(+)	0	0
Bone marrow	++	++	++	++	+	(+)	((+))	((+))	0

0, no radioactivity; +++++, very high radioactivity;
0<((+))<(+)<+<+(+)<++<++(+)<+++<+++(+)<++++<++++++

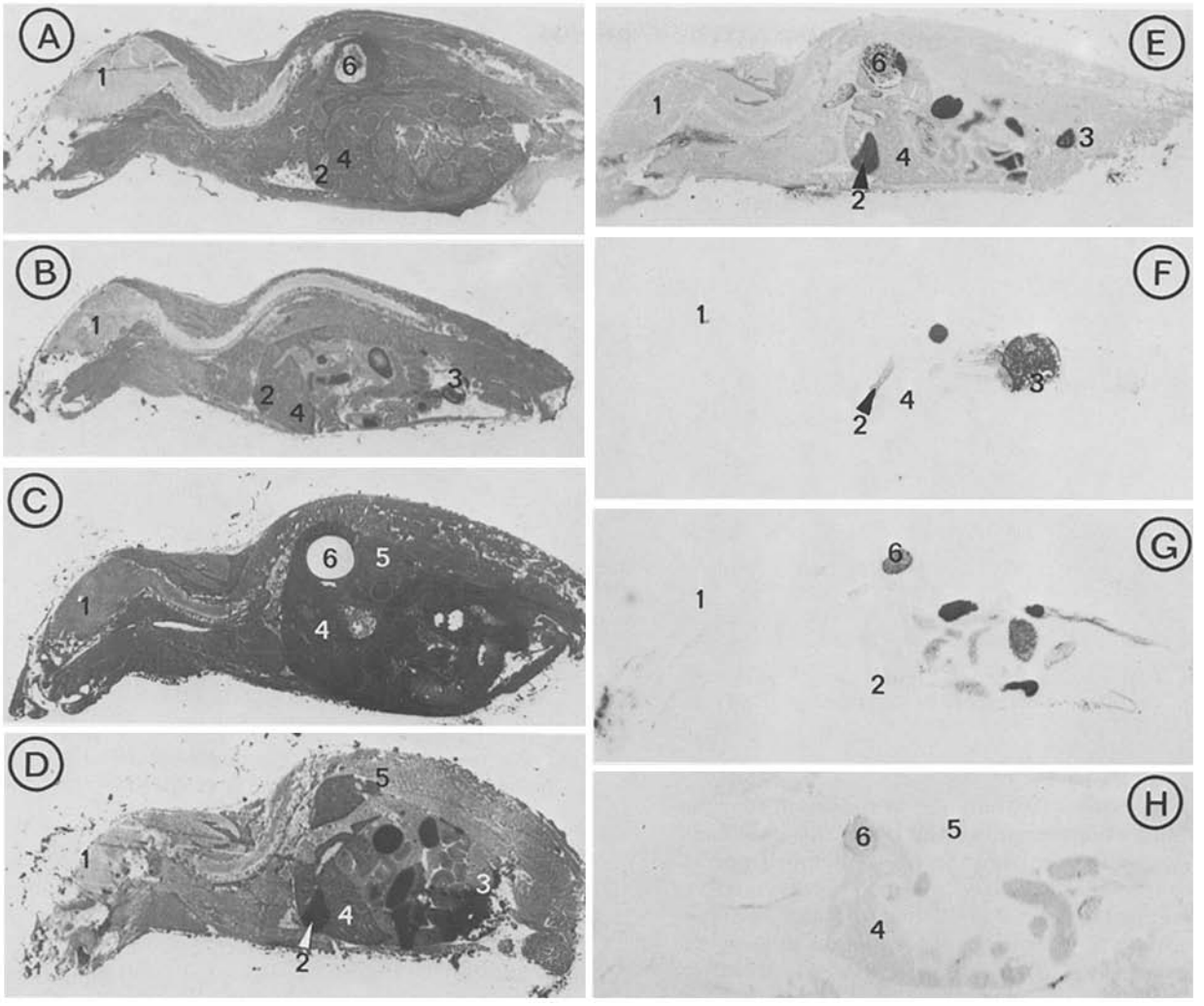

Fig. 2 A–H. Whole-body autoradiogram of mice sacrificed 5 min **(A)**, 15 min **(B)**, 30 min **(C)** 2 h **(D)**, 4 h **(E)** 8 h **(F)**, **12 h (G)** and 24 h **(H)** after [^{14}C]misonidazole administration. *1*, brain; *2*, gallbladder; *3*, urinary bladder; *4*, liver; *5* kidney; *6*, stomach

