

Thimerosal positivities: the rôle of SH groups and divalent ions

B. SANTUCCI, C. CANNISTRACI, A. CRISTAUDO, E. CAMERA AND M. PICARDO

San Gallicano Dermatological Institute, Via San Gallicano 25a, 00153 Rome, Italy

For a better understanding of the mechanistic details of the interactions of organomercury compounds inside the skin, 32 subjects who previously had given positive patch-test reactions to thimerosal (TH) and negative reactions to thiosalicylic acid, were divided into 2 groups. 16 subjects were repatch tested to ethylmercury chloride (EtHgCl) and to solutions containing EtHgCl mixed with L-cysteine and glutathione, respectively. The remaining 16 were repatch tested to EtHgCl and to solutions containing EtHgCl mixed with chlorides of Zn, Mg, and Mn, respectively. The results showed that whilst L-cysteine, glutathione and ZnCl₂ were able to abolish or to reduce the positive reactions to EtHgCl, chlorides of Mg and Mn were unable to do so. Patch tests revealed that in causing positive reactions to TH, EtHg probably interacted with thiol groups and with Zn ions, as in biological systems when causing toxic effects. The limited number of TH reactions in the general population, the constant presence of concomitant positive reactions to EtHgCl and MeHgCl, and the lack of cross-reactivity with other organic or inorganic mercury compounds, lead us to speculate that reactions to TH are due to organomercury alkyl compounds, and that positive subjects have a constitutively reduced capability to metabolize organomercury compounds, rather than to reveal previous exposure.

Key words: thimerosal; organomercury alkyl compounds; ethylmercury chloride; SH-groups; divalent ions; chemical basis. © Munksgaard, 1998.

Accepted for publication 6 May 1998

In a previous paper (1), we showed that a group of subjects reacting to only thimerosal (TH) when patch tested to the same amount of both ethylmercury chloride (EtHgCl) and methylmercury chloride (MeHgCl) were consistently able to give concomitant positive reactions. Since we previously showed that in weak acidic solutions, TH easily released EtHg moieties (2), we suggested that the reactions to TH were due to organomercury alkyl compounds. Mercury is not an essential metal and there is not a biological requirement. Consequently, the application of substantial amounts of organoalkylmercury compounds to the back might present a hazard. The reason for cutaneous reactions to TH is not clear (3) and so there is a need for a better understanding of the chemical species and mechanisms which initiate cell damage, leading to the harmful effects.

The chemistry of degradation of TH in biological systems is complex. Recently, it has been shown that TH in solution degrades in 2 stages (4). The 1st is the dissociation of TH. The 2nd, a redox reaction involving the oxidation of thiosalicylic acid to produce dithiosalicylic acid, with a corresponding reduction of ethylmercury chloride to

metallic mercury. This 2nd step determines the degree of degradation. Organomercury alkyl compounds are resistant to environmental degradation and, due to their lipophilicity are significantly absorbed on skin contact (5) and will easily cross cellular membranes (6, 7). Once they penetrate into the skin, they may act as xenobiotic compounds undergoing processes of cellular metabolism and removal. In biological systems, organomercury alkyl compounds are partially converted into inorganic mercury (6, 7). The microsome fraction in rat liver (8) and the intraphagosomes of phagocytic cells, such as polymorphonuclear leukocytes, macrophages, monocytes and eosinophils (collected from humans, rats, guinea pigs, and rabbits), are reported to degrade MeHg and EtHg into inorganic mercury (9).

Metals have many complex actions on different cellular structures which are poorly understood (10). Like other transition metals, mercury and its specific chemical forms exert their harmful effects by binding to the essential parts of molecules, altering their normal cellular function. In addition, they may interact with other ions present in solution. In living systems, mercury and its compounds

predominantly form complexes with sulfhydryl (SH) groups (6, 7). Because of the abundance and the different affinity of the individual thiol groups, mercury probably distributes itself irregularly among the various molecules to which it binds. Thiol groups may act as endogenous chelating substances and play a defensive rôle, thereby limiting the access of the metal to the cells and its toxic effects (11).

The aim of the present paper was to ascertain whether either some SH-containing substances or divalent ions influenced the cutaneous reactions to EtHgCl contained in TH. Therefore, we patch tested our TH-positive patients to solutions containing EtHgCl mixed with L-cysteine, glutathione, and with chlorides of divalent ions such as Mg, Mn, and Zn, respectively.

Materials and Methods

32 subjects who previously gave positive patch test reactions to sodium ethylmercurythiosalicylate 0.1% pet. (Hermal) and negative reactions to 8 μ l of thiosalicylic acid 0.05% aq./eth. (50:50), gave their consent to this study. The patients were di-



Fig 1. Positive patch test reactions to EtHgCl were inhibited by mixed solutions containing L-cysteine and glutathione.

