The Influence of Selenium on the Level of Mercury and Metallothionein in Rat Kidneys in Prolonged Exposure to Different Mercury Compounds

J. Chmielnicka, E. A. Brzeznicka

THE INFLUENCE OF SELENIUM ON THE LEVEL OF MERCURY AND METALLOTHIONEIN IN RAT KIDNEYS IN PROLONGED EXPOSURE TO DIFFERENT MERCURY COMPOUNDS[x]

by J.CHMIELNICKA, E.A.BRZEŻNICKA

Department of Toxicological Chemistry, Institute of Environmental Research and Bioanalysis, Medical Academy of Łódź, Narutowicza 120 a, 90-145 Łódź

In rat kidneys metallothionein binds mercury derived from the supplied inorganic mercury /WIŚNIEWSKA-KNYPL et al. 1970, PIOTROWSKI et al. 1974a, 1974b/ and from elementary mercury /SAPOTA et al. 1974, CHERIAN and CLARKSON 1976/. It also binds mercury derived from phenylmercury acetate /PIOTROWSKI and BOLANOWSKA 1970, ELLIS and FANG 1971, FANG 1973/, and ethylmercuric chloride /FANG, 1973/. In the latter cases, however, metallothionein probably binds inorganic mercury which forms as the result of biodegradation of aryl - and alkyl mercury compounds. To a lesser extent metallothionein binds also mercury supplied in form of methylmercury compounds the yield of this process being inversely proportionate to the dose /CHEN et al. 1973/. This also may be due to the biodegradation of methylmercury which yields inorganic mercury /CLARKSON, 1972/.

In mercury poisoning the protective role of both metallothionein /PIOTROWSKI et al. 1973a, Mac GREGOR and CLARKSON 1974/ and of selenium /GANTHER et al. 1972, GROTH et al.1973, GANTHER and SUNDE 1974, PARIZEK and OSTADALOVA 1967/ is considered. Selenium does not stimulate the biosynthesis of metallothionein /PIOTROWSKI and SZYMAŃSKA 1976/ and when administered jointly with mercury it removes this element from metallothionein-like proteins /CHEN et al. 1975, KOMSTA-SZUMSKA et al. 1976/ and eliminates the effect of stimulation of metallothionein biosynthesis caused by mercury /KOMSTA-SZUMSKA and CHMIELNICKA in press/.

The object of the present paper is to investigate changes in the level of metallothionein in the kidneys and liver of rats in prolonged exposure to various mercury compounds and the influence of sodium selenite upon this phenomenon.

---

x/ The study was partly subsidised by the Polish-American agreement 05-009-2 with the National Institute of Occupational Safety and Health, PHS, USA, Chief investigator-prof.dr hab. J.K.PIOTROWSKI.

Bulletin of Environmental Contamination & Toxicology, 0007-4861/78/0019-0183 $1.60

MATERIALS AND METHODS

Animals. 36 female Wistar rats, average body weight 200 g, fed standard LSM diet and supplied with water ad libitum were examined. The animals were divided into 9 groups of 3 rats each and 1 control group of 9 animals. Animal groups 1,2,3,4 received orally water solutions or suspension of examined mercury compounds of 1 % DL_{50} /0,4-0,6 ml vol/ three times a week. Group 1 was given a fluid seed dressing preparation "0,8" containing 0,8 % Hg in form of methylmercurycyanguanidine /Chemical Works, Azoty, Jaworzno/ - 0,46 mg Hg/kg /Met-Hg/, group 2-ethylmercuric chloride /BDH .Laboratory Reagents/ - 0,23 mg Hg/kg /Et-Hg/, group 3-phenylmercuric chloride /BDH Laboratory Reagents - 0,23 mg Hg/kg /Ph-Hg/, group 4-mercuric chloride /POCh, Gliwice /-0,23 mg Hg/kg /$HgCl_2$/. Three times a week animals from parallel groups 1a, 2a, 3a, 4a were given the same doses of mercury compounds and water solution of sodium selenite /POCh, Gliwice/ 0,18 mg Se/kg alternately. Group 5 was a control and the rats of the parallel group 5a were given the water solution of sodium selenite /0,18 mg Se/kg/ three times a week. After 14-week-exposure the animals were killed in ether narcosis liver and kidneys were taken for analysis.

Determination of mercury. The level of inorganic and total mercury was determined in non-mineralized samples of biological material by means of cold atomic absorption /MAGOS, 1971; slightly modified, BALCERSKA and al 1977/ using the mercury vapour detector /Handrey Relays Type E 34 72/.

