

*GENOTOXIC EFFECTS OF A NEW ORGANOTIN
COMPOUND $Et_2SnCl_2 \cdot L$ { $L=N-[p-(2-$
 $pyridylmethylene) methylbenzenamine]$ } IN
MAMMALIAN SYSTEM*



BY

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Declaration

I, Christine Syng-ai, do hereby declare that the subject matter of this thesis is a record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other university/institute.

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Introduction

Since its discovery in 1963, (Frust *et al*, 1993) metal chelation continues to play an important role in the cure and cause of malignancy. One of the most outstanding recent developments in the field of metal compounds in medicine was Rosenberg's accidental discovery that platinum complexes possess anti-tumor activity (Rosenberg, 1969). The most widely used chemotherapeutic regimen includes platinum compounds. (McGuire *et al* 1993) However, drug resistance of tumor cells (Johnson *et al* 1993), toxicity (nephrotoxicity, ototoxicity, neurotoxicity) and side effects (emesis) frequently limit the clinical usefulness of cisplatin. Chemical modifications of the drug have been carried out to reduce toxic effects and improve their antitumor efficacy and pharmacokinetic properties (Perez 1991). Many cisplatin analogs have been investigated in recent years (Carnetta *et al* 1996). Moreover, several non-platinum metal complexes have been extensively investigated in recent years (Moebus *et al* 1997) and antitumor activity has been found in derivatives of rhodium, indium and palladium and other compounds containing copper, gallium, titanium, tin and germanium were tested for their biological activity in human cancer cells (Keppler 1993, Gielen 1990, Respondek *et al* 1996.).

Organotin compounds show a spectrum of biological effects and have been extensively studied and used commercially as fungicides, bactericides, acaricides and wood preservatives (Blunden *et al* 1985), anti-fouling coating, insecticides and plastics stabilizers (Sasaki *et al* 1993). The biological effects of organotins have been well investigated (Boyer 1989), these include skin toxicity, neurotoxicity, immunotoxicity, and carcinogenicity (Innes, 1969). Organotins have also been widely studied as environmental pollutants in both land and water. Triphenyltin have been extensively investigated as a major seawater pollutant in Japan (The Environment Agency of Japan, 1991). But there is virtually no report on the interaction of organotin compounds with the DNA. Early studies showed that they are inactive towards transplanted mouse cancers (Krause 1969). However in 1972, Brown demonstrated that triphenyltin acetate, but not chloride, when

administered in the food or by injection retarded tumor growth in mice. Only scanty and scattered information is available on their activity against cancer (Evans *et al* 1985; Saxena 1987).

genes The development of cancer is no longer a mystery. During the past two decades, investigators have made astonishing progress in the deepest bases of the process - those at the molecular level, but translation of new understanding into clinical practice is complicated, slow and expensive. The term "cancer" refers to more than 100 forms of the disease. Almost every tissue in the body can spawn malignancies some even yield several types. What is more, each cancer has unique features. The 30 trillion cells of the normal, healthy human body live in a complex, interdependent condominium, regulating one another's proliferation. Indeed, normal cells reproduce only when instructed to do so by other cells in their vicinity. Such unceasing collaboration ensures that each tissue maintains a size and architecture appropriate to the body's need. Cancer cells, in stark contrast, violate this scheme; they become deaf to the usual controls on proliferation and follow their own internal agenda for reproduction. They also possess an even more insidious property - the ability to migrate from the site where they began, invading nearby tissues and forming masses at distant sites in the body. Tumors composed of such malignant cells become more and more aggressive over time, and they become lethal when they disrupt the tissues and organs needed for the survival of the organism as a whole.

its first Over the past 25 years, scientists have uncovered a set of basic principles that govern the development of cancer. We now know that the cells in a tumor descend from a common ancestral cell that at one point - usually decades before a tumor becomes palpable - initiated a program of inappropriate reproduction. Further the malignant transformation of a cell comes about through the accumulation of mutations in specific classes of the genes within it. These genes provide the key to understanding the processes at the root of human cancer. Two classes of gene, which together constitute only a small proportion of the full genetic set, play major roles in triggering

cancer. In their normal configuration, they choreograph the life cycle of the cell - the intricate sequence of events by which a cell enlarges and divides. Proto-oncogenes encourage such growth, whereas, Tumor suppressor genes inhibit it. Collectively these two gene classes account for much of the uncontrolled cell proliferation seen in human cancers (Weinberg 1996).

The history of chemotherapy is rather unusual. Although some Egyptian writings from around 1600 B.C. described the use of crude drugs to treat ulcerating skin tumors, and the first documented case of cancer chemotherapy dates back to 1865 (Lissauer 1865), modern cancer chemotherapy is only about five decades old. Sulfur mustard, a toxic chemical was developed during World War I for use by the army in the battlefield. It caused burns in the eyes, on the skin and in the respiratory tract of the soldiers exposed to it and incapacitated them. As early as 1931, attempts were made at using sulfur mustard for treating squamous carcinomas in humans, but it was too toxic for systemic use (Adiar and Bagg 1931) It was several years before Gilman and colleagues discovered that nitrogen mustard inhibited the growth lymphosarcoma in mice, and later initiated the first clinical trial of nitrogen mustard on patients with lymphosarcomas (Gilman and Philips 1946). It was not until after World War II that Gilman's work were published (Gilman and Philips 1946), marking the beginning of modern day cancer chemotherapy. A derivative of this compound, nitrogen mustard, was made later. It had anti-tumor properties. Its first trial on patients was done in 1942. Since then hundreds of thousands of chemicals have been synthesized and tested for anti-cancer action.

