Bull. Environm. Contam. Toxicol. 24,627-633 (1980)

Absorption, Distribution, and Excretion of Methylmercury in Mice

Meena Mehra and Kuldip C. Kanwar

Department of Biophysics, Panjab University, Chandigarh 160014, India

The biotransformation of inorganic mercury to methylmercury in aquatic food-chains has become recognized as a serious environmental hazard. The use of methylmercury as a seed preservative is another major source of environmental pollution by this organometallic contaminant. It is on record that in Japan and Iraq the consumption of methylmercury-contaminated shell fish (MATSUMOTO et al. 1965) and grains (BAKIR et al. 1973) had led to serious outbreaks involving methylmercury poisioning. Severe yet varied neurological disorders (BERGLUND & BERLIN 1969, BERLIN et al. 1975, KOOS & LONGO 1976) and teratogenic effects (KHERA 1973, SU & OKITA 1976, FUYUTA et al. 1979) have also been reported following exposure to methylmercury compounds. However, not much is known about the uptake, retention and excretion of methylmercury. SWENNSON et al. (1959), ULFVARSON (1969), and ANSARI et al. (1973) are a few workers who have studied distribution of this metallo-contaminant.

The present report embodies integrated observations on the uptake, distribution and excretion of $CH_3\,^{203}Hg$ in the varied tissues of the mice following single oral as well as intraperitoneal administration.

MATERIALS AND METHODS

Male swiss albino mice, each weighing 25-30 g, were sacrificed for the present studies. $CH_3\,^{203}Hg$ as $CH_3\,^{203}Hg\,Cl$ (Spec. act. 3.44 mCi/mg of Hg)[3] was obtained from New England Nuclear. The mice were divided into two sets of eight groups each, each group comprising four animals. A single dose of MeHg (20 uCi/100 g body weight) was fed orally to each animal belonging to set I and an identical dose of the radioisotope was administered intraperitoneally to each animal belonging to set II. The isotope-treated animals from both the sets were anaesthetised with nembutal (5 mg/100 body weight) and sacrificed at varied intervals ranging from 2 to 72 h. The blood was drawn directly from the heart of the experimental animals, whereafter the body cavity was opened, heart removed for instantaneous cessation of blood supply to other organs and the various organs dissected out in the following order: the testes,

0007-4861/80/0024-0627 $01.40

seminal vesicles, epididymes, brain (cerebrum, cerebellum, pons), intestine, spleen, lungs, liver and the kidney. Faeces were also collected and analyzed for determining the faecal excretion of MeHg. The tissue were carefully weighed and digested in 30% KOH at 90°C for 10 min. The digested samples were monitored for ^{203}Hg counts employing a X-ray medical spectrometer having a well type detector.

RESULTS and DISCUSSION

The present observations clearly indicate that the uptake and distribution patterns of MeHg depend on the route of administration (Table 1 and Table 2). The uptake of orally administered MeHg (Table 2) was rather poor as compared with the uptake following single intraperitoneal administration (Table 1). However, the relative uptake of MeHg in the varied tissues was similar following both the routes of administration barring the intestine, which obviously showed distinctly higher uptake following oral administration. The incorporation of MeHg proceeded at a faster rate following parenteral administration as compared with oral feeding. Further, faecal excretion rates were markedly higher in case of oral exposure suggesting an over-all poor absorption of the radioisotope by the gastrointestinal tract.

Whole blood and the erythrocytes showed quick incorporation of MeHg following intraperitoneal administration. The higher levels of MeHg in the erythrocytes were attained as early as 2h post-administration, whereas the peak level in the blood was recorded 6h post-administration. In contrast, the highest levels of the radiomethylmercury were recorded 12 and 24h post-administration in the erythrocytes and whole blood, respectively, following oral feeding. The present observation lend support to the view that majority of the blood methylmercury is contained in the erthrocytes alone, wherein the former is exclusively bound to haemoglobin (SWENNSON et al. 1959, LUNDGREEN et al. 1967, SUZUKI et al. 1971, NORDBERG & SKERFVING 1972, ROTHSTEIN 1973, GARCIA et al. 1974). Moreover, the present observations also suggest the existence of more than one compartment in which MeHg is localised in the blood, inasmuch as that there is no temporal synchronization between the peak levels of MeHg in the erythrocytes and the whole blood.

