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Changes in Ultraweak Luminescence from Living Fish Induced by Three Chemicals

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Ultraweak luminescence is a ubiquitous phenomenon in biological systems, which differs from bioluminescemce of luciferin-luciferase. This low-intensity emission is inherently associated with the following important processes such as oxidative metabolism, cell division, carcinogenesis, photosynthesis, and cell death. In general, ultraweak luminescence may be classified as two kinds, namely spontaneous and induced (Slawinska and Slawinski 1985a). Zebra fish is a recommended specimen for toxicity and toxicological test (laaie 1977). The purpose of this, the changes before and after the treatment with three chemicals : UO_2, NaN_3 or cyclophosphamide® and their correlations between the dose and effect.

MATERIALS AND METHODS

Adult zebra fish (Brachydanio rerio) purchased from Guanyuan-Aquarium (Beijing China). The fish were acclimated in our laboratory for at least 1 week prior to the start of the experiment. they were kept in aerated tap water at $25 \pm 1°$ C and fed with the granulated fish feed (Beijing fish feed Inc. China). Sodium azide (NaN_3) was purchased from Sigma, USA. Uranium dioxide (UO_2) and cyclophosphamide® (CP) were products in China.

Ultraweak luminescence from fish was measured with a high sensitive single-photon counting system which made in China (Shen Xun 1988). It could measure the intensity of photon emission from living fish in the wavelength range of 400–700nm. The photomultiplier EMT 9659 QB used in the system was cooled by liquid nitrogen to reduce the background down to 40 cps, In this way, the equipment was able to detect a current density of $0.3 h\nu \cdot s^{-1} \cdot cm^{-2}$ at a significance level of 99.9% within 6 hr.

Fish samples (five of each sample) from which the self-controlled (i.e., the background value which exposured to none of chemicals) photon count rates ($h\nu \cdot s^{-1}$) had been measured, were exposured in the solution prepared fish were washed 3 times with the distilled water and then measured again to obtain the observed rates. All measurements were carried out at 1400 mV, 400 times, 100mV on the counting equipment. Finally, the length (cm) of each zebra fish was measured. The average values of total boby surface area of each sample (5 fish) could be figured out from body surface area of one side of fish which had been found out by the length of fish from

Table 1. Meanwhile, the difference of photon count rate was figured out from that the observed rate minus the background rate of selfcontrol and quartz cuvette-water, Finally, the ultraweak luminescence intensity (ULI) was calculated from that the difference of photon count rate of each sample was divided from the average value of the total body surface area, and expressed in $h\nu \cdot s^{-1} \cdot cm^{-2}$.

Table 1. Surface area of zebra fish body.

Lenggh (cm)	Area (cm^2)	Length (cm)	Area (cm^2)	Length (cm)	Area (cm^2)
2.0	0.7497	2.7	1.2048	3.4	1.8740
2.1	0.7570	2.8	1.2965	3.5	1.9320
2.2	0.8710	2.9	1.3701	3.6	2.0116
2.3	0.8957	3.0	1.4142	3.7	2.1586
2.4	1.0222	3.1	1.4686	3.8	2.3473
2.5	1.0602	3.2	1.6647	3.9	2.4832
2.6	1.1672	3.3	1.7454	4.0	2.7073

RESULTS AND DISCUSSIONS

Zebra fish emit the spontaneous ultraweak luminescence in the living state. Based upon the statitics from 100 fish, the average value of the natural mean of ULI was 25 ± 1.68 $h\nu \cdot s^{-1} \cdot cm^{-2}$ and thus had an intensity of the order of $10^{-10} h\nu \cdot s^{-1} \cdot cm^{-2}$. Table 2 further shows that the intensity of the spontaneous photon emission is relevant to the season and so increases with raising temperature of seanon.

Table 2. Ultraweak luminescence intensity from zebra fish under the various seasan

ULI ($h\nu \cdot s^{-1} \cdot cm^{-2}$)	Winter	Spring	Summer
Average	14	27	49
$\pm$SE	1.57	2.58	5.23

In this experiment, the photon count rates from zebra fish treated with UO_2 were less than the relevant self-controlled rates. ULI of between selfcontrolled rates observed ones treared with UO_2 increased with the increasing of UO_2 concentration, i.e., the rates from UO_2 -treated fish decreased with the increase of UO_2 concentration, reflecting the degree of UO_2 damaging the luminescence function from fish enhanced with the raise of UO_2 concentrations. When the concentration of UO_2 was over 0.3 $\mu g/ml$, all fish died.

