INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 17, no. 2 (S), 123-128 (2004)

REVERSIBILITY OF THYMULIN PRODUCTION IMPAIRMENT BY L-ARGININE SUPPLEMENTATION IN MICE EXPOSED TO INORGANIC MERCURY

L. SANTARELLI[1], M. VALENTINO[1], M. BRACCI[1], V. RAPISARDA[1], L. SOLEO[2] and E. MOCCHEGIANI[3]

[1]Institute of Occupational Medicine, University of Ancona, Polo Didattico Via Tronto 10, Ospedale di Torrette, 60020 Ancona, Italy; [2]Department of Internal Medicine and Public Medicine, Section of Occupational Medicine, University of Bari. Italy; [3]Immunology Center, Gerontology Research Department Italian National Research Center on Aging (INRCA), Via Birarelli 8, Ancona, Italy

Immunotoxicological effects of mercury on peripheral immune system are known. We had previously in vitro found that mercuric chloride inhibits thymulin production in mouse thymus cultures at concentrations as low as 10^{-8} M. In this study, thymus efficiency, assessed as production of active and total thymulin, was evaluated in vivo using young mice that were injected sc every 3 days for 4 weeks with saline containing mercuric chloride at different concentrations (0 -controls-, 0.001 or 1.0 mg $HgCl_2$/kg body weight). The results show that both the doses are able to cause a significant reduction in active and total thymulin production. Since arginine enhances immune efficiency some of the animals also received a diet supplemented with arginine in order to evaluate a possible role of arginine during mercury intoxication. The data show that arginine has a protective effect on thymic endocrine efficiency. Mice, treated with the lowest dose of mercury and receiving an arginine supplemented diet, produced active and total thymulin like mercury untreated mice. Arginine is an aminoacid which may be found in various amounts in different foods, some foods are particularly rich in arginine i.e. peanuts, stock fish. We suggest that the daily arginine intake may account for an individual susceptibility to the mercury-induced immunological effects which are found in mercury occupationally exposed workers.

The effects of inorganic mercury on the immune system of man and experimental animals have been largely discussed. It is well documented that $HgCl_2$ may compromise the immune response, damaging or stimulating a particular cell line or involving more than one of the cell types responsible for the right functioning of the immune system. Mercury can interfere with both humoral and cell-mediated immunity (1-2) and exposure of animals to mercurial compounds decreases their thymus weight and cell number (3-4). Thymic epithelial cells produce total thymulin and active thymulin, a zinc-dependent nonapeptide, which has immunoregulatory activities (5). Plasma concentration of this active zinc-linked thymulin is strictly related at an optimal peripheral immunological efficiency.

We had previously demonstrated that in vitro addition of very low amounts, 10^{-8}M, of inorganic mercury to thymuses cultures, reduced kinetics of thymulin production at all time intervals considered (1, 2, 4, 5 and 6 hours) as compared to kinetics of thymulin production in thymuses from control mice (6).

Key words: mercury, immunotoxicity, thymulin, arginine diet

Mailing address: Dr. Lory Santarelli
Clinica di Medicina del Lavoro, Università di Ancona
Polo Didattico Torrette, via Tronto 10/A 60020 Ancona, Italy.
Tel 0039 71-2206063, Fax 0039 71-2206062
e-mail: l.santarelli@univpm.it

0394-6320 (2004)

The aim of the present study has been to evaluate if inorganic mercury exposure may affect the in vivo thymulin production. Groups of mice were treated with saline sc injections with different doses of $HgCl_2$ for 4 weeks, before sacrifice, in order to measure plasma thymulin concentrations after the intoxication.

Since it has been proven that arginine enhances immune efficiency and increases thymus weight (7-9), some of the animals also received a diet supplemented with arginine in order to evaluate a possible role of arginine during mercury intoxication.

MATERIALS AND METHODS

Animals

Thirty male Balb/c mice Iffacredo aged 3 months were housed, 2 or 3 animals per metabolic cage, with ad libitum access to normal pellet food and water. Mice elapsed a period of 7 days in the metabolic cages before treatments for adaptation to new condition to exclude stress due troubles. Treatments and euthanasia were in line with the Guidelines of Principles in the Use of Animals in Toxicology (Society of Toxicology, 2002).

