A Critical Review of the Therapeutic Potential of Dibenzyl Trisulphide Isolated from *Petiveria alliacea* L (Guinea hen weed, anamu)

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ABSTRACT

The data compiled in the present review on dibenzyl trisulphide (DTS) isolated from Petiveria alliacea *L (the guinea hen weed or anamu) revealed that the compound and its derivatives could be of tremendous pharmaceutical interest. The mode of action elucidated for DTS revealed that it is a mitogen activated protein extracellular regulated kinases 1 and 2 (MAPKinases erk1 and erk 2) signal transduction molecule. Dibenzyl trisulphide caused hyper-phosphorylation of growth factor induced MAPKinases (erk 1and erk 2) phosphorylation, a process critical for the improvement of long term memory, and is implicated in neuronal growth. Dibenzyl trisulphide and its derivatives exhibited potent anti-proliferation/cytotoxic activity on a wide range of cancer cell lines. The cytotoxic activity of DTS was increased by 70 – 1000 fold when bound to albumin* in vitro. *Dibenzyl trisulphide seems to have a cytokine switching mechanism in which it down regulates cytokines from the Type 1 helper cells (Th -1 cell) pathway which contained several pro-inflammatory cytokines and up-regulates those on the Type 2 helper cells (Th-2) pathway. The trisulphide up-regulates some reticuloendothelial system parameters eg granulocyte counts and increased thymic and Peyer's patches masses via cell proliferation processes which are known to be regulated* via *the MAPKinase signal transduction pathway. When the zygotes of* Asternia pectinifera *(Starfish) were exposed to DTS at concentration of 10 mM, a dose lethal to all cancer cells tested, it was observed that the sensitive process of protein biosynthesis was not affected. Similarly, the proliferation of the HOFA human fibroblast, a non-cancerous cell line, was not severely affected by DTS at 8.9 μM over seven days, a concentration also lethal to most cancer cell lines tested. The implications of the findings will be highlighted in the present review.*

Un Estudio Crítico del Potencial Terapéutico del Trisulfuro de Dibencilo Aislado a Partir de *Petiveria alliacea* L (yerba de Guinea, anamú)

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RESUMEN

Los datos compilados en el presente estudio sobre el trisulfuro de dibencilo (TSD) aislado a partir de Petiveria alliacea *L (yerba de Guinea, ó anamú) revelaron que el compuesto y sus derivados podrían tener extraordinario interés farmacéutico. El modo de acción esclarecido en el TSD, reveló que se trata de una molécula de transducción de señales de proteínas kinasas 1 y 2 (MAP quinasas ERk 1 y 2) reguladas extracelularmente y activadas por mitógenos. El trisulfuro de dibencilo causó hiperfosforilación de la fosforilación de las quinasas MAP (Erk 1 y 2) inducidas mediante factor de crecimiento, un proceso crítico para el mejoramiento de la memoria a largo plazo, y que está implicado en el crecimiento neuronal. El trisulfuro de dibencilo y sus derivados mostraron una poderosa actividad citotóxica y antiproliferativa en una amplia gama de líneas celulares de cáncer. La actividad citotóxica del TSD se incrementaba de 70 á 1000 veces, cuando se vinculaba a la albúmina* in vitro. *El trisulfuro de dibencilo parece poseer un mecanismo conmutador citoquínico que regula por decremento las*

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citoquinas provenientes de la vía de las células auxiliares de tipo 1 (células Th-1), que contiene varias citoquinas pro-inflamatorias y regula por incremento las de la vía de las células auxiliares de tipo 2 (Th-2). El trisulfuro regula por incremento los parámetros del sistema reticuloendotelial, p.ej. los conteos de granulocitos y el aumento tanto de las masas tímicas como de las masas de placas de Peyer, a través de los procesos de proliferación celular, de los cuales se sabe que son regulados mediante la vía *de la transducción de señales de la quinasa MAP. Cuando los cigotos de* Asternia pectinifera *(estrella de mar) fueron expuestos al TSD a una concentración de 10 mM – una dosis letal para todas las células cancerosas sometidas a prueba – se observó que el proceso sensible de biosíntesis de la proteína no era afectado. De modo similar, la proliferación del fibroblasto humano HOFA – una línea celular no cancerosa – no fue afectada severamente por el TSD a 8.9 μM en siete días – una concentración letal para la mayoría de las líneas celulares cancerosas sometidas a prueba. Las implicaciones de los hallazgos se pondrán de relieve en el presente estudio.*