Fig. 3. Enlargement of a whole-body autoradiogram of mouse after [^{14}C]misonidazole, showing the homogeneous distribution of radioactivity within central nervous system. Note the retention of isotope in the hypophysis *(arrow)*

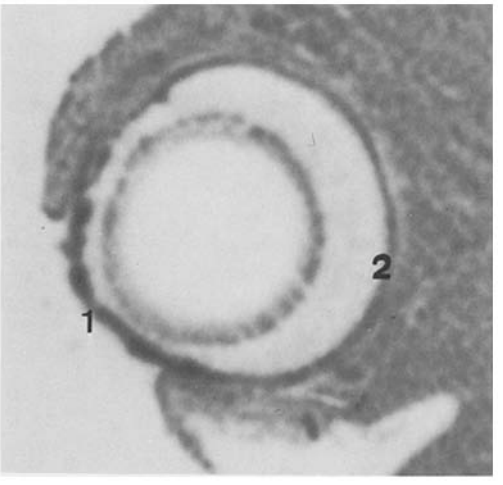

Fig. 4. Detail of an autoradiogram of mouse after [^{14}C]misonidazole and sacrificed 30 min later. Note the weak uptake in the eye. *1*, cornea; *2*, uveal tract

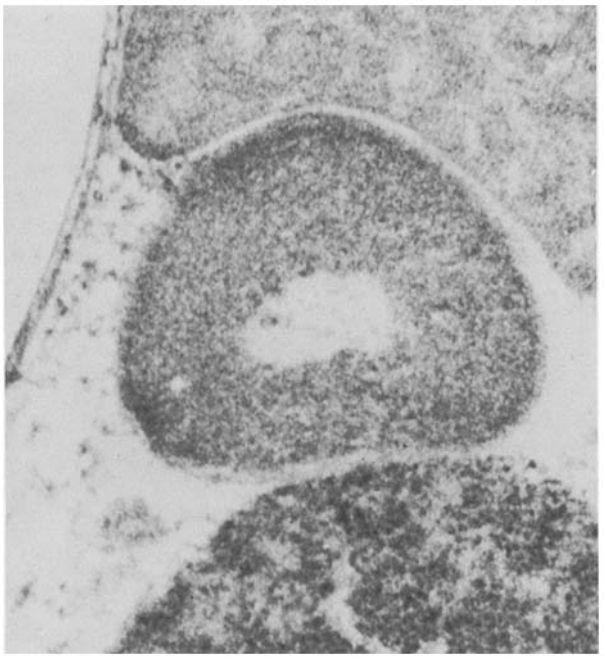

Fig. 5. Detail of an autoradiogram of mouse after [^{14}C]misonidazole and sacrificed 30 min later, showing the retention of radioactivity on the adrenal gland.

