# Triphenyltin chloride inhibits superoxide production by human neutrophils stimulated with a surface active agent

Hisao Matsui, Osamu Wada, Yoshio Ushijima* and Takafumi Akuzawa

*Department of Hygiene, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi 371* and **Clinical Laboratory, Gunma University Hospital, 3-39-15 Showa-machi, Maebashi 371, Japan*

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Treatment of human neutrophils with triphenyltin chloride (TPTCl)-inhibited superoxide ($O_2^-$) production stimulated with phorbol myristate acetate (PMA). TPTCl was more potent as inhibitor of $O_2^-$ production than other phenyltin compounds. The $O_2^-$ production by the xanthine oxidase–acetaldehyde system was not inhibited by TPTCl. This finding indicates that TPTCl does not itself react with $O_2^-$. Furthermore, TPTCl did not influence the isolated NADPH oxidase at all, though $O_2^-$ production of neutrophils stimulated with PMA in the presence of TPTCl was inhibited. These results indicate that TPTCl inhibits the activation process of the $O_2^-$ generating system.

*Triphenyltin chloride* *Superoxide* *Phorbol myristate acetate* *Neutrophil* *NADPH oxidase*

## 1. INTRODUCTION

The biological effects of organotin compounds are of some concern because applications of the chemical and physical properties and the biocidal properties of these compounds may result in exposure of man and other mammals to organotin [1]. In the course of the toxicological study of triphenyltin chloride (TPTCl), we found that this compound inhibited chemiluminescence production by rabbit neutrophils stimulated by particulate and soluble stimuli [2]. To clarify the inhibitory effects of TPTCl upon a burst of oxidative metabolism, we studied the effect of the inhibitor on superoxide ($O_2^-$) production by human neutrophils. $O_2^-$ production of leukocytes stimulated with phorbol myristate acetate (PMA) was inhibited by TPTCl, though the inhibitor did not influence the NADPH oxidase ($O_2^-$-forming enzyme) isolated from leukocytes stimulated with PMA in the absence of TPTCl. These results indicate that TPTCl inhibits the activation process of the $O_2^-$-forming system.

## 2. MATERIALS AND METHODS

Triphenyltin chloride (TPTCl) was obtained from Tokyo Kasei Chemical Co.; phorbol myristate acetate (PMA) and cytochrome *c* type VI from Sigma; xanthine oxidase and acetaldehyde from E. Merck. Stock solutions of TPTCl were made in ethanol and diluted 1:99 with Hanks' balanced salt solution immediately before use. The final ethanol concentration in the reaction mixture was 0.1% (v/v). PMA was dissolved in dimethylsulfoxide (DMSO) and stored at −20°C before use.

### 2.1. *Preparation of neutrophils*

Neutrophils were obtained from healthy adult donors by dextran sedimentation, as in [3]. Contaminated red cells in neutrophil fraction were removed by hypotonic lysis. The leukocytes (80–90% neutrophil) were suspended in Hanks' balanced salt solution.

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2.2. *Measurement of $O_2^-$ production by intact leukocytes*

Release of $O_2^-$ was measured by superoxide dismutase (SOD)-inhibitable reduction of cytochrome *c*, using an extinction coefficient oxidized *vs* reduced of 21.1 $mM^{-1}$ at 550 nm [4]. Reaction mixtures contained 1 × $10^6$/ml cells, 0.1 mM ferricytochrome *c* with 0.1% ethanol (control) or TPTCl. The suspension was preincubated for 3 min at 37°C in a cuvette which was placed in a Hitachi 557 dual wavelength spectrophotometer. The reaction was started by the addition of PMA. The time course of the absorbance change at 550 nm relative to that at 540 nm was followed on a recorder.

2.3. *Preparation of particulate fraction*

The particulate fraction was prepared from PMA-activated neutrophils as in [5]. The homogenate was diluted 5-times with 0.34 M sucrose and centrifuged at 450 × *g* for 15 min to remove cell debris and nuclei. The supernatant was centrifuged at 16900 × *g* for 35 min and the sedimented particles were resuspended in chilled 0.34 M sucrose.

2.5. *NADPH oxidase activity*

NADPH oxidase was assayed at 37°C by recording the rate of decrease in absorbance at 340 nm. The assay mixture consisted of 0.1 mM NADPH, 0.9 ml of 0.05 M phosphate buffer (pH 7.5) in a final volume of 1.0 ml.

