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Microcalorimetric studies on *Tetrahymena pyriformis* Part 2. Photosensitizer toxic effect on *Tetrahymena pyriformis*

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Abstract

Using a microcalorimetric method, the effects on *Tetrahymena pyriformis* of photosensitizers YHPD, TSPP and TAMP metabolic activity have been determined. The experimental results indicate that the prophyrin compounds TSPP and TAMP strongly affect *T. pyriformis* metabolism, but inhibition due to YHPD is slight. The photosensitization effects follow the sequence TAMP > TSPP > YHPD. These results are consistent with the results of SEM and TEM methods, and show that photosensitizers in *T. pyriformis* cells lead to similar cellular structural damage in the same sequence.

INTRODUCTION

Tetrahymena pyriformis belongs to the protozoa family, is distributed widely, in general is collected from polluted water, and can be separately grown under bacteria-free conditions because it is easily cultured and preserved in the laboratory. It can be used as a biological indicator in freshwater biology and environmental pollution and has also been used widely as a "test organism" to monitor and evaluate toxins, nutrients, antibiotics, carcinogenic compounds and anti-cancer medicament [1]. Because, some porphyrin compounds are photosensitizers, when they accumulate in a tumour they can be used for clinical diagnosis and photodynamic treatment of cancer.

Studies of the toxic effects of photosensitizers *T. pyriformis*, can help elucidate the effects in other biological systems. The results will give useful information about the alteractions to biochemical processes resulting from photosensitizer action and how to apprecite the effectiveness of the photosensitizer.

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The fundamental growth thermograms of *T. pyriformis* can be determined by the microcalorimetric method. These thermograms reflect the active changes of *T. pyriformis* under photosensitizer action. Therefore one can use them as a "valuable standard" to evaluate the photosensitization effects of medicaments.

INSTRUMENT AND MATERIALS

Instrument

An LKB 2277 Bioactivity Monitor was used to obtain the growth thermograms of *T. pyriformis*. The performance of this instrument and the details of its construction have been described previously [2,3].

Materials

Tetrahymena pyriformis B J 4, mononuclear was provided by the Department of Biology, Beijing University.

Medium. The medium solution contained the following nutrients (wt.%): peptone, 1%; beef extract, 0.1%; glucose, 0.5%.

Photosensitizers used were heliotherapy porphyrin injection (YHPD) produced by the Yangzhou Biochemical Reagent Factory, Yangzhou, China, meso-tetra(*p*-sulfophenyl)porphyrin (TSPP) and tetra(*p*-trimethylammoniumphenyl)porphyrin (TAMP); the structural formula of TSPP and TAMP is shown in Fig. 1. These last two porphyrins were synthesised by the Organic Chemistry Research Group, Department of Chemistry, Wuhan University.

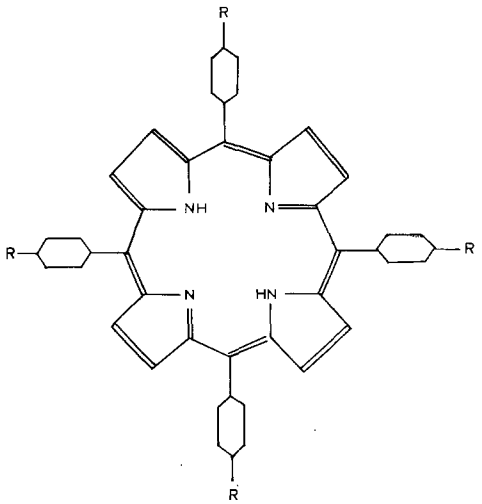

Fig. 1. Structural formula of TSPP and TAMP: TSPP, R = $-SO_3H$; TAMP, R = $-N^+Me_3$.

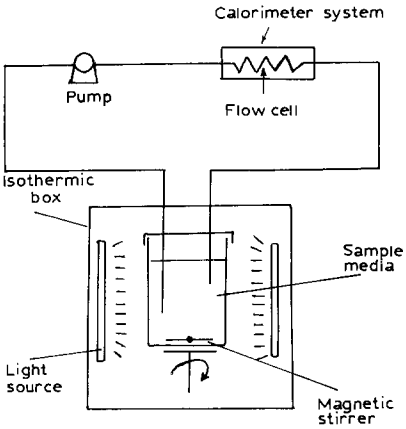

Fig. 2. Schematic diagram of apparatus.

