Influences of methyl-, phenyl-, ethylmercury and mercury chloride on lymphocyte proliferation and cytokine expression in harbour seals

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Introduction

Marine mammals as top predators take Hg by consumption of contaminated fish and are endangered by Hg influences on health. Although methyl-Hg is the predominant form of organic Hg found in the environment, all three forms (methyl-, ethyl-, phenyl-Hg) have been produced as industrial compounds, primarily as biocides, and an intake in the marine ecosystem could not be ruled out. Both organic and inorganic Hg compounds have been demonstrated to exert immunotoxic properties.

Animals and methods

• Results of lymphocyte transformation tests (LTT) obtained in previous studies on harbor seals (Kakuschke et al., 2005, 2006a, 2006b) were analyzed in respect to different mercury compounds and concentrations.
• Data from 21 adult and juvenile harbour seals caught along the Wadden Sea and from animals of the Seal Station Friedrichskoog, Germany, were used.
• The animals were grouped in juveniles (animals 1-4 months old) and adults (perennial seals).

Brief description of LTTs:
• The lymphocytes were cultured (Fig. 1) with methyl- (Me-Hg), ethyl- (Et-Hg) and phenymercury (Ph-Hg) as well as mercurychloride (HgCl).
• The cells were cultured without and with Hg-compounds in two concentrations: 0.25 µg/mL and 0.5 µg/mL.
• After 5 days, the cells were incubated with methyl-3H-thymidine and the radioactivity measured in a scintillation counter.
• The stimulation index was calculated as below:
  stimulation index (SI) = Proliferation of Hg-incubated cells - Proliferation of non-incubated cells
SI<0.1 is regarded as a strong immunosuppressive or cytotoxic influence.

In a preliminary study mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), interleukin-2 (IL-2), -4 (IL-4) and -6 (IL-6) of Me-Hg-incubated lymphocytes of two seals of the Seal Station Friedrichskoog was analyzed using RT-qPCR.
• Following primers were used:
  GAPDH s: GGGGCCATCACAGCTGCTTCT
  GAPDH as: GACAAAGGTGCTGGTGAGTG
  IL-2 s: CACTGAACCACACATCTCC
  IL-2 as: CTTGGGATGCGCTGTG
  IL-4 s: CGCCACAATGTTCTGCT
  IL-4 as: CTTCCCAACTATTECAACT
  IL-6 s: CAGAAGAGGTCTGCTGTG
  IL-6 as: GGACACACTATTGACAG
• The cytokine production is measured as number of copies and finally calculated as follows:
  IL-2: 1E+02
  IL-4: 1E+03
  IL-6: 1E+05

Results

• In juvenile seals, a significantly decreased lymphocyte proliferation was found after Me-Hg- (for both concentrations) and Et-Hg-incubation with the higher concentration (0.5 µg/mL), whereas incubation with HgCl did not reduce significant lymphocyte proliferation (Fig. 2).
• In adult seals, mercury compounds tested did not significantly reduce the lymphocyte proliferation.

Fig. 2: Proliferation of seal lymphocytes after incubation with 0.25 µg/mL and 0.5 µg/mL of different Hg-compounds.

• Lymphocytes incubated with 0.25 µg/mL MeHg showed a reduced IL-2 expression, but no changes for the IL-4 and IL-6 expression (Fig. 3). The higher concentration of Me-Hg induced a reduction of all three tested cytokines IL-2, -4, and -6.

Fig. 3: Cytokine mRNA expression of seal lymphocytes after incubation with 0.25 µg/mL and 0.5 µg/mL Me-Hg (• with Me-Hg) and without Me-Hg (○ without Me-Hg).

Conclusion

This study showed an age-dependent immunosuppressive influence of mercury compounds. The cellular immunity of young animals in particular appears to be susceptible to the immunotoxic effect of mercury. Furthermore, the chemical form of mercury was important in respect to immunotoxicity. All three organic Hg compounds (0.5 µg/mL) seem to be more immunotoxic than inorganic compounds and between the three organic Hg compounds tested, Me-Hg showed the strongest effect.

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