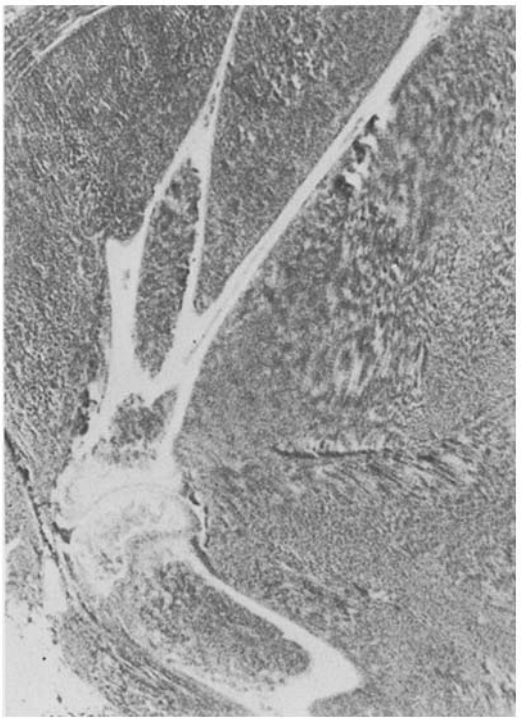

Fig. 6. Detail of an autoradiogram of mouse after [^{14}C]misonidazole and sacrificed 30 min later, showing the absence of the isotope in cartilage and bone except in the bone marrow

Central nervous system. In brain and in spinal chord, rather low activities were present within 5 min after treatment (Fig. 3). This activity seemed to increase with time from 2 h after injection, so that the activity in central nervous system, blood or muscle were in the same order of magnitude. By 24 h later only trace amounts were present in these tissues. In all cases, the distribution of the isotope within these tissues was homogeneous. Moreover, a low uptake of the isotope was noted in the hypophysis of all animals sacrificed between 5 min and 24 h after treatment.

Eyes. As illustrated in Fig. 4, autoradiograms revealed that all the anatomical portions of the eyes contained radioactivity. However, this activity was very low and decreased rapidly.

Miscellaneous. Several glands, such as the adrenal gland (Fig. 5), thymus, pancreas, thyroid, Harderian gland, spleen (red pulp), and skin retained activity. In all these organs, the maximum [^{14}C] activity seemed to occur between 30 min and 2 h after the injection. The isotope was not detectable in cartilage or bone, except in the bone marrow (Fig. 6).

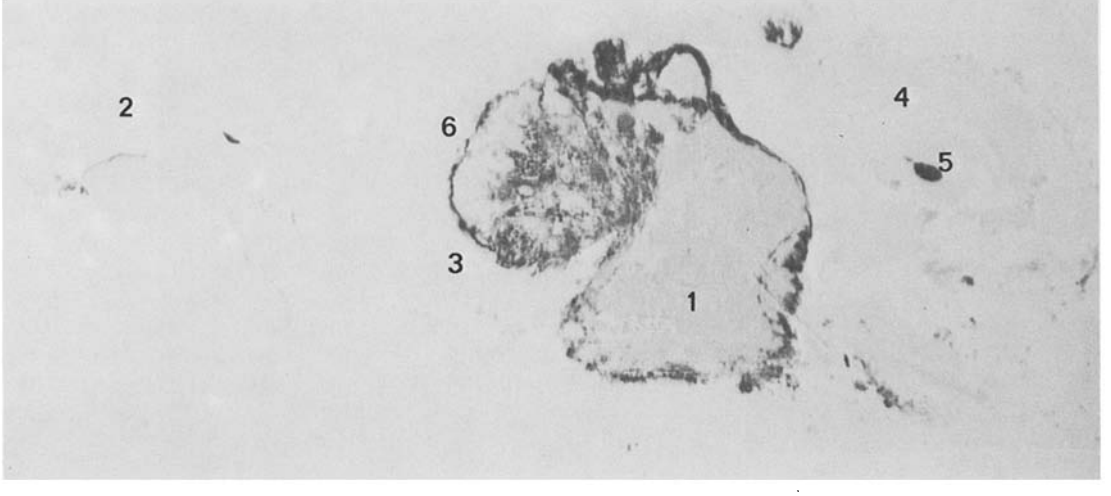

Fig. 7. Whole-body autoradiogram of Wistar rat bearing rhabdomyosarcoma tumour, injected with [^{14}C]misonidazole and sacrificed 12 h later. Note the higher uptake and the heterogeneous distribution of radioactivity within the tumor. *1*, tumor; *2*, brain; *3*, liver; *4*, muscle; *5*, urinary bladder; *6*, lung

Rats

Results pertaining to the distribution of radioactivity within the body of control rats were very similar to those described above for mice.

