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# The Study of Anti-tumor Activity of Trichosanthin by Cyclic Voltammogram

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**Abstract:** The anti-tumor activity of Trichosanthin (TCS) has been frequently reported in recent years. In our experiments, electrochemical methods were applied to detect the effects of TCS on human leukemia cells U937. 50μg/ml TCS treatment for 40 hours can cause irreversible negative effects on the viability of U937 cells. This effect largely depends on the concentration of TCS and the time period of treatment.

**Keywords:** Trichosanthin (TCS), U937 cells, cyclic voltammogram, Ribosome Inactivating Proteins (RIPs).

It has been reported that Trichosanthin (TCS), isolated from *T. kirilowii*, has anti-tumor[1-4] and anti-viral activities[5-7]. Some anti-tumor remedies, which are designed from TCS, are being tested and developed[8-11]. Studying the biological phenomenon by electrochemical methods is a new hot research spot emerging in recent years[12]. Many results showed that not only the enzyme system such as succinate dehydrogenase displayed the diode-like behavior during the electron transfer process[13], but also the highly organized organelle, for example, mitochondria, and even intact cells[14-16] had similar behavior. The cyclic voltammogram showed that intact cells have apparent faradaic response and display a unidirectional oxidation peak, whose strength indicates the cell health state[14-16]. The results of this new method correspond to that of the morphological observation but are more sensitive. In our experiments, the electrochemical method was applied to detect the effects of TCS on human leukemia cells U937.

TCS was extracted from the tuber root of *Trichosanthes kirilowii Maxim*. Ammonium sulfate fractional precipitation was carried out after pulverization and the Sepharose Fast Flow Chromatography was used for further purification. The graphite electrode used in our experiments had both hydrophilic and hydrophobic functional groups, which made it easy for cells to be attached[17-18]. The cells were washed three times carefully to make sure to get rid of the culture medium and re-suspended in physiological saline (0.9% NaCl) before measurement.

**Figure 1** Cyclic voltammogram of U937 cells (scan rate: 50 mV/s.)

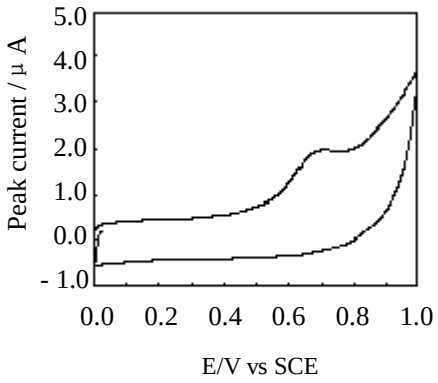
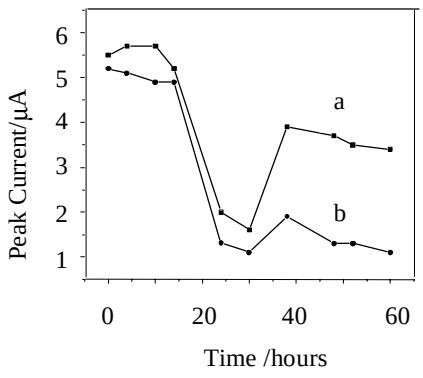



**Figure 1** showed the typical voltammogram of U937 cells. The scan rate was 50mV/s *vs* SCE. In this figure, we could see that the cells were ready to discharge electrons (0.68V *vs* SCE) but refused to be reduced. The peak current had a positive relationship with the cell viability described by Trypan Blue exclusive test during cell culture course. The result agreed with the previous reports about the voltammetric behavior of the cells *T. Shanghaiensis*, *S. cereviase*, HL60 and HEL[15-16] *etc*.

**Figure 2** Time course of the voltammetric behavior of U937 cells (scan rate: 50 mV/s).




a. without TCS treatment; b. with 50 μg/ml TCS treatment.