There are two strategies for the development of new anti-tumor drugs. The first is to search for agents that exert cytotoxicity (Kanzawa *et al* 1990, Kanzawa *et al* 1995), and second is to screen new agents for their effectiveness against tumor cells (Kanzawa 1981, Horichi *et al* 1990, Ohmori *et al* 1990). The type of chemicals tested seems to come from every possible source. Most are synthesized in laboratories. In addition, natural products isolated from plants and animals, antibodies made by organisms from

different habitats and products mentioned in various indigenous systems of medicine undergo rigorous testing. Only a handful of them make it to the final stage of treating patients. Thus, finding a new effective anti-cancer drug is an extremely expensive task.

The effect of a new compound is first tested on mouse tumors. If it kills these tumors cells, it has then to undergo tests for toxic effects on different systems. In this process it is often necessary to change the structure of the original molecule so that it can kill cancer cells but not have undesired effect on the rest of the body. The entire process may take a very long time, up to ten years from the first tests in mice to the time the drug comes, for what is called, phase one trial.

Certain advantages in the chemotherapy of infectious diseases (bacterial, fungal or parasitic) do not exist to aid in the treatment of cancer. Most important, the concept of selective toxicity is usually not operative. The often-significant biochemical differences between the cells of the host and the invading organism simply do not exist between the cancerous cells and normal cells of the same tissue. The great majority of anticancer drugs bring about a general nonselective interference in cellular processes and cell growth. In fact, many of these cytotoxic agents are almost equally detrimental to normal and neoplastic tissues. Therefore it is not surprising that these drugs produce serious and often debilitating side effects. The usually high doses of these cytotoxic agents that are needed to arrest tumor growth or to obtain a temporary remission of symptoms will also depress bone marrow function; causing drastic reductions in the number of platelets, leukocytes and lymphocytes, which in turn lead to greater susceptibility to infection and internal bleeding. One of the basic tenets of cancer chemotherapy is that all neoplastic cells be eradicated, if a cure is to be achieved. Whether the modality used is surgery, radiation or chemotherapy, or most often a combination of these, total cell kill is the objective of treatment (Gringauz, 1997).

(Crowe) Many organotin compounds have been tested by the United States' National Cancer Institute. Surprisingly and interestingly enough, the percentage of active compound against P-388 lymphocytic leukemia in mice was indeed very high, up to almost 50% for those complexes, which contain diorganotin - dihalides (Gielen 1986). During the period 1973-1977, the Institute for Organic Chemistry TNO, Utrecht, The Netherlands carried out a wide variety of tests on organotin compounds including anti-tumor activity, some 115 complexes were found to exhibit reproducible activity against P-388 lymphocytic leukemia in mice. The screenings results indicate that diethyltin dihalide adducts tend to show the highest activity (Crowe 1987).

A consideration of the screening results for a series of complexes (where $R=C_n$, $n=1-6$, $X=Cl, Br, I$) reveals that many more dibromo-complexes are active than dichloro- or diiodo- compounds, whilst for R the diethyl- and / or diphenyl- tin complexes usually possess the highest activity. However, no real link between the acceptor strength of the parent organotin halide and activity can be discerned (Crowe 1984). The majority of the ligands used were bidentate to ensure that the resulting octahedral complex possessed cis-halogens, which has been shown in the case of platinum compounds, to be an essential requirement for activity (Cleare 1974).

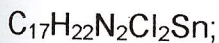
2.56A Another study of metallocene dichloride (VI) has shown that the anti-tumor activity of such compounds, as well as that of cisplatin, may be dependent upon the Cl-M-Cl bond angle and hence the corresponding non bonding Cl---Cl distance (bite). Only those compounds for which the Cl-M-Cl angle is $<95^\circ$, giving a bite size of $<3.6\text{\AA}$ are active (Kopf *et al* 1983). But the X-ray structural parameters of some of the diorganotin dihalide complexes show that the Cl-M-Cl bond angles of both active and inactive compounds are all of the same magnitude. This suggested that the mode of action for the formation of metal-base cross-links for the organotin depends more on the Sn-N bond lengths rather than the Cl-M-Cl bond angle (Crowe 1984) The structure / activity relationship for diorganotin-dihalide complexes is that the Sn-N bond lengths appear to determine the anti-tumor activity

(Crowe 1984). The more stable complexes exhibit lower activity. Those with an average Sn-N bond length larger than 2.39\AA are active, whereas those with bond length lesser than 2.39\AA are inactive. This implies that a predissociation of the (bidentate) nitrogenous ligand might be a crucial step in the formation of tin-DNA complex (Gielen 1986).