The determination of metallothionein in the homogenates of liver and kidneys was performed by radiochemical method according to PIOTROWSKI et al./1973b/modified by ZELAZOWSKI and PIOTROWSKI /in press/. Metallothionein standard was obtained in our laboratory/ZELAZOWSKI et al. in press/ from the cortex of horse kidneys, by means of a technique similar to that of PULIDO et al. /1966/. The standard contained about 3 groups of SH/mg protein; in conditions of analysis 1 mg protein bound about 200 /ug Hg.

RESULTS AND DISCUSSION

The level of total mercury in the organs examined was dependent on the mercury compound administered /Table 1/. After oral administration of ethylmercuric chloride, methylmercurycyanguanidine, phenylmercuric chloride and mercuric chloride the content of total mercury in the kidneys was respectively: 11,1; 2,7; 4,3 and 0,7 % of the cumulative dose. The low level of mercury after the administration of mercuric chloride is the result of the low rate of absorption of mercuric chloride from the gastro-intestinal tract /FRIBERG and VOSTAL, 1972/. A considerable accumulation of inorganic mercury in kidneys has been found after the administration of alkylmercury compounds. As far as the methylmercurycyanguanidine is concerned our results approximate those obtained

TABLE 1

The level of organic and inorganic mercury in the organs of rats exposed to different mercury compounds without and with selenium.

Compounds	Cumulative dose mg Hg/kg	Hg / /ug/g tissue/				Kidneys / Liver /ug Hg/g/	
		Kidneys		Liver			
		Organic	Inorganic	Organic	Inorganic	Inorganic	Organic
Met-Hg	20.0	45.0 /42.0- 47.0/	26.0 /25.0- 28.0/	6.50 /4.37- 7.12/	0.42 /0.40- 0.50/	62	7
Met-Hg + Se[x]		31.0 /25.2- 35.0/	15.2 /14.0- 16.0/	6.70 /6.50- 6.80/	0.46 /0.46- 0.46/	33	5
Et-Hg	10.0	78.0 /69.0- 79.0/	64.6 /60.3- 69.6/	1.50 /1.30- 1.70/	0.59 /0.50- 0.65/	110	52
EtHg + Se[x]		78.0 /65.8- 89.0/	63.0 /55.8- 73.7/	1.78 /1.60- 1.90/	1.05 /0.90- 1.20/	60	44
Ph-Hg	10.0	-	55.0 /45.0- 64.5/	-	0.43 /0.35- 0.50/	128	-
Ph-Hg + Se[x]		-	27.5 /26.5- 29.0/	-	0.60 /0,47- 0.65/	46	-
$HgCl_2$	10.0	-	11.3 /8.4 - 12.4/	-	0.10 /0.07- 0.14/	113	-
$HgCl_2$ + Se[x]		-	5.5 /4.0 - 6.8 /	-	0.12 /0.08- 0.16/	46	-

- bellow detectability of this method /50 ng Hg/g tissue/
x - cumulative dose of selenium - 8.0 mg Se/kg

by MAGOS and BUTLER /1976/ for methylmercuric chloride. Of interest is the high level of mercury accumulated in the kidneys after exposure to ethylmercuric chloride /142 ug Hg/g/. In liver, regardless of the compound administered, only small percentage of the cumulative dose of mercury was present amountig to 1,2-1,4 % for alkyl mercury compounds and much less for the remaining ones.

Sodium selenite caused a decrease of inorganic mercury in kidneys by 50 percent with the exception of ethylmercuric chloride. Only for the latter compound an elevation of the level of inorganic mercury in liver was stated under the influence of selenium.

Binding of inorganic mercury by metallothionein paralles the levels of this protein, since the latter is induced by mercury /PIOTROWSKI et al. 1974a/. Hence in our experiment the process of binding mercury by metallothionein was examined in an indirect way through the level of metallothionein. Prolonged exposure of rats to mercury compounds used in the experiments stimulated an increase of metallothionein level in rat kidneys in comparison with the control group /Figure 1a/.