In rhabdomyosarcoma-bearing rats, the presence of tumor did not change the distribution of the isotope in the whole body (Fig. 7). In tumor tissues a very low fixation occurred 5 min after treatment, but the activity was rather higher in the animals sacrificed 12 h after injection. The distribution of the isotope was heterogeneous within the tumor.

Discussion

The results presented in this paper give an idea of the distribution of [^{14}C] in the body of rodents dosed with [^{14}C]misonidazole. Moreover, they enhance our knowledge of the elimination pathways and the organs in which the isotope is retained.

In mice, the maximum activity in the blood is reached 15 min after administration. These results confirm the pharmacokinetic data of Workman et al. [29], which were recorded in BALB/c mice. These authors, who used HPLC analysis, reported a blood concentration peak occurring 20–30 min after injection. Then the concentration fell with half-lives of 0.73 h and 2.5 h respectively for injected doses of 0.5 mmol/kg and 5 mmol/kg.

Our results indicate that several aspects are important for pharmacological and toxicological purposes. As proposed by Ash et al. [2], it is important to note that radioactive misonidazole is a freely diffusible substance, which penetrates easily into tumor tissue. Our study clearly demonstrated radioactivity in all tissues in both mice and rats, as reported also by Chin and Rauth [5].

In the liver the [^{14}C] concentration rises rapidly; the isotope distribution is very homogeneous. The liver activity is the most persistent within the body of mice and rats. This fact does not agree with biochemical analysis by HPLC which has shown a rapid decrease of MISO concentration in liver [1]. This discrepancy can be explained by the experiment of Pedersen et al. [18], which demonstrated a rapid degradation of MISO in various tissues excised and left at room temperature. So, it is necessary to assay or freeze tissues immediately after removal to obtain accurate estimates of concentrations and to avoid drug degradation. The high activity in the liver could be explained by the role of liver in the biotransformation process of misonidazole [15, 16]; Schoemaker et al. [21] showed the importance of this organ in the demethylation process either in vivo or in vitro. Moreover, the liver plays an important metabolic role with its biliary function. Our autoradiograms demonstrate that there is a rapid and significant excretion of radioactivity in the bile of mice.

In the initial phase, the radioactivity in the kidney is also similar to that detected in the liver. The distribution in this organ is homogeneous in the hours immediately after administration. Then there is higher activity in renal medulla, with a decrease of activity in the renal cortex. This observation could be related to the importance of renal function in the elimination process of misonidazole and its metabolites. Effectively, the renal elimination of [^{14}C] is very important. It begins rapidly (after 5 min), with the appearance of a high radioactivity in urine, and is achieved between the 12th and 24th hours. These observations were confirmed by the studies of urinary excretion of MISO and metabolites in mice [28, 29] and were rather similar to results obtained with other xenobiotics, such as L-(1,2-ethyl-[^{14}C])ethionine [7]. Fecal excretion is also very significant, since 48 h after injection trace amounts of radioactivity are still present in the cecal and rectal contents.

Koch et al. [13] showed the role of the intestinal flora in the metabolism of misonidazole. The radiation sensitizer misonidazole is metabolized to its derivative 1-(2-aminoimidazole-1-yl)-3-methoxypropan-2-ol in pure or mixed cultures of the intestinal microflora. This metabolite appears in the excreta of conventional rats, but is not detectable in the excreta of germfree rats. Thus, its formation appears to be due to the activity of the intestinal flora both in vivo and in vitro. In addition, the flora can liberate [$^{14}CO_2$] from 2-[^{14}C]misonidazole, and it appears that 1-(2-aminoimidazol-1-yl)-3-methoxypropan-2-ol and urea are intermediates in this metabolic pathway.

This fecal excretion is a result of the biliary elimination demonstrated in mice. The results obtained in mice show that this elimination appears early; maximum elimination appears after 2 h and is complete after 24 h. Under these conditions, an enterohepatic cycle probably does not exist.