EXPERIMENT AND RESULTS

Experimental method

A schematic representation of the experimental apparatus is shown in Fig. 2. The complete cleaning and sterilization procedure for the flow tubing has been described previously [2]. Once the system was cleaned and sterilized, 60 ml liquid medium were added to the cycle-flow system (flow rate 20 ml h^{-1}). The temperatures of the calorimeter system and the isothermic box were controlled at 28°C. When the stabilization baseline had been obtained, 20 ml of *T. pyriformis* sample (population density about 4000 cells ml^{-1}) was added to the cycle-flow system, and the monitor continued to record the thermograms of *T. pyriformis* growth. Throughout the growth period the culture was irradiated by a light source (illuminance, 3000 lux).

RESULTS

The thermograms of *T. pyriformis* cultures containing different concentrations of TSPP are shown in Fig. 3, those containing TAMP are shown in Fig. 4 and those containing YHPD are shown in Fig. 5.

These experimental results indicate that the log phase of the growth thermogenesis curve obeys the equation $P_t = P_0 \exp(kt)$, or $\ln P_t = \ln P_0 + kt$. So in making use of data $\ln P_t$ and t taken from the log phase of the growth curve to fit a linear equation, one can obtain the rate constant k. For example, the data of curves (a), (b) and (c) of Fig. 3. are shown in Tables 1–3; the corresponding equations are as follows:

Curve (a) (without TSPP)
$\ln P_t = -0.6756 + 0.01059\ T\ k = 0.01059\ \text{min}^{-1}\ r = 0.9984$

Curve (b) (TSPP conc. 2.5×10^{-5} M)
$\ln P_t = 1.5985 + 0.006163\ t\ k = 0.006163\ \text{min}^{-1}\ r = 0.9976$

Curve (c) (TSPP conc. 1.0×10^{-4} M)
$\ln P_t = 2.0527 + 0.00240\ t\ k = 0.002430\ \text{min}^{-1}\ r = 0.9471.$

The different rate constants at different concentrations of photosensitizer reflect the changes in metabolic activity of *T. pyriformis*. Therefore we

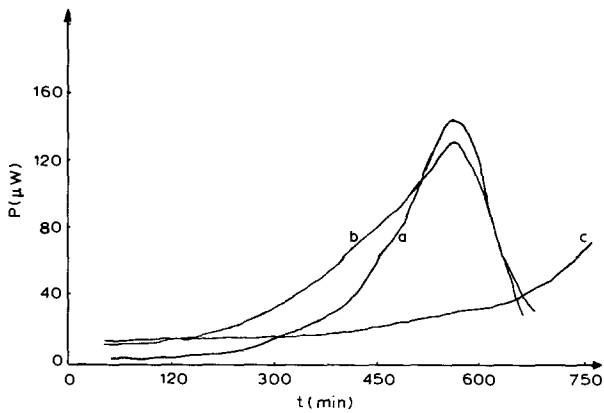

Fig. 3. Thermogenesis curves for *T. pyriformis* culture containing the photosensitizer meso-tetra(*p*-sulfophenyl)porphyrin TSPP: (a) without TSPP; (b) 2.5×10^{-5} M (TSPP); (c) 1.0×10^{-4} M TSPP.

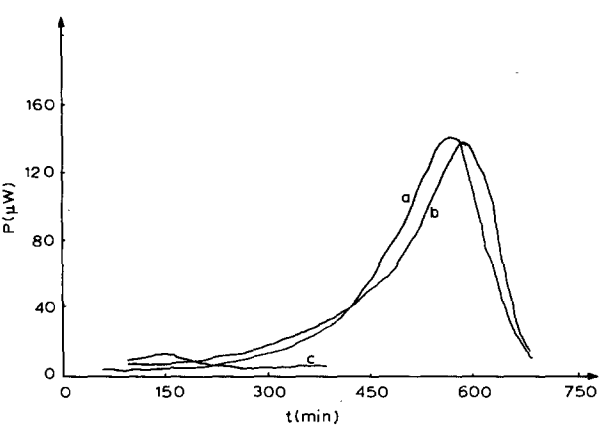

Fig. 4. Thermogenesis curves for *T. pyriformis* culture containing the photosensitizer tetra(*p*-trimethylammoniumphenyl) porphyrin (TAMP). (a) without TAMP; (b) TAMP concentration, 1.0×10^{-5} M; (c) TAMP concentration, 2.5×10^{-5} M.