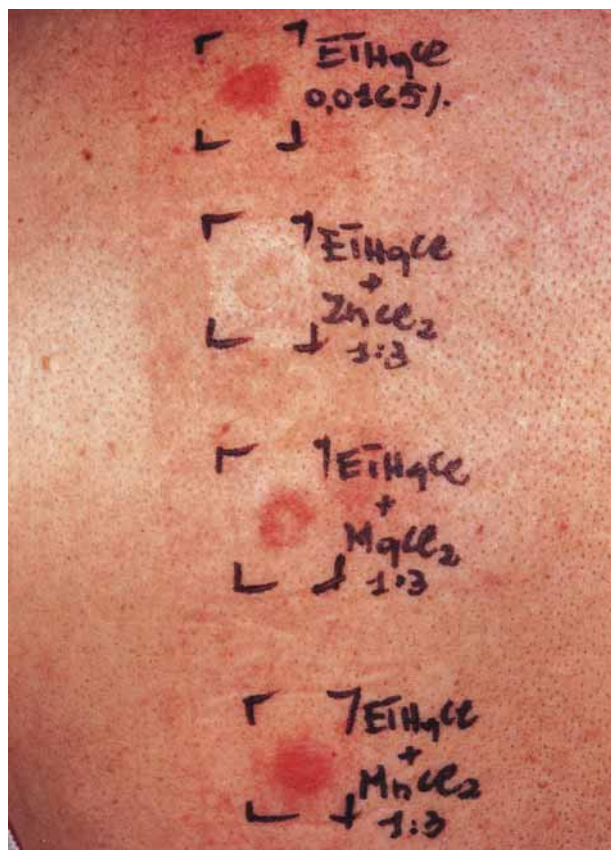


Fig 2. Positive patch test reactions to EtHgCl were inhibited only by mixed solutions containing ZnCl₂.

vided in 2 groups and repatch tested in accordance with the following plan:

Group 1: 16 subjects were tested with 8 μ l of EtHgCl 0.0165% eth. and to 8 μ l of 2 mixed solutions containing EtHgCl 0.0165%+L-cysteine 0.058% (molar ratio 1:3) and EtHgCl 0.0165%+glutathione 0.114% (molar ratio 1:3), respectively.

Group 2: The remaining 16 subjects were tested to 8 μ l of EtHgCl 0.0165% eth. and to 8 μ l of 3 mixed solutions containing respectively EtHgCl 0.0165%+ZnCl₂ 0.075% (1:3 w/w), EtHgCl 0.0165%+MgCl₂ 0.075% (1:3 w/w), and EtHgCl 0.0165%+MnCl₂ 0.075% (1:3 w/w). The ratio has been reported as 1:3 with respect to TH, because we have considered the % of EtHgCl contained in the TH molecule.

Patch tests were carried out using Al-test on Leucotest for all the solutions. The panel containing the Al-test was arranged in a vertical row and applied to the back. The distance between the patches was 3 cm. Patch tests were read in all subjects at 2 and 4 days. Since there were no significant variations in the reactions, the results were scored at D2 in accordance with ICDRG guidelines. Visual

scoring was used in comparing the differences between the positive responses found to EtHgCl and those to the mixed solutions.

The substances used were: sodium ethylmercurithiosalicylate 0.1% pet. (Hermal), thiosalicylic acid (Aldrich, purity 97%, M.W. 154.19), EtHgCl (Alfa, purity 97.7%, M.W. 265.13), L-cysteine hydrochloride anhydrous (Fluka, purity 99.5, M.W. 157.62), glutathione reduced (Merck, purity 98%, M.W. 307.33), $\text{MgCl}_2 \times 6\text{H}_2\text{O}$ (Merck, purity 99.5%, M.W. 120.4), $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ (Fluka, purity, 99%, M.W. 197.91), and ZnCl_2 (Fluka, purity 98%, M.W. 136.29).

Results

Group 1: All subjects gave a positive reaction to EtHgCl. The mixed solutions containing L-cysteine abolished the EtHgCl reactions in 11 subjects and reduced the reactions in the remaining 5 subjects. The mixed solutions containing glutathione abolished the EtHgCl reactions in 14 subjects (Fig. 1) and inhibited the reactions in the remaining 2 subjects.

Group 2: All subjects gave a positive reaction to EtHgCl. The mixed solutions containing ZnCl_2 abolished the positive reactions to EtHgCl in 14 subjects (Fig. 2) and reduced the reactions in the remaining 2 subjects. The mixed solutions containing MgCl_2 reduced the reactions in 2 subjects, and had no effect in the remaining 14 subjects. The mixed solutions containing MnCl_2 reduced the reaction in 1 subject, and had no effect in the remaining 15 subjects.