The kidneys and the liver together, as usual, accounted for the bulk of MeHg present in the body; the kidneys incorporated five times more radioactivity than the liver following intraperitoneal administration whereas the ratio of kidney MeHg to liver MeHg never exceeded 3:1 in case of oral administration. It is

TABLE 1

$CH_3^{203}Hg$ distribution in mice (20 uCi/100 g body weight)

%age uptake of intraperitoneally administered dose ± S.D.

	2h	4h	6h	12h	24h	36h	48h	72h
Blood	3.2±0.6	3.5±0.9	3.6*±0.2	3.2±0.2	2.3±0.2	2.1±0.1	1.3±0.2	0.74±0.02
Erythrocytes	2.3*±0.8	1.7±0.3	1.0±0.1	0.94±0.09	0.86±0.08	0.54±0.14	0.32±0.06	0.21±0.01
Kidneys	21±1	22.6±1.8	26.1±1.0	32.4±1.7	41±2	49*±2	46±2	39±1
Liver	4.6±0.9	4.6±0.5	6.2±1.0	8.1±1.0	9.1*±0.6	8.4±1.2	5.2±1.0	3.6±1.2
Spleen	3.7*±0.6	2.8±0.5	2.5±0.6	1.9±0.3	1.7±0.4	1.3±0.5	0.9±0.2	0.74±0.09
Heart	3.4±0.4	4.2*±0.5	3.6±1.0	2.4±0.4	2.1±0.4	2.0±0.4	1.9±0.3	1.2±0.2
Lungs	3.2±0.9	3.2*±0.7	2.6±0.8	1.9±0.5	1.8±0.5	0.7±0.1	0.49±0.09	0.32±0.06
Intestine	1.5±0.5	2.1±0.5	2.3*±0.4	2.1±0.4	1.7±0.2	1.4±0.4	0.94±0.07	0.62±0.09
Cerebrum	0.17±0.02	0.21±0.04	0.82±0.12	1.2±0.5	1.3±0.4	1.4±0.4	1.4±0.3	1.5±0.4
Cerebellum	0.20±0.01	0.25±0.01	0.69±0.17	0.99±0.09	1.3±0.3	1.5±0.4	1.6±0.5	1.9±0.3
Pons	0.10±0.01	0.14±0.01	0.26±0.10	0.61±0.06	0.98±0.12	1.1*±0.2	1.0±0.5	1.0±0.1
Testes	0.95±0.11	1.0±0.6	1.2±0.3	1.3*±0.4	0.74±0.09	0.64±0.10	0.52±0.06	0.24±0.09
Epididymes	0.72±0.08	0.82±0.08	0.94±0.14	1.1*±0.1	1.1±0.2	0.92±0.09	0.71±0.12	0.31±0.10
Seminal Ves	0.94±0.09	1.0±0.1	1.1*±0.5	0.96±0.08	0.84±0.12	0.71±0.11	0.42±0.09	0.21±0.08
Faeces	-	-	-	-	2.5±0.5	-	1.4±0.3	1.1±0.5

*indicates time period in which the highest $CH_3^{203}Hg$ activity was recorded.

± S.D. was calculated from the data on 4 animals at each time interval.

TABLE 2

$CH_3^{203}Hg$ distribution in mice (20 uCi/100 g body weight)

%age uptake of orally administered dose ± S.D.