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INTRODUCTION

The urgent need to find effective and safe therapeutic agents to treat cancers and auto-immune diseases such as Type 1 diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis has been a challenge for the pharmaceutical companies (1). Recent studies have revealed that of the top 150 propriety drugs used in the western hemisphere, 57% contained at least one major active compound derived from natural sources (2). However, one of the major aims of the therapeutic industry is to find small molecules which regulate the biochemistry of disease cells *via* signal transduction modes of action (3). Dibenzyl trisulphide (DTS) is one such molecule (Figure). Dibenzyl trisulphide

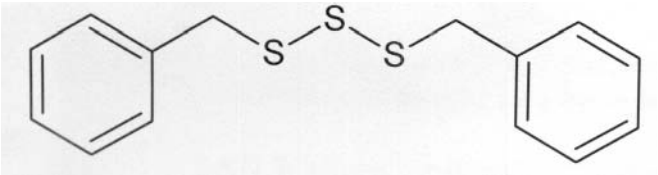

Figure: Dibenzyl trisulphide

was first coded at DBTS when its insecticidal/repellent activities were discovered (4) and re-coded as DTS when its therapeutic potential was found (5). The signal transduction pathways regulate cell biological processes *eg* gene expression, transcription, differentiation, cell division and apoptosis generated from interaction/binding of molecules to cell membrane receptors. One of the most intensely investigated therapeutic signal transduction pathway is that which regulates the process of apoptosis or programmed cell death. The apoptotic signal transduction cascade is implicated in several cancers, Alzheimer's, Parkinson's, transplant rejection, auto-immune disorder such as diabetes, acquired immune deficiency syndrome and Hodgkin's lymphoma (6).

Implication of DTS in the treatment of cancers

The anti-proliferation/cytotoxic activity of DTS was first elucidated by Rosner's group (7) using the human SH-SY5Y neuroblastoma cells. The cell differentiation effects on HL-60 promyelocytic cells has also been reported (8). Subsequently, the IC_{50} values in μM concentrations (data in parentheses) were reported for DTS on several human cancer cell lines using different bioassay techniques (9,10): SH-SY5Y neuroblastoma (0.43 μM) (9), MCF-7 mammary carcinoma (2.24 μM and 6.6 μM) (9;10), IPC-melanoma (2.90 μM) (9), A549 small cell lung cancer (15.85 μM) (9), A637 primary bladder carcinoma (18.84 μM) (9), Jurkat leukaemia (0.35 μM) (10), ovarian A2780 and OVCAR4 (0.40 μM and 1.4 μM, respectively (10), fibrosarcoma HT1080 (1.9 μM) (10), non-small cell lung cancer H460 (5.1 μM) (10), breast M231 (2.4 μM) (10) and adenocarcinoma HeLa (2.5 μM) (10). In addition, it was reported that 0.56 μM of DTS gave 44.67 % anti-proliferation/cytotoxic activity on the human TE-671 sarcoma cell line (9). The cytotoxic action of DTS on the human SH-SY5Y neuroblatsoma cell line was enhanced by complexing DTS to Bovine Serum Albumin by 70 fold *in vitro* (11, 12). Current research has revealed that this enhancement in cytotoxicity can be as great as 1000 fold. Based on modes of action studies, DTS could be useful against prostate cancers for the following reasons:

(i) DTS was found to enhance the binding of mitogen-activated protein kinases phosphatase-1 (MKP-1) to its substrate 3-O-methyl-fluorescein phosphate cyclohexyl ammonium salt (OMFP) *in vitro* (12). MKP-1 is a dephosphorylator of various MAPkinases including extracellur regulated kinases 1 and 2 (erk1/2) (13).

(ii) it is known that in prostate cancer the phosphorylation of erk 1/2 are markedly increased, up to 1600% have been reported (14). Thus, if DTS enhanced the activity of the dephosphorylator MKP-1, it could be implicated in the treatment of prostate cancers as a possible down regulator of the phosphorylation on erk1/2. Moreover, MKP-1 is now recognized as a potential therapeutic target in cancer chemotherapy (15).

The final common pathway in the anti-cancer activity of DTS appears to be apoptotic cell death (9). IPC-melanoma cells exposed to 1.0 μM of DTS undergo nuclear fragmentation to produce micronuclei, indicating mitotic catastrophe (9). The fragmentation of the nucleus in cells exposed to cytotoxic agents is one of the diagnostic features of

apoptosis. The fact that DTS has a strong binding affinity for albumin and red blood cells (RBCs) may have implications for the treatment of cancers in the central nervous system. These multiple mechanisms clearly indicate the potential utility of DTS in the treatment of a range of cancers.