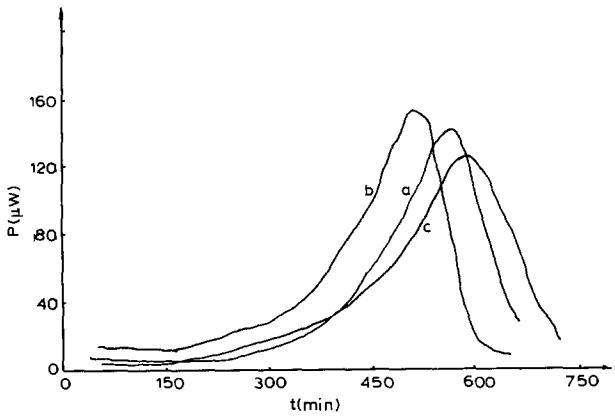

Fig. 5. Thermogenesis curves for *T. pyriformis* culture containing photosensitizer heliotherapy porphyrin (YHPD). (a) without YHPD (b) YHPD conc., 10 μg ml^{-1}; (c) YHPD conc., 100 μg ml^{-1}.

can use the rate constant data as a measure of the inhibitory effect of the photosensitizer.

Using the same method, from the data of P_t versus t in Figs. 4 and 5 the rate constants of all experiments can be obtained, and these are shown in Tables 4 and 5.

CONCLUSION

Differential thermograms reflect the changes of metabolic activity of *T. pyriformis* under the action of photosensitizer, and the changes of rate

TABLE 1

$P_t - t$ data for Fig. 3 curve (a)

t (min)	P_t (μw)	ln P_t [a]
110	1.5	0.4055
160	3.2	1.163
210	4.5	1.504
245	6.0	1.792
260	8.0	2.079
310	15.0	2.708
335	18.0	2.890
360	24.0	3.178
383	30.0	3.401
410	38.0	3.638
430	48.0	3.871
450	60.0	4.094
460	68.0	4.220
495	90.0	4.500

[a] ln $P_t = -0.6756 + 0.01059t$, $k = 0.01059$ min^{-1}, $r = 0.9984$.

TABLE 2

$P_t - t$ data for Fig. 3 curve (b)

t (min)	P_t (μw)	ln P_t [a]
100	10.0	2.303
150	12.0	2.485
200	15.8	2.760
250	21.6	3.073
300	32.0	3.466
365	47.9	3.869
410	65.0	4.174
430	75.0	4.317
460	84.0	4.431
507	105.2	4.656

[a] $\ln P_t = 1.5985 + 0.006163t$, $k = 0.006163\ \text{min}^{-1}$, $r = 0.9976$.

TABLE 3

Data of $P_t - t$ for Fig. 3 curve (c)

t (min)	P_t (μw)	ln P_t [a]
50	11.0	2.398
100	12.0	2.485
175	13.0	2.565
285	12.7	2.542
400	16.0	2.773
440	17.8	2.879
450	20.0	2.996
500	24.0	3.178
540	28.3	3.343
600	32.0	3.466
630	35.1	3.558
650	39.0	3.664
700	49.5	3.902
750	68.5	4.227

[a] $\ln P_t = 2.0527 + 0.002430t$, $k = 0.002430\ \text{min}^{-1}$, $r = 0.9471$.

TABLE 4

k and TAMP concentration data for *T. pyriformis*

TAMP conc. (M)	k (min^{-1})	r
0.0	0.01059	0.9984
1.0×10^{-5}	0.006328	0.9932
2.5×10^{-5}	0.0000	0.9815

TABLE 5

k and YHPD concentration data for *T. pyriformis*

YHPD conc. (μg ml^{-1})	k (min^{-1})	r
0	0.01059	0.9984
10	0.007535	0.9978
100	0.007065	0.9903

constants (k) can be used as a valuable standard to evaluate the inhibition level of medicaments.

The inhibition levels of the photosensitizers examined follow the sequence TAMP > TSPP > YHPD on *T. pyriformis*. The experimental results indicate that the relation between TAMP concentration and rate constant (k) is inversely proportional; when TAMP concentration increases from zero to 1.0×10^{-5} M and then 2.5×10^{-5} M, the rate constant k decreases from 0.01059 min^{-1} to 0.006328 min^{-1} and then 0.0000 min^{-1} (see Table 4).

The inhibitory action of YHPD is very small, as the experimental results indicate. When YHPD concentration increases from 10 to 100 μg ml^{-1}, k decreases only from 0.007535 min^{-1} to 0.007065 min^{-1} (see Table 5).

The toxic effects of these photosensitizers on *T. pyriformis* can be measured by a microcalorimetric method, and the changes of metabolic activity reflect the structural damage to the cell. Results obtained by scanning electron microscopy and transmitting electron microscopy show this damage; these experimental results will be reported later.

ACKNOWLEDGEMENT

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