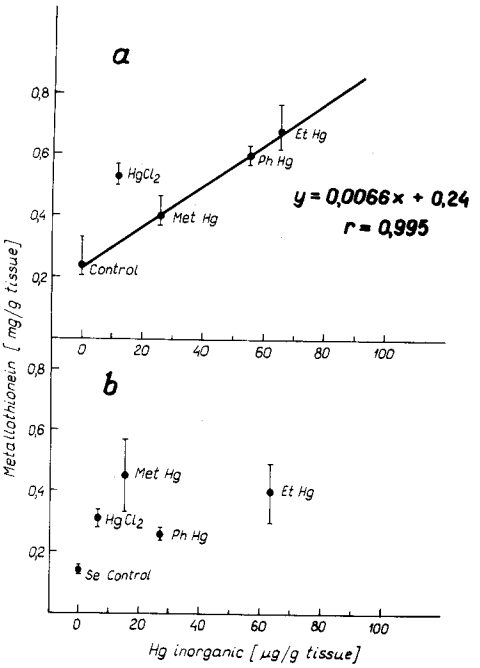

Figure 1. The levels of metallothionein and inorganic mercury in kidney of rats exposed to different mercury compounds without /a/ and with sodium selenite /b/.

This process is probably caused by inorganic mercury which constituted about 40 percent of mercury accumulated in the kidneys for methylmercurycyanguanidine and ethylmercuric chloride as well. In the liver of rats exposed the level of metallothionein did not deviate from the physiological level and amounted in all cases to 0,06-0,12 mg/g on the average. There is a correlation between the amount if inorganic mercury accumulated in the kidneys and the level of metallothionein /Figure 1a/. It is highly significant for all organic mercury compounds /r=0,995/. The group which received mercuric chloride departed from this dependence. The latter deviation may be conditioned upon differences in subcellullar distribution of inorganic mercury depending on whether inorganic mercury entered the cell directly or was formed inside of the cell through the biodegradation of organic mercury compounds. The highest concentration of inorganic mercury followed the supply of ethylmercuric chloride /65 ug Hg/g/ and it was accompanied by the highest metallothionein level /0,68 mg/g/.

Sodium selenite /Figure 1b/ supplied in the absence of mercury compounds caused a decrease of the level of metallothionein in kidneys from 0,25 down to 0,14 mg/g, but in the presence of mercuric chloride and phenylmercuric chloride the content of this protein returned to physiological level.

In rats exposed to phenylmercuric chloride and mercuric chloride sodium selenite caused a decrease of the level of inorganic mercury in kidneys which was accompanied by a drop in the level of metallothionein.

No distinct diversion of mercury from the kidneys to the liver was noted as observed in our previous experiments where inorganic mercury was administered intravenously /KOMSTA-SZUMSKA and CHMIELNICKA in press/. The latter phenomenon has not been found in case of alkylmercury compounds either, which is in agreement with the data of OHI et al. /1975/ as well as of POTTER and MATRONE /1974/ who performed investigations in conditions similar to ours.

It is possible to infer that the effect of mercury and selenium interaction in vivo depends not only on the molar ratio of both elements supplied /GROTH et al. 1976/ but also on the way of administration. Howewer regardless of the way in which it is administered sodium selenite eliminates the stimulation of the biosynthesis of metallothionein caused by inorganic mercury in rat kidneys.

SUMMARY

Mercuric chloride, phenylmercuric chloride, ethylmercuric chloride /0,23 mg Hg/kg/ and methylmercurycyan guanidine /0,46 mg Hg/kg/ were orally administered to rats every second day for 14 weeks. The same doses of the above mentioned mercury compounds were administered alternately with sodium selenite /0,18 mg Se/kg/ to parallel groups of rats at the same time. The level of total and inorganic mercury and of metallothionein was determined. All mercury compounds

increased the level of metallothionein in rat kidneys. In rats which received only selenium the level of metallothionein was twice lower in the kidneys in relation to the physiological level of this protein. Selenium eliminated the stimulation of biosynthesis of metallothionein by mercury.

ACKNOWLEDGMENTS

We are deeply indebted to Prof.dr J.K.PIOTROWSKI for the critical discussions.

REFERENCES

BALCERSKA, I., BRZEŹNICKA, E.A., and CHMIELNICKA, J.: Bromat.Chem.Toksykol. 10 ,103/1977/.

CHEN, R.W., GANTHER, H.E., and HOEKSTRA, W.G.; Biochem. Biophys.Res. Commun. 51, 383 /1973/.

CHEN, R.W., LACY, V.L., and WHANGER, P.D.: Res. Comm. Chem. Path. and Pharm. 12 , 297 /1975/.