Discussion

Although patch tests are thought to represent a reliable method for identifying substances causing allergic or irritant contact dermatitis, in our opinion, when correctly performed and interpreted, they may give at least an idea about the mechanisms underlying the positive reactions. In previous work (12, 13), the patch test procedure was successfully adopted in identifying ionic interactions between nickel and other divalent ions, and showed that different metals were able to exert antagonistic or synergistic rôles in nickel reactions. It should be stressed that the observed findings revealed that the same factors responsible for certain nickel effects in experimental systems were also operative in human skin reactions. In TH-positive subjects, patch test results do not account for the fact that the skin may be so permeable to the applied EtHgCl that it may simply accumulate up to a level high enough to cause skin damage.

The fact that positive reactions to EtHgCl were inhibited or reduced by the simultaneous administration of EtHgCl mixed with cysteine or glutathi-

one, confirms that these substances act as chelating agents in aqueous solutions, modifying the reactivity of EtHgCl. We have previously shown (2) that in vitro cysteine and glutathione increased the cleavage of TH by binding the EtHg moiety and that the reaction is dependent on the concentration of SH-containing substances. Since L-cysteine and glutathione also play a rôle as endogenous chelating agents in the uptake and the extrusion of mercury from the cells (14), the inhibiting effect may also suggest that these subjects have an impaired ability of their cutaneous SH ligands to bind to the penetrating organomercury alkyl compound. The consequence may be the accumulation of the compound at sites where it exerts toxic effects and/or from which it is difficult to remove.

There are individual factors that affect deposition, transport, and excretion of organomercury alkyl compounds and, consequently, their toxicity (3, 4). They are mostly related to the specific chemical form, to the number, affinity and accessibility of thiol groups, and to genetic differences in thiol metabolism (15). Moreover, the behaviour of our subjects may be consistent with some of the reported experimental findings and could confirm the opinion expressed by Möller that "*the high incidence of the positive cutaneous tests to merthiolate in healthy subjects might be explained as being an inborn error of metabolism, rather than an allergic reaction*" (16).

New questions arise as to the nature of the effects exerted by the mixed solutions containing ZnCl_2 . At the concentrations tested, only ZnCl_2 was among the mixed salts of divalent metals, able to abolish (or reduce) positive reactions to EtHgCl. Even if we cannot exclude that ZnCl_2 interacted in vitro with EtHgCl, making it less harmful, we can speculate that, as shown in other biological systems, Zn exerts an in vivo action exploiting at least 3 different possibilities.

(i) Zn may exert an antinflammatory effect, reducing some keratinocyte activation markers (ICAM-1 and $\text{TNF-}\alpha$) as reported by Gueniche et al. (17).

(ii) Since there is a loss of power of the physiologically active chelants, the removal of EtHgCl must follow different pathways involving the binding to thiol groups with differing affinities present in the biomolecules of the skin. An important mechanism which regulates the disposition of mercury and protects against its toxic effects, may be represented by the level of metallothioneins, a group of low molecular mass proteins with a high cysteine content. These compounds exist in various tissues (18) under physiological conditions, and may be induced further (either by inorganic mercury or by cadmium and zinc (19)), demonstrating

a detoxifying rôle for the heavy metals (18–20). It follows therefore, that the simultaneous administration of higher amounts of Zn may reduce the harmful effects of EtHgCl, inducing specific genes which enhance the levels of metallothionein-containing cysteine groups, thereby favouring the binding of alkylmercury compounds.

(iii) Mercury and its compounds exert their toxic effects by substituting Zn in some biomolecules in which it is weakly bound (21). Zn is an essential component of many proteins, enzymes, transcription factors and hormone receptors (22). Therefore, the simultaneous administration of higher doses of Zn may counteract the action of mercury.

In conclusion, patch test results show that both thiol groups and Zn ions may be involved in the cutaneous reactions to EtHgCl. Patch test results do not allow clarification of all the cellular mechanical interactions which lead to toxic effects, and so more work needs to be done in order to understand these points. However, the limited number of positive reactions in the general population, their lack of clinical relevance, the experimentally moderate sensitizing capability of thimerosal, and the fact that the positive subjects react consistently to methylmercury chloride (and inconsistently to organic and inorganic form of mercury (2)), lead us to speculate that the reactions are caused predominantly by organomercury alkyl compounds.

The patch test procedure adopted shows that once they have penetrated into the skin, organomercury alkyl compounds exert toxic effects, probably according to the pathways utilized in other biological systems (7, 8). Furthermore, the positive reactions may also reflect a constitutive inability to metabolize organomercury alkyl compounds rather than previous exposure.

Acknowledgements

The study was financially supported by Ministero Sanità, Progetto 95/01/G/49.