Organ	2h	4h	6h	12h	24h	36h	48h	72h
Blood	0.12±0.03	0.96±0.10	1.2±0.3	2.2±0.7	2.6*±0.2	2.4±0.7	2.1±0.1	1.3±0.2
Erythrocytes	0.09±0.01	0.21±0.07	0.84±0.02	0.98*±0.10	0.96±0.06	0.86±0.12	0.66±0.09	0.52±0.04
Kidneys	5.3±0.3	10.7±0.6	18.8±1.2	19.5*±1.0	16.2±1.2	12.6±0.8	10.4±0.6	8.7±0.7
Liver	1.6±0.2	2.4±0.5	3.4±0.5	5.4±0.6	6.0*±0.8	3.4±0.5	2.4±0.1	2.1±0.1
Spleen	0.94±0.09	0.96±0.09	1.1±0.3	1.7±0.4	2.1*±0.3	2.0±0.3	1.9±0.2	-
Heart	0.84±0.06	0.91±0.14	1.2*±0.6	1.1±0.2	0.96±0.16	0.36±0.05	0.24±0.09	-
Lungs	0.15±0.01	0.62±0.08	0.74±0.08	0.96±0.12	1.1*±0.1	0.54±0.08	0.32±0.06	-
Intestine	1.2±0.1	3.1±0.6	3.6±0.7	4.9±0.9	5.2*±1.0	4.7±0.5	4.2±0.3	-
Cerebrum	0.09±0.00	0.09±0.00	0.14±0.00	0.26±0.01	0.29±0.02	0.32*±0.01	0.28±0.01	-
Cerebellum	0.06±0.00	0.08±0.01	0.15±0.02	0.28±0.09	0.31±0.10	0.43*±0.04	0.30±0.06	-
Pons	0.08±0.01	0.08±0.01	0.11±0.00	0.16±0.01	0.19*±0.09	0.14±0.00	0.16±0.00	-
Testes	0.12±0.01	0.14±0.01	0.21±0.01	0.32±0.03	0.61*±0.04	0.46±0.04	0.39±0.02	-
Epididymes	0.09±0.00	0.09±0.00	0.14±0.01	0.18±0.01	0.26±0.01	0.31*±0.01	0.18±0.00	-
Seminal Ves	0.10±0.00	0.10±0.01	0.20*±0.01	0.14±0.01	0.11±0.00	0.13±0.00	0.11±0.00	-
Faeces	-	-	-	-	6.42±0.2	-	3.4±0.3	2.5±0.1

* indicates time period in which the highest $CH_3^{203}Hg$ activity was recorded.

± S.D. was calculated from the data on 4 animals at each time interval.

rather intriguing that the kidneys show faster accumulation of MeHg (Table 2) following oral exposure as compared with intraperitoneal dose in which case the peak activity in the kidney was recorded 36h post-administration as against 12h after oral administration. The peak levels of MeHg in liver were however, recorded 24h post-administration following both the routes of administration. The gradual but extremely high retention of MeHg in the kidneys is suggestive of specialized selective compartment in this tissue accounting for such a phenomenon. Metallothionein has been implicated in the retention of inorganic mercury in the kidney (cf. CHERIAN & GOYER 1978) but the mechanism of MeHg retention in the tissues is not clear, though sulphydryl compounds, particularly SH- amino acids, have been implicated in MeHg retention in the liver (BEIJE & ARRHENIUS 1978).

The spleen, heart and the lungs showed quicker incorporation of MeHg in contrast to the liver and the kidneys; but the amount of the radiomethylmercury incorporated in the spleen, the heart and the lungs was markedly less than that recorded in the kidneys. Following intraperitoneal exposure, the highest MeHg levels in the spleen were recorded as early as 2h post-administration whereas the heart and the lungs attained peak radioactivity 4h post-administration. In case of oral feeding, the peak MeHg levels were sufficiently delayed i.e. 24h post-administration in both the spleen and the lungs. The heart recorded highest MeHg levels 6h post-administration.

Of all the tissues studied, the intestine showed unique pattern of MeHg uptake which depended on the route of administration. The intestine recorded peak MeHg levels only 24h post-administration following oral dose in comparison to the peak activity being attained at 6h post-administration following parenteral administration. The extent of uptake following oral administration was, however, 2.5 times higher when compared with intraperitoneal dosing. Higher incorporation following oral exposure results due to the direct absorption of this organometallic contaminant from the gastrointestinal tract. Following parenteral exposure, however, most of the available MeHg is trapped up by the liver and the kidneys and hence poor MeHg activity in the intestine.

The cerebrum, the cerebellum and the pons were rather very slow in incorporating MeHg and the radioactivity in these brain components steadily increased till 72h post-administration following single intraperitoneal dose. The present observations also indicate differential uptake of MeHg by the varied regions of the brain - the cerebellum accounted for the maximum

activity of MeHg, whereas the pons incorporated the least . Such regional variations in the uptake of methylmercury by the different parts of the brain have been observed by other authors too (cf. BUTTER WORTH et al. 1978). The regional affinity of brain for organomercurials has been interpretted in terms of the neurological disorders produced in the Minamata disease. The uptake of MeHg by brain and consequently the neurotoxic effects produced, have recently been explained by the interaction of MeHg with the lipid components (ANDO et al. 1979).

