THE EFFECT OF THIOMERSAL ON IN VITRO SWINE LYMPHOCYTE MITOGENESIS. A NOTE.

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Abstract: Thiomersal (Th) was added ab initio in 72 h blood cultures, from 3 piglets, in 1640 RPM I medium supplemented with 10% fetal bovine serum and gentamicin (50 µl/ml). Lymphocyte mitogenesis was induced with phytohaemagglutinin (PHA-M, 30 µg/ml). The lymphocyte response was evaluated by microscopy (mitosis on slide). As compared with only PHA- stimulated variant, the proliferation of lymphocytes stimulated with PHA in Th-added medium represented 0; 0; 0; 0; 6; 35,1 and 95,8% for final Th concentrations of $10^{-2}$, $10^{-3}$, $10^{-4}$, $5 \times 10^{-4}$, $1,5 \times 10^{-5}$, $2 \times 10^{-5}$, $2,5 \times 10^{-5}$ resp., in culture medium (w/ v). At the Th final concentrations of $3 \times 10^{-5}$, lymphocyte proliferation was normal. According to experimental results, Thiomersal elicits a strong and progressive toxic effect on pig lymphocyte mitogenesis at concentrations higher than $3 \times 10^{-5}$ in culture medium.

INTRODUCTION

Thiomersal (Thimerosal/ Mertiolat/ Mercurothiolate/ Sodium ethylmercurythiosalicylate/ Mercury-[(o-carboxyphenil)thio]ethyl sodium salt/ 2-[(Ethylmercurio)thio]benzoic acid disodium salt/ C$_9$H$_9$HgO$_2$SNa) is a mercury-contained compound used as a preservative to prevent bacterial and fungal growth in some biological products and also as an inactivating agent in some killed vaccines (2,4).

In the body, Thiomersal is broken down in ethylmercury and thiosalicylate. Ethylmercury binds to blood cells or other tissues and is rapidly converted to inorganic mercury. Ethylmercury is excreted in the faeces and bile, mainly as inorganic mercury. Due to this fact, Thiomersal is less toxic than methylmercury, an other organic form of mercury, which is present in the environment and concentrated in some fishes (1).

Thiomersal has been used in vaccines for over 60 years. In man, the only evidence of harm due to thiomersal in vaccines is a small risk of hypersensitivity (skin rashes or local swelling at the site of injection (2) but there is no evidence of toxicity or long-term adverse effects (2, 4).

To evaluate the direct action of Thiomersal on immune system, the pig lymphocyte mitogenesis in Thiomersal-added medium was tested. The results of this experiment are presented.

MATERIAL AND METHODS

Lymphocyte culture
A method of lymphocyte culture from peripheral, whole blood was used (3).
The sterile blood samples (on heparin), from 3 weaned pigs, were cultured 1/10 in 1640 RPMI medium, supplemented with 10% fetal bovine serum and gentamicin (50 µg/ml), in the following variants:

- stimulated with phytohaemagglutinin (PHA-M), 30 µg/ml medium (reference variant);
- stimulated with PHA (30 µg/ml) and treated with Thiomersal, equivalent to final concentrations of 10^-2, 10^-3, 10^-4, 5 x 10^-4, 10^-5, 1,5 x 10^-5, 2 x 10^-5, 2,5 x 10^-5 and 3 x 10^-5 in culture medium (w/ v);
- treated with Thiomersal in final concentrations of 10^-4 and 10^-5;
- unstimulated and untreated (negative control).

The cultures were incubated at 37,5°C, for 72 hours and then prepared for obtaining the slides, by: - treatment with colchicin (12 µg/ml), in the last 90 minutes of incubation time – red cells lysis by hypotonic solution (0.5% KCl) – two fixations (acetic acid 1p+ methanol 3p) - spread on slides – fixative burning – Giemsa (5%) staining – washing – air draying.

The slides were read at microscope.

Stimulation indices (SI) were calculated as report between the numbers of mitosis (metaphases) identified in stimulated and/or treated variants and those observed in negative control of each sample.

Lymphocyte proliferation, in stimulated and treated variants, was calculated, as percentage from PHA-stimulated variant, which are considered 100%, indifferent SI values.

RESULTS AND DISCUSSIONS

The effects of Thiomersal on PHA-stimulated lymphocyte mitogenesis are presented in table 1.

Table 1. Relative proliferation (%) of PHA- stimulated pig lymphocytes in Thiomersal-added medium.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>PHA-M (30 µg/ml medium) + Thiomersal (final concentrations in culture medium, w/ v)</th>
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<tbody>
<tr>
<td></td>
<td>10^-2</td>
</tr>
<tr>
<td></td>
<td>S.I. %</td>
</tr>
<tr>
<td>1613</td>
<td>30 µg/ml medium</td>
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<tr>
<td>1614</td>
<td></td>
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<tr>
<td>1615</td>
<td></td>
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<tr>
<td>Average</td>
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The addition of Thiomersal in culture medium, at final concentrations of 10^-2 - 10^-5, (w/ v) have produced a complete suppression of proliferation of PHA- stimulated lymphocytes (S.I. = 0). For concentrations of 1,5 x 10^-5 Thiomersal in medium, a restart of lymphocyte mitogenesis was observed. So, the mean proliferation of PHA- stimulated lymphocytes at
Thiomersal concentrations of $1.5 \times 10^{-5}$, $2 \times 10^{-5}$ and $2.5 \times 10^{-5}$ in medium represented 63.1 and 95.8% respectively, from reference PHA-variants.

At concentration of $3 \times 10^{-5}$ Thiomersal, mitogenetic activity of PHA- stimulated lymphocytes was a normal one, as compared with reference PHA-variants.

No proliferation was noted in treated – unstimulated variants.

According to experimental data, Thiomersal elicits a strong and progressive toxic effect on pig lymphocyte mitogenesis at final concentrations higher than $3 \times 10^{-5}$ in culture medium (w/ v).

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