NaN, enhanced the photon emission from zebra fish, but its

Table 3. Ultraweak luminescence intensity from zebra fish induced by uranium dioxide.

	concentration (μg/ml)			
	0.05	0.1	0.2	0.3
ULI ($h\nu \cdot s^{-1} \cdot cm^{-2}$)	9	13	19	27
±SE	3.27	3.26	2.48	1.77

magnitude was so lowered that when NaN_3 concentration was up to 0.2 mg/ml, ULI approximated to 1 $h\nu \cdot s^{-1} \cdot cm^{-2}$. The enhanced ULI decrseased with the rise of NaN concentrations.

Table 4. Ultraweak luminescence intensity from fish induced by sodium azide.

	Concentration (mg/ml)		
	0.05	0.1	0.2
ULI ($h\nu \cdot s^{-1} \cdot cm^{-2}$)	5	3	1
±SE	1.20	0.55	0.58

Table 5. Ultraweak luminescence intensity from zebra fish induced by cyclophosphamide.

	Concentration (mg/ml)			
	0.4	0.8	1.6	2.4
ULI ($h\nu \cdot s^{-1} \cdot cm^{-2}$)	32	20	13	6
±SE	6.47	2.05	3.25	2.10

As with NaN_3, CP enhanced the photon emission from fish. The enhanced ULI also decreased with the rise of CP concentrations. The data to be presented in Table 6 shows that the correlation between the dose of chemicals and effect of their inductions is as follows: for UO_2 or NaN_3, it is very sigmificant ($p<0.01$), and for CP, it is significance ($P<0.05$).

Zebra fish emit ultrawesk luminescence (Ruth 1978), because of fish's survival after measuring, it may serve as self-control for the toxicity and toxicological test to prove the accuracy of the experimental results. UO_2 induces a decrease of photon emission from the living fish and its induction increases with the increase of UO_2 concentrations. By contrast, NaN_3 or CP induces an increase in luminescence intensity, and its induction decreases with an increase of the concentration of either NaN_3 or CP. Moreover, there

Table 6. Correlation between the dose of chemicals and changes of ultraweak luminescence intensity from fish.

Chemicals	Regression equatio $\hat{Y}=a+BX$	Regression coefficient (r)	Significant test (t test)
UO_2	$\hat{Y}=-6-71X$	-1.000	$P<0.01$
NaN_3	$\hat{Y}=3-5X$	-0.993	$P<0.01$
CP	$\hat{Y}=33-12X$	-0.959	$P<0.05$

are signficant or very significant correlations between the doses of chemical toxicants and changes of ultraweak luminescence induced by chemicals. Based upon the generalized model for the different types of biological chemi-luminescence (Slawinska and Slawinski 1985a), fish is an emitter of excited carb onyl compounds (>C=O*)and dimoles $O(^1O_2)$. Three chemical toxicants may affect the formation of activated oxygen free radicals in biological system to disturb the luminescence function of zebra fish. For example. UO_2 affects on the formation of active species such as ROO·, $^1O_2$, H_2O_2 and O_2 because the oxidation of uranium may directly interfere to the activity of the above free radicals (Pettersson 1977). NaN_3 is well known to be a high seneitive quencher for $^1O_2$(Abeles 1986). As for CP, it is a cytostatic agent that causes the inhibition of cell growth and, for photon emission is in close relationship with cell division, affects on the luminescence function of fish. Although the changes of ultraweak luminescence have been known to be relevant to the formation and/or quenching of activated oxygen, the ultraweak luminescence from objects may conceivably have various origins (Candenas 1984). Hence, the exact mechanism of ultraweak luminescence from the whole zebra fish needs further research. Obviously, the results of this study will be useful for the theoretical aspects as well as the practical utilization of ultraweak luminescence and its technique (Slawinska and Slawinski 1985b).

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