## 3. RESULTS AND DISCUSSION

3.1. *Effect of TPTCl on $O_2^-$ production*

Cell suspensions in cuvettes were preincubated with various concentrations of TPTCl for 3 min, before PMA (1 μg/ml) was added. $O_2^-$ production by neutrophils was inhibited by TPTCl in a dose-dependent fashion (fig.1).

We examined the action of TPTCl on the $O_2^-$ production of the xanthine oxidase–acetaldehyde system. The $O_2^-$ production was not impaired by TPTCl as shown in fig.2. This indicates that TPTCl does not itself react with $O_2^-$.

3.2. *Effect of other phenyltin compounds on $O_2^-$ production*

When the other phenyltin compounds were used, the relative potencies of inhibitory effect were in the order: TPTCl > diphenyltin dichloride > phenyltin trichloride > tetraphenyltin on a molar basis (fig.3).

3.3. *Effects of TPTCl on NADPH oxidase isolated from PMA-stimulated neutrophils*

Because the production of $O_2^-$ by neutrophils is a result of two phenomena: the activation of an $O_2^-$ generating system and the activity of this enzyme system [6], the results of the above experiments could be the results of an effect of TPTCl on the

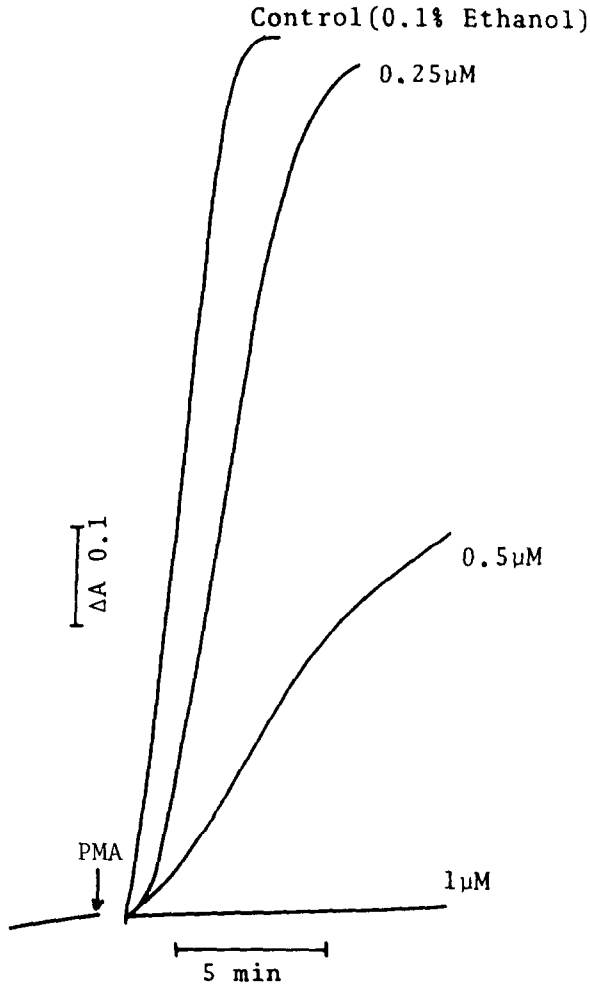


Fig.1. The inhibitory effect of TPTCl on $O_2^-$ production by human neutrophils. $O_2^-$ production by neutrophils was induced by PMA (1 μg/ml). Cell suspensions were preincubated with TPTCl for 3 min at 37°C before addition of PMA.