The reproductive tissues, viz the testes, epididymes and the seminal vesicles showed a rather poor MeHg uptake and the peak levels recorded were as low as 1% of the administered dose/g wet tissue. To the best of our knowledge no direct effects of MeHg on reproductive organs have been reported so far. It is worth noting that the testes incorporated significantly higher MeHg as compared with the epididymes or the seminal vesicles.

The studies on faecal excretion of MeHg carried out during the course of present investigations show that the amount of MeHg excreted via faeces is the highest on first day and the excretion decreases steadily during the next two days. The total proportionate amount of MeHg excreted via faeces was, however, much lower than the faecal excretion of Hg^{2+} (MEHRA & KANWAR 1979) or Cd^{2+} (KANWAR et al.). Further higher rate of faecal excretion following oral administration as compared with parenteral administration is suggestive of poor absorption of this compound by the gastrointestinal tract.

ACKNOWLEDGEMENT

This research was, in part, financed by a senior research fellowship of Council of Scientific and Industrial Research, India to Meena Mehra.

REFERENCES

ANDO, T., A. NAKANO and I. WAKISAKA: Bull. Environm. Contam. Toxicol. 22, 214 (1979)

ANSARI, M.S., W.J. MILLER., R.P. GENTRY, M.W. NEATHERY, P.E. STAKE: J. Anim. Sci. 36, 415 (1973)

BAKIR, P., S.F. DAMULJI, L. AMIN-ZAKI, M. MURTADHA, A. KHALIDI, N.Y. AL-RAWI, S. TIKRITI, H.I. DHAHIR, T.W. CLARKSON, J.C. SMITH and R.A. DOHERTY: Science 181, 230 (1973)

BEIJE, B., and E. ARRHENIUS: Chem-Biol. Interact. 21, 167 (1978)

BERGLUND, F., and M. BERLIN: In "Chemical Fallout" (M.W. MILLER, and G.G. BERG, Eds), 258 (1969)

BERLIN, M., C. A. GRANT, J. HELLBERG, J. HELLSTROM, and A. SCHUTZ : Arch. Environ. Hlth. 30, 340 (1975)
BUTTERWORTH, R. F., M. Gonce, and A. BARBEAU: Can. J. Neurological Sci. 397 (1978)
CHERIAN, M. G., and R. A. GOYER: Life Sci. 23, 1 (1978)
FUYUTA, M. T. FUJIMOTO, and E. KIYOFUJI: Acta Anat. 104, 356 (1979)
GARCIA, J. D., M. G. YANG, J. H. C. WANG, and P. S. BELLO Proc. Soc. Exp. Biol. Med. 146, 66 (1974)
KANWAR, K. C., S. C. KAUSHAL, and R. K. MEHRA : Bull. Environm. Contam. Toxicol. 24, 321 (1980)
KHERA, K. S. : Teratology 8, 293 (1973)
KOOS, B. J., and LONGO, L. D. : Am. J. Obstet. Gynec. 126, 390 (1976)
LUNDGREEN, K. D., A. SWENNSON, and U. ULFVARSON : Scand. J. Clin. Lab. Invest. 20, 164 (1967)
MATSUMOTO, H. G. KOYA., and T. TAKEUCHI : J. Neuropathol Exp. Neurol. 29, 563 (1965)
MEHRA, M., and K. C. KANWAR : Bull. Environm. Contam. Toxicol. 21, 733 (1979)
NORDBERG, G. F., and S. SKERFVING : Metabolism. In : Mercury in the environment, edited by L. FRIBERG., and J. VOSTAL. Cleveland, Ohio, CRC Press, 1972, p. 29
ROTHSTEIN, A.: Mercaptans, the biological Targets for mercurials. In : Mercury, mercurials, and mercaptans, edited by M. W. MILLER, and T. W. CLARKSON. Springfield, Ill. C. C. Thomas, 1973, p. 68
SU, M. Q., and G. T. OKITA : Toxicol. Appl. Pharmacol. 38, 207 (1976)
SUZUKI, T. T. MIYAMA, and H. KATSUMUMA : Bull. Environm. Contam. Toxicol. 5, 502 (1971)
SWENNSON, A., K. D., LUNDGREEN, and O. LINDSTROM : Arch. Ind. Health. 20, 432 (1959)
ULFVARSON, U. : Toxicol. Appl. Pharmacol. 15, 525 (1969)