CHERIAN, M.G., and CLARKSON, T.W.: Chem.-Biol. Interactions 12 , 109 /1976/.

CLARKSON, T.W.: Annual Review Pharm. 12 , 375 /1972/.

ELLIS, R.W., and FANG, S.C.: Toxicol. Appl. Pharmacol. 20, 14 /1971/.

FANG, S.C.: p. 277. In MILLER, M.W., and CLARKSON, T.W. /ed/. Mercury, Mercurials and Mercaptans. C.C. Thomas, Springfield, Illinois /1973/.

FRIBERG, L., and VOSTAL, J.: Mercury in the Environment. C.R.C. Press, Cleveland, Ohio /1972/.

GANTHER, H.E., GOUDIE, C., SUNDE, M.L., KOPECKY, M.J., WAGNER, P., OH, S.H., and HOEKSTRA, W.G.: Science 175 , 1122/1972/.

GANTHER, H.E., and SUNDE, M.L.: J.Food Sci. 39 , 1 / 1974/.

GROTH, D.H., VIGNATI, L., LOWRY, L., MACKAY, G., and STOKINGER, H.E.: Trace Subst. Environ. Health 6 , 187 /1973/.

GROTH, D.H., LOYD, S., and MACKAY, G.: p. 527. In NORDBERG, G.F., /ed/. Effect and Dose-Response Relationships of Toxic Metals. Elsevier, Amsterdam /1976/.

KOMSTA-SZUMSKA, E., CHMIELNICKA, J., and PIOTROWSKI, J.K.: Biochem.Pharmacol. 25 , 2539 /1976/.

KOMSTA-SZUMSKA, E., and CHMIELNICKA, J.: /in press/.

MACGREGOR, J.T., and CLARKSON, T.W.: p. 463. In FRIEDMAN, M. /ed/. Protein-Metal Interactions. Plenum Press, New York /1974/.

MAGOS, L.: Analyst 96 , 847 /1971/.

MAGOS, L., and BUTLER, W.H.: Arch. Toxicol. 35 , 25 /1976/.

OHI, G., NISHIGAKI, S., SEKI, H., TAMURA, Y., MAKI, T., MAEDA, H., OCHAI, S., YAMADA, H., SHIMAMURA, Y., and YAGYU, H.: Toxicol. Appl. Pharmacol. 32 , 527 /1975/.

PARIZEK, J., and OSTADALOVA, I.: Experientia 23 , 142 /1967/.

PIOTROWSKI, J.K., and BOLANOWSKA, W.: Med. Pracy 21 , 338 /1970/.

PIOTROWSKI, J.K., TROJANOWSKA, B., WIŚNIEWSKA-KNYPL, J.M., and BOLANOWSKA, W.: p. 247, In MILLER, M.W., and CLARKSON, T.W. /ed/. Mercury, Mercurials and Mercaptans. C.C.Thomas, Springfield, Illinois /1973a/.

PIOTROWSKI, J.K., BOLANOWSKA, W., and SAPOTA, A.: Acta Bioch. Pol. 20 , 207 /1973b/.

PIOTROWSKI, J.K., TROJANOWSKA, B., WIŚNIEWSKA-KNYPL, J.M., and BOLANOWSKA, W.: Toxicol. Appl. Pharmacol. 27 , 11 /1974a/.

PIOTROWSKI, J.K., TROJANOWSKA, B., and SAPOTA, A.: Arch. Toxicol. 32 , 351 /1974b/.

PIOTROWSKI, J.K., and SZYMAŃSKA, J.A.: Toxic. Environ.Hlth. 1 , 991 /1976/.

POTTER, S., and MATRONE, G.: J. Nutr. 104 , 638 /1974/.

PULIDO, P., KAGI, J.H.R., and VALLEE, B.L.: Biochemistry 5 , 1768 /1966/.

SAPOTA, A., PIOTROWSKI, J.K., and BARAŃSKI, B.: Med.Pracy 25 , 129 /1974/.

VIŠNIEWSKA-KNYPL, J.M., TROJANOWSKA, B., PIOTROWSKI, J.K., and JAKUBOWSKI, M.: Toxicol. Appl. Pharmacol. 16 , 754 /197(

ŻELAZOWSKI, A.J., and PIOTROWSKI, J.K.: /in press/.

ŻELAZOWSKI, A.J., PIOTROWSKI, J.K., MOGILNICKA, E.M., SZYMAŃSKA, J.A., and KASZPER, W.B.: /in press/.