The mode of action of cisplatin and its analogues in their anti-tumor activity appears to be fairly well established; the X-ray structure clearly shows that cisplatin cross-links the DNA and that the binding sites are the N-7 nitrogen atom on two adjacent guanine rings of the same chain. Furthermore, to accommodate the platinum atom in this structure, the guanine molecules must be tilted away from the DNA helix, and so interfering with replication. Since, the tin complexes were structurally similar to those of platinum; it was expected that their mode of action would also be similar (Crowe *et al* 1984).

Based on these facts we thought it worthy to undertake this investigation on the Genotoxic effect as well as its anti-tumor potentiality of a new organotin compound $\text{Et}_2\text{SnCl}_2 \cdot \text{L}$ {L=N-[p-(2-pyridylmethylene) methylbenzenamine]} in mammalian system. The advantage of this new complex is that it has Sn-N bond lengths of Sn-N (1) = 2.45\AA and Sn-N (2) = 2.56\AA which is higher than 2.39\AA . Another advantage is that this new organotin compound is a diethyltin dihalide adduct. It is therefore believed to be quite unstable and in turn facilitates the formation of a tin-DNA complex.

Formula:



Properties:

Mol. wt. 444.0; Pale yellow in color. Soluble in ethanol (<2%). Hereafter called OTC

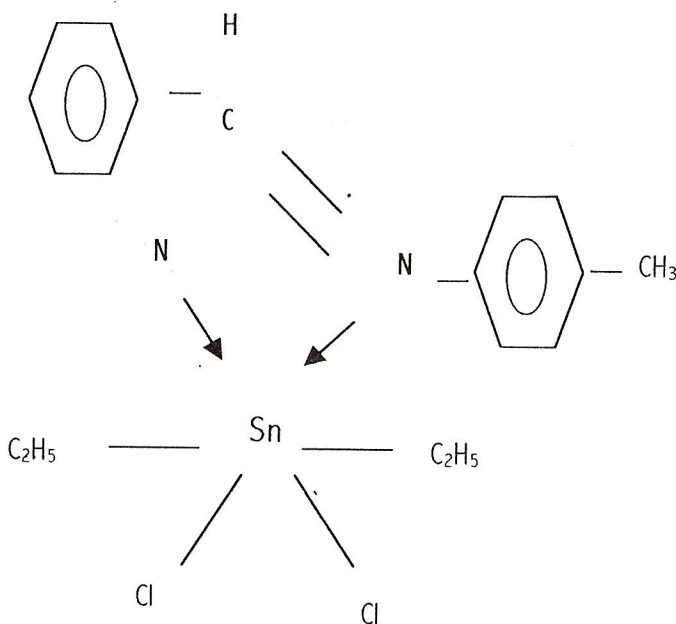
From the background, it is expected this new organotin compound can form better organotin-DNA complex. Therefore the objective of this project is to investigate:

Objective:

1. Genotoxic potentiality of $\text{Et}_2\text{SnCl}_2 \cdot \text{L}$ { $\text{L} = \text{N}$ -[*p*-(2-pyridylmethylene) methylbenzenamine]} in both *in vivo* and *in vitro* systems :

2. Establish a relationship between genotoxic effects and the endogenous GSH levels. Since the outcome of the sample exposure to organotin compound is determined by the detoxification status, the level of glutathione-S-transferase and glutathione will be estimated.

3. Anti-tumor activity in the mammalian system.



Structure of OTC

Determination of Lethal dose

Most anticancer drugs have a relatively poor therapeutic index. For this reason the majority of antiproliferative agents in current use are given at very close to the maximum tolerated dose (MTD). This precludes the use of healthy volunteers that makes such studies potentially hazardous. It has been the standard practice to establish the MTD in rodents and extrapolates to man using some form of stepwise escalation to reach a maximum tolerant dose (Phase I), which can be applied in tumor-specific studies of anti-tumor activity (Phase II).

A number of different schemes have been used for determination of a safe starting dose based on animal toxicology. The NCI, US reviewed the data from mice and dogs and concluded that one-tenth the LD₁₀ (Lethal dose₁₀ – This is the dose of drug which will kill 10% of the exposed animals within 30 days) in mice is a safe starting dose provided this is tolerated in dogs in Phase 1 clinical trial (Judson 1995).

In this study, a few preliminary experiments were carried out so as to determine an appropriate dose for use in all further studies. Some experiments were also carried out with buthionine sulfoximine (BSO; Glutathione depleting agent). (The importance of BSO will be discussed in the following chapter).

Table 1. To determine the LD_{10/30} for a colony of mice exposed to OTC.

Dose	No. of animals exposed	No. of dead animals(%)
0	10	0
5	20	0
15	20	3(15)
30	20	8(40)
BSO+15	20	10(50)
BSO+30	20	13(65)

Therefore, in all further experiments, 15mg kg^{-1} was used, as it appears to be close to the $\text{LD}_{10/30}$. Although, in some experiments 30 mg kg^{-1} was also used.