We noted the presence of the isotope in the central nervous system and in various glands, e.g. hypophysis, thyroid, pancreas, spleen and adrenal gland, but not in cartilage. This result is very different from that yielded by the work of Langler et al. [14], who showed that rabbit ear cartilage had a misonidazole concentration 70% that of blood.

The fixation of misonidazole or its metabolites in the central nervous system, also established with a conventional HPLC technique [1, 30], might be related to severe neuropathies which have been described in humans [26]. This neurotoxicity has been the main limiting factor in clinical trials. While the exact mechanism of misonidazole toxicity is unknown, it has been suggested, as for other nitro-aromatic compounds, that it is due to the reduction of the nitro group to a reactive metabolite [11, 27].

In sarcoma-bearing animals the presence of tumor does not modify the distribution of the isotope within the rat body. However, tumor content is persistent, and 12 h after injection tumor concentrations are elevated. The isotope is present not only at the periphery of the tumor but also in the core. Some variations in isotope activity have been found within the tumor and are also described by other authors [2, 4]. This could be related to the hypoxic state within the tumor. Chapman et al. [5] administered [14C]-labeled MISO to tumor-bearing mice and analyzed the pattern of binding by autoradiography. The rate of binding to intact cells within a few cell layers of necrotic regions was always much greater than that to cells further from necrosis or to necrotic material. In another work, Franko and Chapman [10] found that the rate of binding to chronically hypoxic cells at the edge of the necrotic centre was 20 times less than the similar cells in other spheroids made maximally hypoxic with N_2.

The various data obtained in this autoradiographic study give some idea of the distribution of [^{14}C]misonidazole in the body of rats and mice. This study emphasizes the importance of hepatic, renal, and intestinal tissues in the metabolism and excretion of this hypoxic cell radiosensitizer.