Animals were treated according to Hu et al. (10) with some modifications. Briefly, three groups of ten mice received respectively sc injections of 0 - controls, 0.001 or 1.0 mg $HgCl_2$/kg body weight diluted in 0.84% sterile NaCl to a final volume of 100 µl, every 3 days for four weeks. Five mice in each group received a normal diet while the remaining mice received a diet supplemented with arginine in drinking water at a final dose of 1.4×10^{-3} g/mouse/die.

Animals were sacrificed under ether anesthesia after one month. Heparinized blood was collected by cardiac puncture; plasma was obtained by centrifugation and stored at –70°C until use for the determination of thymulin.

Plasma thymulin determination

This bioassay, extensively described elsewhere (11), is based on the ability of thymulin to restore the inhibitory effect of azathioprine on the formation of spleen T cell rosettes from young thymectomized mice. Results are expressed as $\log_{-2}$ of the reciprocal maximal plasma dilution that induces this phenomenon.

Mercury urinary concentrations

Urines from each metabolic cage were collected before and after 7, 15, 28 days from the beginning of mercury treatment. Urines were stored at –20°C until analysis.

Urinary mercury was measured by direct cold vapour atomic absorption spectrometry. (Spectometer model 460 and MHS-1 iride generator, Perkin-Elmer) (12). The laboratory of occupational toxicology, where all mercury measurements were assessed, participates regularly in national and international external quality programs.

Statistical analysis

The difference between the three doses of mercury treatment was analysed with one way ANOVA test. The difference between two mice groups was analysed with unpaired t Student test. Statistical significance difference was considered for $p < 0.05$.

RESULTS

All mice survived until the sacrifice.

Figure 1 shows urinary mercury concentrations in urines collected from the metabolic cages. No significant difference was found between the two cages which housed the five mice treated with the same mercury dose and fed with the same diet.

Mice which received the highest dose of mercury had detectable levels of urinary mercury after 7 days while those treated with the lowest dose of mercury had amounts of mercury in urine similar to those found in untreated mice (Figure 1). There were not differences in mercury excretion related to arginine supplemented diet (data are not shown).

We found that both active and total thymulin are decreased in mercury treated mice (Figure 2); high dose mercury treated mice produced the lowest amounts of thymulim which however were not significantly different from those roduced by the low dose mercury treated mice.

It is known that arginine increases thymus weight and cellularity in normal rats (8) and for this reason some animals received a diet supplemented with arginine. In these mice (Figure 3) thymulin production remained impaired only in the group which had been treated with

Fig. 1. *Mercury urinary concentrations (ng/ml) in mice treated with sinjections of saline containing different doses of mercury.*