Implication of DTS in the treatment of auto-immune diseases

Data obtained from Human Mixed Lymphocytes Responses and CD3 dependent activation revealed that DTS down regulates Th-1 cytokines (11) to which the pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), IL-1 (b) and IL-8 belong. While the Th-2 cytokines such as IL-4 were up-regulated. The preferential up-regulation of the IL-4 group of cytokines is an important observation since they are known to regulate the reticulo-endothelial system (bone marrow functions) via the MAPkinase signalling pathway. Dibenzyl trisulphide caused an increased in the production of granulocytes and erythrocytes in mice (5, 11) suggesting that it has an effect on bone marrow activity-possible on stem cells themselves. Elevation in the levels of pro-inflammatory cytokines are associated with the onset of autoimmune diseases such as Type 1 diabetes, rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus (16, 17). The anti-infective activity of DTS was highlighted by the fact that the molecule prevents the establishment of murine hepato-actinomycosis which is a cell mediated/humoral immune and phagocytosis down regulating infection (11).

Dibenzyl trisulphide caused an increase in thymic weight in old mice by 52.29% (5, 11); histological analyses of the thymic sections revealed that there was a proliferation of cells in the cortical region infiltrating the medulla (11). It is recognized that mitogenesis (MAPKinase/p21^{ras}) signalling pathway activated from the immuno-receptor tyrosine based activating motifs (ITAMS) within the CD3-TCR receptor complex is critical for positive/negative thymic cell production (18). Cancer patients' ability to generate T-lymphocytes is inversely related to their age, suggesting an indirect contribution from thymic involution (19). Similarly, the recovery of CD4+ cells is inversely related to age and was enhanced in patients with an enlarge thymus after chemotherapy (19). The functional status of the thymus is regulated by an immuno-endocrine-neurological input emerging from the hypothalamus-pituitary axis (19). These findings are not surprising because of the fact of the interconnectivity of the immune, hormonal and nervous systems via the Zn^{2+} dependent activating Sigma receptor which is mediated via a selective up-regulation of Th-2 cytokines *eg* IL-4 and IL-10 in conjunction with thymulin. Thus, the potent cytotoxic effect of DTS on melanoma cells which carry Sigma receptors may support the fact that DTS could be a Sigma receptor agent. Presently, the Sigma receptor group of therapeutic drugs are being critically evaluated for treating/managing various forms of human leukocyte antigen-DR (HLA-DR) immune system dysfunction diseases such as diabetes, osteoarthritis, acquired immune deficiency syndrome (AIDS), bronchial asthma and cancers. In addition, the loss of a functional thymus or its absence is common among individuals with congenital immunodeficiency diseases *eg* DiGeorge Syndrome (DGS) and Chromosomal Breaking Syndromes (CBSs). The thymus is one of the sites producing cytotoxic lymphocytes (CTLs) which produced the serine protease enzyme, granzyme B, a potent inducer of apoptosis in cancer cells. Thus, the thymic enlargement effect of DTS is worthy of detailed investigations.

The manipulation of various homing factors *eg* L-selectin-mAb LM1-3 and addressin-mAdCAM-1 and integrins such as alpha4beta7 as therapeutic targets on Peyer's patches dendritic cells is a rapidly expanding field in drug development against intestinal bowel diseases (IBD) *eg* colitis, colorectal carcinoma and Chrohn's disease (20; 21). DTS caused an enlargement in Peyer's patches possibly by an activation of cell proliferation by 45.65% (5, 11). In view of this finding, we proposed that the effect of DTS on the Peyer's patches should be elucidated with the hope of developing drugs against IBD.

Implication of the up-regulation of cadherin-5 (VE-cadherin)

Dibenzyl trisulphide up-regulate the expression of cadherin-5 (vascular endothelial cadherin [VE-cadherin]) in human SH-SY5Y$_{TRK-A}$ neuroblastoma cells pre-treated with nerve growth factor (NgF) (11). Cadherin-5 is one of the important factors responsible for the stabilization of tight junctions. The loss of cadherin-5 mediated adhesion has been known to play an important role in the transition of epithelial tumours from a benign to an invasive state (22), and also in diabetic retinopathy. Thus, beside the direct toxic effect that DTS has on various forms of cancer cells, it also has the ability to inhibit their metastasis via the up-regulation of cadherin-5.

Implication for the inhibitory effect of DTS on glycation

From 1D ^{1}H NMR studies, DTS interact with Bovine Serum Albumin (BSA) at two main sites (a) at 1.6 – 3.4 ppm (multiplet) and (b) at 6.8 ppm (11). The first site could be attributed to an interaction on the redox sensitive *episolon*-lysine envelope or on threonine. The second interaction signal is associated with the tyrosine envelope of the BSA (11). Dibenzyl trisulphide was found to inhibit the binding of glucose to BSA (O'Connar personal communication). From the above findings, the following hypothesis is proposed: that DTS is interacting with the *episolon*-lysine residue in the BSA which suggest that the Amadori re-arrangement binding interaction was blocked. The Amadori interaction (glycation) between proteins and glucose is central to the generation of free radicals and destruction of proteins in the body and is the cause of various degenerative diseases such as arterial

stiffening, cataract and neurological impairment (23). Therefore, it would appear that DTS could be developed as an anti-gly-cation molecule to halt the rapid progression of some degenerative diseases associated with free radicals and stress. It is also worthy of note that DTS is able to protect BSA from denaturation by 50.83% at 500 ng/mL (0.5 µg/mL) at pH 6.0 (11). This is a feature of non-steroidal anti-inflammatory drugs (24, 25).