References

1. Santucci B, Cannistraci C, Camera E, Cristaudo A, Picardo M. Thimerosal positivities: the rôle of organomercury alkyl compounds. *Contact Dermatitis* 1998; 38: 325–328.
2. Camera E, Cannistraci C, Cristaudo A, Santucci B, Picardo M. Studies on the mechanism of thimerosal sensitivity. *Int J of Immunopathology and Pharmacol* 1997; 10: 49–51.
3. Möller H. All these positive tests to Thimerosal. *Contact Dermatitis* 1994; 31: 209–213.
4. Tleugabulova D, Gonzalez Perez I. Reversed-phase high-performance liquid chromatographic study of thimerosal stability in Cuban recombinant hepatitis B vaccine. *J of Chromatography* 1996; 729: 219–227.
5. Wahlberg J E. Percutaneous toxicity of methyl compounds. A comparative investigation in guinea pigs. *Arch Environ Health* 1965; 11: 201–204.
6. Suzuki T, Imura N, Clarkson T W. Overview. In: Suzuki T, Imura N, Clarkson T W (eds). *Advances in mercury toxicology*. New York: Plenum Press, 1991: 439–457.
7. Miura K, Naganuma A, Himeno S, Imura N. Mercury toxicity. In: Goyer A, Cherian G (eds). *Toxicology of metals: biochemical aspects*. Berlin-Heidelberg: Springer-Verlag, 1995: 163–188.
8. Suda I, Hyrayama K. Degradation of methyl and ethylmercury into inorganic mercury by hydroxyl radical produced from a rat liver microsomes. *Arch Toxicol* 1992; 66: 398–402.
9. Suda I, Totoki S, Uchida T, Takahashi H. Degradation of methyl and ethylmercury into inorganic mercury by various phagocytic cells. *Arch Toxicol* 1992; 66: 40–44.
10. Dawson D C, Ballatori N. Membrane transporters as sites of action and route of entry for toxic metals. In: Goyer A, Cherian G (eds). *Toxicology of metals: biochemical aspects*. Berlin-Heidelberg: Springer-Verlag, 1995: 53–76.
11. Jones M M. Chemistry of chelation: Chelating agent antagonists for toxic metals. In: Goyer A, Cherian G (eds). *Toxicology of metals: biochemical aspects*. Berlin Heidelberg: Springer-Verlag, 1995: 279–304.
12. Santucci B, Cannistraci C, Cristaudo A, Picardo M. Interactions of sulfates of divalent metals in nickel sulfate sensitive patients. *Exp Dermatol* 1996; 5: 79–84.
13. Santucci B, Cannistraci C, Cristaudo A, Picardo M. Nickel chloride/chlorides of divalent metals interactions in nickel sensitive subjects. *Exp Dermatol* 1996; 5: 254–259.
14. Naganuma A, Tanaka T, Urano T, Imura N. Role of glutathione in mercury disposition. In: Suzuki T, Imura N, Clarkson T W (eds). *Advances in mercury toxicology*. New York: Plenum Press, 1991: 111–119.
15. Doi R. Individual difference of methylmercury metabolism in animals and its significance in methylmercury toxicity. In: Suzuki T, Imura N, Clarkson T W (eds). *Advances in mercury toxicology*. New York: Plenum Press, 1991: 11–119.
16. Möller H. Merthiolate allergy: a nationwide iatrogenic sensitization. *Acta Dermatovener* 1977; 57: 509–51.
17. Gueniche A, Viac J, Lizard G, Charveron M, Schmitt D. Protective effect of Zinc on keratinocyte activation markers induced by interferon or nickel. *Acta Dermato-venereologica* 1995; 75: 19–23.
18. Cherian M G. Methallothionein and its interaction with metals. In: Goyer A, Cherian G (eds). *Toxicology of metals: biochemical aspects*. Berlin-Heidelberg: Springer-Verlag, 1995: 121–137.
19. Tohyama C, Ghaffar A, Nakano A, Nishimura N, Nishimura H. Immunohistochemical localization of methallothionein in organs of rats treated with either cadmium, inorganic or organic mercurials. In: Suzuki T, Imura N, Clarkson T W (eds). *Advances in mercury toxicology*. New York: Plenum, 1991: 155–165.
20. Foulkes R C. Metallothionein and glutathione as determinants of cellular retention and extrusion of cadmium and mercury. *Life Sciences* 1993; 52: 1617–1620.
21. Yamane Y, Fukino H, Imagawa M. Suppressive effect of Zn on the toxicity of mercury. *Chem Pharm Bull* 1977; 25: 1509–1518.
22. Vallee B L, Auld D S. New perspectives on zinc biochemistry: co-catalytic sites in multi-zinc enzymes. *Biochemistry* 1993; 32: 6493–6500.

Address:

Baldassarre Santucci
S. Gallicano Dermatological Institute
Via S. Gallicano 25 A
00153 Rome
Italy