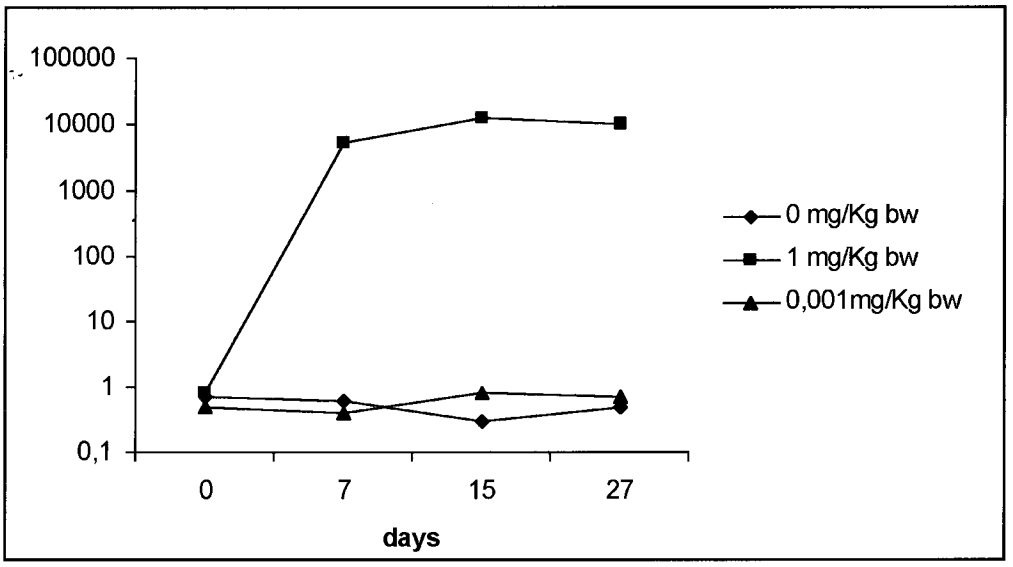
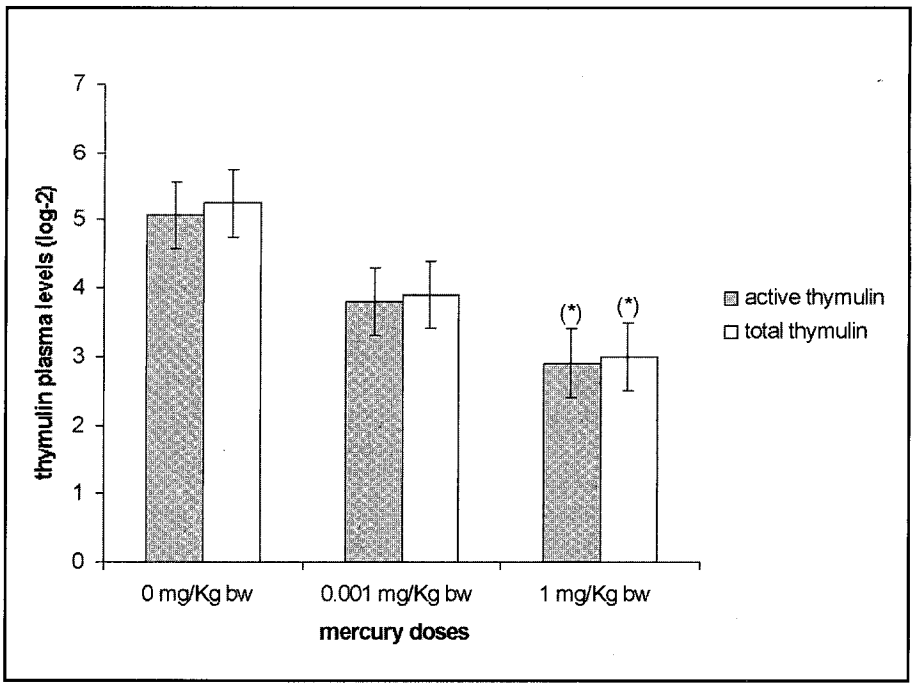


Fig. 2. *Thymulin Plasma levels in control and mercury treated mice fed with a normal diet.*



**p < 0.05 Bonferroni t test: active thymulin, total thymulin compared with group treated with 0 mg/Kg bw*
[Fi=12 t=3.431 active thymulin; F=12 t=3.558 total thymulin]

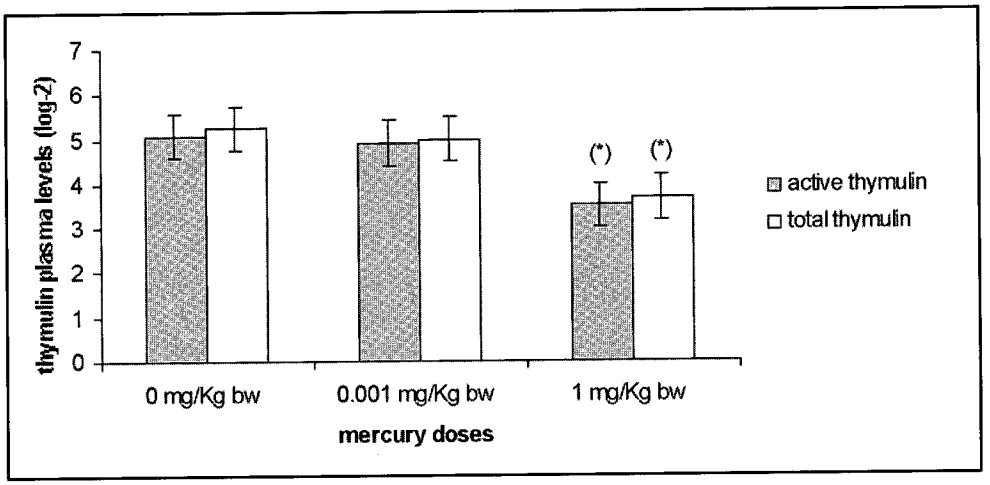


Fig. 3. *Thymulin Plasma levels in control and mercury treated mice fed with an arginine supplemented diet.*
**pc 0.05 Bonferroni test: active thymulin, total thymulin compared with group treated with 0 mg/Kg^{bw}*
[Fi=12 t=2.467 active thymulin; F=12 t=2.482 total thymulin]