**Figure 2** shows time course of the voltammetric behavior of U937 cells. The initial concentration of the U937 cells is $2\times10^5$/ml and TCS concentration in test group is 50 μg/ml (b). During the beginning 15 hours, though the viability of the cells in control group (a, without TCS) increased a little while the test group (b) decreased continuously, there was no significant difference between them. After 15 hours, with depletion of nutrition, the faradaic response of both groups was attenuating. But when the nutrition

was replenished at 35 hours, the viability of the control group cells became to recover and stay at high level for a long while. While at the same period, the viability of the test group remains in low status. The results indicated that after long run treatment (40 hours) of TCS under 50 μg/ml, some irreversible negative effects were caused on U937 cells, which was reflected as that the faradaic response of test group cells could not come back to the initial health state even after nutrition replenishment. Microscope observation showed that most cells in the test group demonstrated obvious evidence of necrobiosis: broken-down cell membranes, pieces of cells and organelles while the control group cells remained in good conditions.

**Figure 3** Effectiveness of TCS on the cells U937 (From left to right for both 8 and 40 hours treatment, the TCS concentrations are 0, 5, 25, 50, 75, 100,150 and 200 μg/ml respectively. The scan rate: 50 mV/s)

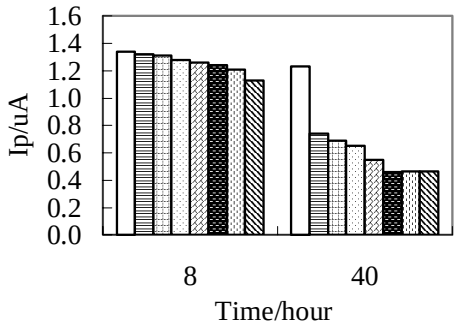



**Figure 3** showed the effects of different concentration of TCS on U937 cells. The initial concentration of U937 cells is $1\times10^5$/ml this time and other treatment was the same as done in **Figure 1**, including adding nutrition at 35 hours. The experimental results showed that the damage caused by TCS on U937 cells not only depended on the time length of treatment but also positively correlated to the concentration of TCS. The longer the time of treatment and the higher the concentration of TCS, the stronger the effects TCS had on U937 cells. Such effects can be observed even at the treatment with 5 μg/ml TCS. When the concentration was over 50 μg/ml, the effects of TCS on U937 cells showed no remarkable difference, which might imply that 50 μg/ml TCS was enough to completely repress the viability of U937 cells.

It has been well known that TCS belongs to Ribosome Inactivating Protein (RIP) family[19]. It can cut the adenine at 4324 site of 28S rRNA thus preventing the protein synthesis in eukaryotic cells[20-21]. However, its anti-tumor activities and high selective cytotoxity can hardly be explained by only indicating TCS is one kind of RIPs. The $ID_{50}$ of TCS to various types of tumor cells fluctuated in a large range[3]. It was also reported that in the cells infected by the hepatitis virus, TCS has little effects on normal protein synthesis but greatly suppressed the synthesis of hepatitis surface antigen[5]. Some evidence showed that the anti-tumor activity of TCS might result from the

interaction between TCS and biomembrane system[3]. The breaking of membranes are commonly seen in the *in vitro* cultured cells with TCS treatment. It is also observed in our experiments that it is very hard to wash TCS down from cell membrane, which usually disturbed the voltammogram of U937 cells. Other evidence showed that TCS might possess this anti-tumor activity only after it has been activated. The process may include some modification of TCS or start other signal pathway to reach the final results. TCS can enter the intact cells in 30 minutes, but in our experiments it can take effects only after a relatively long time (24 hours or longer usually). The more interesting is that we found TCS itself had specific electrical behavior after stimulation of electrochemical scanning. A couple of new oxidation and reduction peaks appeared (figure not shown), which needs more experiments to verify if TCS can be engaged in electron transfer network in tumor cells. This discovery may shed light on the mechanisms of TCS anti-tumor activity.

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