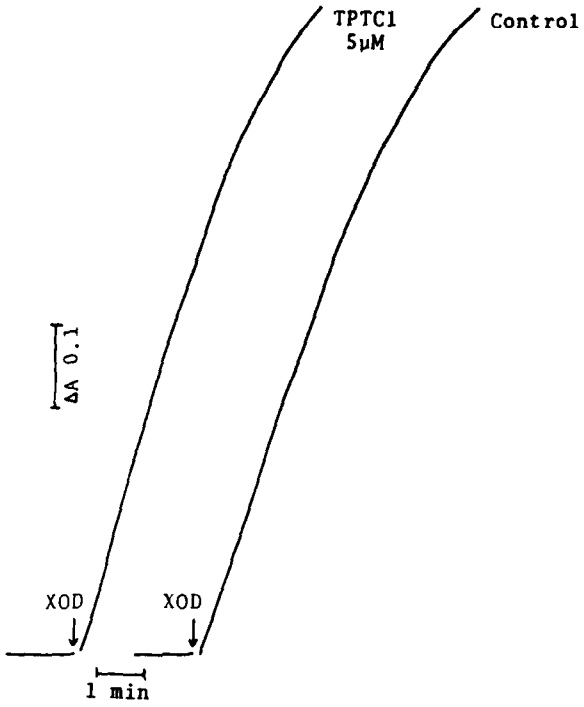


Fig.2. The effect of TPTCl on $O_2^-$ production by the acetaldehyde–xanthine oxidase system. Xanthine oxidase (XOD) was added to the reaction mixture containing 10 mM acetaldehyde and 0.1 mM ferricytochrome *c* with or without TPTCl in Hanks' balanced salt solution.

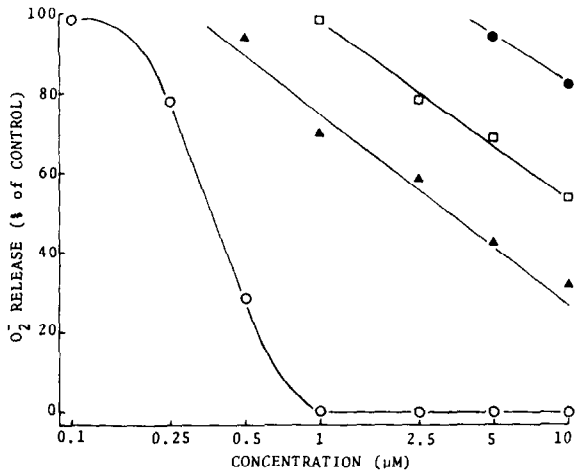


Fig.3. The inhibitory effect of various phenyltin compounds on the $O_2^-$ production by human neutrophils stimulated by PMA (1 μg/ml). Cell suspensions were preincubated with phenyltin compounds for 3 min at 37°C before addition of PMA. (●) Tetraphenyltin; (○) TPTCl; (▲) diphenyltin dichloride; (□) phenyltin trichloride.

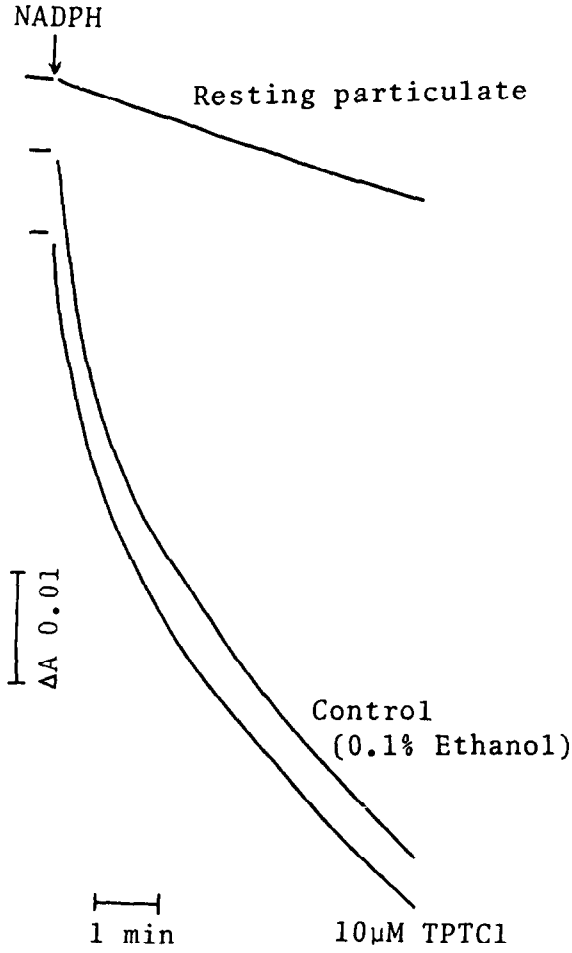


Fig.4. Effect of TPTCl on particulate NADPH-oxidase activity of PMA-stimulated neutrophils. Particulates were preincubated with TPTCl for 5 min at 37°C before addition of NADPH.

activation or the enzyme system itself. To determine which process is affected, NADPH oxidase isolated from PMA-stimulated neutrophils was determined in the presence of TPTCl. No inhibition was observed, indicating that TPTCl inhibits the activation process of the $O_2^-$ generating system.

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