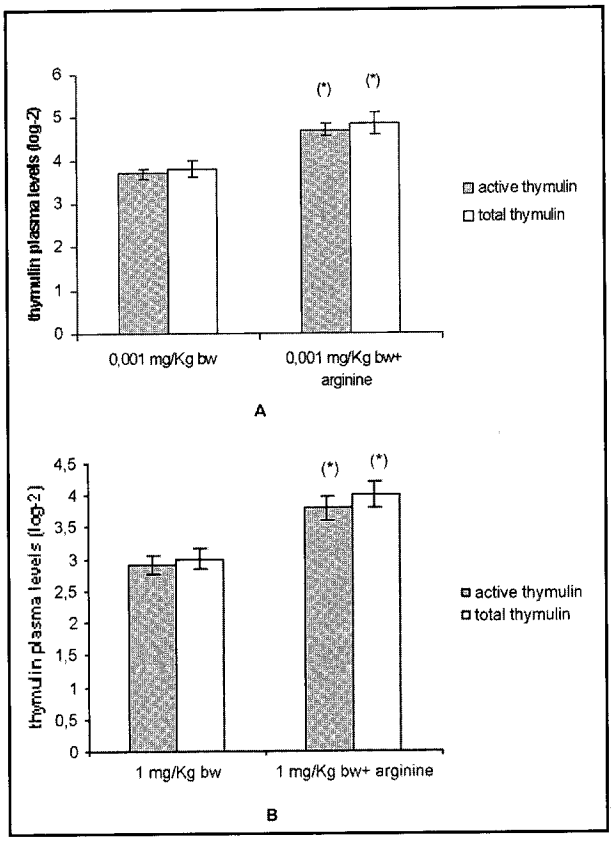


Fig 4. *Thymulin plasma levels in intoxicated mice fed with and without arginine 1.4x 10^{-3}*
**pc 05 t student test: active thymulin levels in treated mice with low mercury dose (A) and high mercury dose (B) compared with groups fed without orginine*

the highest dose of mercury. Arginine was able to restore completely the production of thymulin in mice which had received the lowest dose of mercury.

Significant differences ($p<0.05$) in the production of active and total thymulin were found between mice treated with the same mercury dose but with a different content of arginine in the diet (Figure 4). On the contrary, arginine was not able to increase the production of active and total thymulin in mercury untreated controls which received the arginine supplemented diet (P = N.S.).

DISCUSSION

With this animal model of mercury intoxication immunological effects were found at very low urinary mercury levels. Data show that very low doses of inorganic mercury are able to affect the in vivo thymulin production by thymic epithelial cells. The urinary concentrations which have been found in the mice which had received the lowest dose of mercury are commonly found in mercury occupationally exposed workers (12-13). Total and active thymulin are both inhibited probably because mercury is able to block, as it occurs in vitro in mercury incubated thymuses, the shyntesis

of the hormone at source (6).

It is known that thymulin production inhibition may be responsible of modifications of several immunological parameters (14), some of which have been found to be modified in occupationally exposed workers (1, 12, 15). However in these human studies a clear dose relationship has never been found. In fact the motility of polymorphonuclear leukocytes does not improve after a sensible reduction of workers urinary mercury levels (1). A non dose-response relation was found between urinary mercury and TNFα concentrations which are impaired in mercury exposed workers (12). For this reason Moszcynski et al. (15) have suggested that the mercury duration exposure, and not the mercury dose, is related to the modification of immunological parameters.

On the contrary effects of mercury on neurological functions, which are not dependent on arginine, seem to be dose related. The increase of the level of occupational exposure to mercury is related with an increase of the neuro behavioural toxicological effects which are found in workers (13, 16).

We suggest that the role of arginine may be important in contrasting mercury immunological effects which are found in occupationally exposed workers. Under analogous experimental procedures we found that old mice, which have a thymulin reduced production due to aging, treated with arginine exhibit thymus regrowth with restoration of thymic endocrine activity to the levels of young mice (17). The protective effect of arginine may be explained by the important role which arginine plays in many physiologic and biologic processes, beyonds its role as a protein-incorporated amino acid.