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References

1. Akel G, Canal P, Mockey E, Vandevoorde E, Laurent F, Soula G (1983) Comparison of the fate of misonidazole (Ro 07-0582) and its metabolite desmethylmisonidazole (Ro 05-9963), two hypoxic cell radiosensitizers: penetration properties in tumor-bearing rats. J Pharmacol (Paris) 14: 67
2. Ash DV, Smith MR, Budgen RD (1979) Distribution of misonidazole in human tumours and normal tissues. Br J Cancer 39: 503
3. Benard P, Desplanches G, Macquet JP, Simon J (1983) Whole-body autoradiographic study of the distribution of 195 mPt in healthy and tumor-bearing mice treated with labelled cisplatin. Cancer Treat Rep 67: 457
4. Brown JM, Yu NY, Workman P (1979) Pharmacokinetic considerations in testing hypoxic cell radiosensitizers in mouse tumours. Br J Cancer 39: 310
5. Chapman JD, Franko AJ, Sharplin J (1981) A marker for hypoxic cells in tumours with potential clinical applicability. Br J Cancer 43: 546
6. Chin JB, Rauth AM (1981) The metabolism and pharmacokinetics of the hypoxic cell radiosensitizer and cytotoxic agent, misonidazole, in C3H mice. Radiat Res 86: 341
7. Dische S, Saunders MI, Lee ME, Adams GE, Flockhart IR (1977) Clinical testing of the radiosensitizer Ro 07-0582: experience with multiple doses. Br J Cancer 35: 567
8. Durand S, Benard P (1980) Whole-body autoradiographic study of the distribution of radioactivity in rats given L-[1,2-ethyl-^{14}C] ethionine. Toxicol Appl Pharmacol 53: 204
9. Flockhart IR, Large P, Troup D, Malcolm SL, Marten TR (1978) Pharmacokinetic and metabolic studies of the hypoxic cell radiosensitizer misonidazole. Xenobiotica 8: 97
10. Franko AJ, Chapman JD (1982) Binding of ^{14}C-misonidazole to hypoxic cells in V79 spheroids. Br J Cancer 45: 694
11. Gray AJ, Dische S, Adams GE, Flockhart IR, Foster JL (1976) Clinical testing of the radiosensitizer Ro 07-0582: I. Dose, tolerance, serum and tumour concentrations. Clin Radiol 27: 151
12. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OCA (1953) The concentration of oxygen dissolved in tissue at time of irradiation as a factor in radiotherapy. Br J Radiol 26: 638
13. Josephy PD, Placic B, Skarsgard LD (1981) In vitro metabolism of misonidazole. Br J Cancer 43: 443
14. Koch RL, Beaulieu BB Jr, Goldman P (1980) Role of the intestinal flora in the metabolism of misonidazole. Biochem Pharmacol 29: 3281
15. Langler AI, Budgen RD, Gibson P (1982) The penetration of misonidazole into mature structural cartilage. Br J Cancer 45: 282
16. McManus ME, Monks A, Collins JM, White R, Strong J (1981) Non-linear pharmacokinetics of misonidazole and desmethyl-misonidazole in the isolated perfused rat liver. J Pharmacol Exp Ther 219: 669
17. McManus ME, Lang MA, Stuart K, Strong JM (1982) Activation of misonidazole by liver microsomes and purified NADPH cytochrome C reductase. Biochem Pharmacol 31: 547
18. McNally NJ, Denekamp J, Sheldon P, Flockhart IR, Stewart FA (1978) Radiosensitization by misonidazole: the importance of timing and tumour concentration of sensitizer. Radiat Res 73: 568
19. Pedersen JE, Smith MR, Budgen RD, Peckham MJ (1979) Distribution and tumour cytotoxicity of the radiosensitizer misonidazole (Ro 07-0582) in C57 mice. Br J Cancer 39: 429
20. Phillips TL, Wasserman TM, Johnson RJ, Gomer CJ, Lawrence GA, Levine ML, Sadee W, Penta JS, Rubin DJ (1978) The hypoxic cell sensitizer programme in the United States. Br J Cancer 37: 276
21. Sakamoto K, Aritake S (1981) Effects of misonidazole on tumour cell radiation sensitivity and potentially lethal damage repair in vivo and in vitro. Eur J Cancer Clin Oncol 17: 825
22. Shoemaker DD, McManus ME, Hoerauf R, Strong JM (1982) Studies on the *O*-demethylation of misonidazole by rat liver microsomes. Cancer Treat Rep 66: 1343
23. Smithen CE, Clarke ED, Dale JA, Jacobs RS, Wardman P, Watts ME, Woodcock M (1980) Novel (nitroimidazolyl) alkanolamines as potential radiosensitizers with improved therapeutic properties. In: Brady LW (ed) Radiation sensitizers. Masson New York, 22
24. Sweeney FL, Pot-Deprun J, Poupon MF, Chouroulinkov I (1982) Heterogeneity of growth and metastatic behavior of cloned cell lines derived from a primary rhabdomyosarcoma. Cancer Res 42: 3776
25. Ullberg S (1954) Studies on the distribution and fate of ^{35}S-labelled benzypenicillin in the body. Acta Radiol [Suppl] 118: 1
26. Ullberg S (1977) The technique of whole-body autoradiographic study. Science Tools, Special issue: 2
27. Urtasun RC, Chapman JD, Feldstein ML, Band RP, Rabin HR, Wilson AF, Marynowsky B, Starreveld E, Shnitka T (1978) Peripheral neuropathy related to misonidazole: incidence and pathology. Br J Cancer 37: 271
28. Varghese AJ, Whitmore GF (1980) Binding to cellular macromolecules as a possible mechanism for the cytotoxicity of misonidazole. Cancer Res 40: 2165
29. Workman P (1979) Effects of pretreatment with phenobarbitone and phenytoin on the pharmacokinetics and toxicity of misonidazole in mice. Br J Cancer 40: 335
30. Workman P (1980) Dose-dependance and related studies on the pharmacokinetics of misonidazole and desmethylmisonidazole in mice. Cancer Chemother Pharmacol 5: 27

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