The implication of DTS/growth factor induced hyper-phosphorylation of MAPKinases (erk 1 and erk 2) for memory enhancement

Dibenzyl trisulphide (DTS) hyper-phosphorylated MAP-Kinases (erk 1 and erk 2) signalling induced by growth factors *eg* basic fibroblast growth factor (bFGF) by 30% at 1.0 µM or nerve growth factor (NgF) at 0.5 µM (7, 11). The MAPKinase pathway is required for both long-term recognition memory and is associated with hyper-phosphorylation of erk 1 and erk 2 in different sub-regions of the entorhinal cortex-hippocampal circuitry (26). In addition, the medial division of the medial geniculate nucleus and adjacent posterior intralaminar nucleus (MGm/PIN) cells that project to the lateral nucleus of the amygdala (LA) contribute to memory formation via erk 1 and erk 2 mediated transcriptions (27). This phenomenon leads to the production of acetylcholine at the synaptic junction. This could indeed be a biologically plausible mechanism for the potential role of DTS in attenuating memory loss. It is also important to note that *P alliacea*, from which DTS was isolated is used by the Amerindians of Latin America to improve memory (28) and for headaches and epilepsy in Jamaica.

Implication of DTS induced cell-cell attraction possibly through a polarization effect on ankyrins

Erythrocytes (red blood cells) separated from white blood cells (WBCs) when exposed to DTS in buffered solution induced cell-cell contact with morphological changes without lysis, at concentrations higher than those effective on cancer cells (11, 29). Spectrins are responsible for maintaining the morphological integrity of RBCs. Since DTS interacts with the tyrosyl residues on albumin *eg* bovine serum albumin (7, 11), we inferred that the attraction induced by DTS among the RBCs could be on a polarized tyrosine rich domain such as ankyrins (29) and not a classical agglutination effect. 1D ^{1}H NMR analyses revealed that the interaction between DTS and RBCs gave a similar aromatic signal to that observed for the DTS-BSA interaction (11) which suggest that tyrosine is also involved. Thus, from these observation we hereby proposed that DTS may activate ankyrin domains, located on neurons, which are capable of inducing attraction and growth in these cells by causing their redistribution to the plasma membrane with spectrin involvement (30). The above mentioned phenomenon involve the phosphorylation on tyrosine residues in the ankyrins (31), which is one of the central binding modes of DTS leading to the activation of the MAPKinase pathway (7, 11). MAPKinase phosphorylation emerging from tyrosine residue situated on axons is one of the processes implicated in the regeneration/growth and possible repair of damaged neurons (32).

DTS as a pharmaceutical prototype

Unlike the complex nature of some of the most promising anti-cancer drugs such as taxol and vinblastine, DTS is a simple molecule. Several derivatives of DTS were produced, all having the trisulphide bridge which was proposed as the critical site for biological activity (7) at low cost, some with higher cytotoxic activities (10, 33). In addition, DTS was transformed using 2-mercaptoethanol in methanol to methyl benzyl sulfonic anhydride, a molecule with potent anti-microbial and agrochemical activities (34). Other "simple molecules" which function as signal transduction inhibitors such as Iressa and Gleevec are now offering new hope for cancer victims (3, 35).

Toxicity

Dibenzyl trisulphide at 10 mM did not have any effect on the sensitive process of protein biosynthesis in Starfish (*Asternia pectinifera*) embryos (11). In addition, it seems to have some degree of selectivity to pathological cells, since it was found not to be toxic to the human fibroblast (HOFA), a non-cancerous cell line over seven days(9). In addition, concentrations of up to 34 mg/kg body weight did not cause mortality to mice. Dibenzyl trisulphide seems to activate the bone marrow at 11 mg/kg body weight since granulocytes differential count was increased by 62.75% (5).

CONCLUSION

The authors hereby proposed that DTS is a polysulphide mitogen with a wide range of implications in medicine and therapeutics. The molecule signals *via* the complex MAP-kinase pathway. The fact that its cytotoxic activity is increased by 70 to 1000 fold when bound to albumin suggests that the intravenous use for cancer treatment would be most optimal. The albumin-DTS complex should also be carefully validated intravenously for its cytotoxic efficacy since DTS may bind to other proteins beside albumin in the blood which could compromise its efficacy.

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