Dietary supplementation can regulate endocrine activity and potentiate immune activity (9). It has been calculated that the arginine daily intake in a man of 70 Kg is about 3.2 g. Some uncommonly used foods-i.e. peanuts, seeds, nuts, soia, stock fish - have a very high content of arginine, much more than 3 g/100g food- while several commonly used foods-i.e. milk, egg, pasta, bread, vegetables, fruits- have a very low arginine content, much less than 1g/100g. The lack of a dose-related immunological effect in workers exposed to mercury might be due to the inteference of arginine diet intake which can be very different in individuals.

Our findings point out the attention on the possibility that the diet may modulate immunological effects due to mercury occupational exposure; however other studies are necessary to prove this hypothesis.

REFERENCES

1. **Baginski B.** 1980. Effect of mercuric chloride on microbicidal activities of human polymorphonuclear leukocytes. *Toxicology 50:247.*
2. **Moszczynski P.** 1997. Mercury compounds and the immune system: a review. *Int. J. Occup. Med. Environ. Health 10:247.*
3. **Hirokawa K and Y. Hayashi.** 1980. Acute methyl mercury intoxication in mice effect on the immune system. *Acta Pathol. Jpn. 30:23.*
4. **Ilback N.G.** 1991. Effects of methyl mercury exposure on spleen and blood natural killer (NK) cell activity in the mouse. *Toxicol. 67:117.*
5. **Dardenne M. and W. Savino.** 1994. Control of thymus physiology by peptidic hormones and neuropeptides. *Immunol. Today 15:518.*
6. **Valentino M., L. Santarelli, E. Pieragostini, L. Soleo, E. Mocchegiani.** 2001. In vitro inhibition of thymulin production in mercury-exposed thymus of young mice. *Sci. Total Environ. 270:109.*
7. **Barbul A., H.L. Wasserkrug, E. Seifter, G. Rettura, S.M. Levenson and G. Efron.** 1980. Immunostimulatory effects of arginine in normal and injured rats. *J. Surg. Res. 29:228.*
8. **Daly J.M., J. Reynolds, R.K. Sigal, J. Shou and M.D. Liberman.** 1990. Effect of dietary protein and amino acids on immune function. *Crit. Care Med. 18(S): 86.*
9. **Efron D. and A. Barbul.** 2000. Role of arginine in immunonutrition. *J. Gastroenterol. 35:20.*
10. **Hu H., G. Moller and M. Abedi-Valugerdi.** 1999. Mechanism of mercury-induced autoimmunity: both T helper 1- and T helper 2-type responses are involved. *Immunology 96:348.*
11. **Mocchegiani E., L. Amadio and N. Fabris.** 1990. Neuroendocrine-thymus interactions. I. In vitro modulation of thymic factor secretion by thyroid hormones. *J. Endocrinol. Invest. 13:139.*
12. **Soleo L., A. Vacca, L. Vimercati, S. Bruno, M. Di Loreto, C. Zocchetti, R. Di Stefano, G. Candilio, G.**

Lasorsa, G. Franco and V. Foa. 1997. Minimal immunological effects on workers with prolonged low exposure to inorganic mercury. *Occup. Environ. Med.54:437.*

13. **Echeverria D., N.J. Heyer, M.D. Martin, C.A. Naleway, J.S. Woods and A.C. Bittner.** 1995. Behavioral effects of low-level exposure to elemental Hg among dentists. *Neurotoxicol. Teratol.17:161.*
14. **Dardenne M., J.M. Pleau, W. Savino, A.S. Prasad and J.F. Bach.** 1993. Biochemical and biological aspects of the interaction between thymulin and zinc. *Prog. Clin. Biol. Res. 380:23.*
15. **Moszczynski P., J. Rutowski, S. Slowinski and S. Bem.** 1998. Immunological effects of occupational exposure to metallic mercury in the population of T-cells and NK-cells. *Analyst 123:99.*
16. **Ngim C.H., S.C. Foo, K.W. Boey and J. Jeyaratnam.** 1992. Chronic neurobehavioural effects of elemental mercury in dentists. *Br. J. Ind. Med. 49:782.*
17. **Fabris N. and E. Mocchegiani.** 1992. Arginine-containing compounds and thymic endocrine activity. *Thymus 19:S21.*