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Solasodine Rhamnosyl Glycosides Specifically Bind Cancer Cell Receptors and Induce Apoptosis and Necrosis. Treatment for Skin Cancer and Hope for Internal Cancers

Bill E. Cham
Australasian Medical Research, P.O. Box 7031, Port Vila, Republic of Vanuatu, Australasia

Abstract: Cattle farmers in Australia have proclaimed that crushing and application of the fruit of a weed known as Devil's Apple retarded the progress of ocular squamous cell carcinoma in Hereford cattle. The Devil's Apple plant, Solanum linnaeanum (also known as Solanum sodomaeum and Solanum hermannii), is a member of the same family as eggplant, potatoes and tomatoes. In 1987 it was reported that the fruit of the Devil's Apple plant contained a mixture of solasodine glycosides. The mixture of solasodine glycosides was constant when extracted from the fruits of the Devil's Apple plant and was given the name BEC which consisted of the triglycosides solasonine, solamargine and di-and monoglycosides. All the glycosides contained the same aglycone an alkaloid solasodine and the sugar moiety consisted of rhamnose, glucose and galactose. BEC has antineoplastic properties against a wide variety of human cancers in cell culture, tissue culture and is very effective against terminal tumours in animals. BEC not only prolonged the life of animals with terminal cancers, but the cancers were completely eliminated rendering the animals cancer free for the remainder of their normal life span. BEC in a topical cream formulation is now available clinically for the treatment of non malignant and malignant human skin cancers. BEC is very selective in killing cancer cells without harming normal cells due to a unique mode of action. In addition BEC exerts its antineoplastic activity on cancer cells at proliferative as well as "resting" (nonproliferative) stages of their life cycles. BEC, due to its selectivity and efficacy is superior to other well established antineoplastics. The observations that BEC has good antineoplastic activities, together with a very high safety profile, have paved the way for much work currently being undertaken to establish the potential of BEC as a new antineoplastic agent for internal cancers.

Key words: Cancer, solasodine glycosides, BEC, skin cancer, apoptosis, lysosome, mitochondria

INTRODUCTION

Natural products and drug discovery: Plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2600BC.

Today, approximately 80% of the world's population depends on traditional plant-based medicines for primary health care. The remaining 20% of the world's population also depends on plant products for health care. About 25% of prescription drugs dispensed in the United States of America contain plant extracts or active ingredients derived from plants.

Natural products should continue to be an important part of drug development well into the future. Folklore amongst farmers in Australia indicated that the crushing and application of the fruit of a weed known as Devil's Apple (Solanum linnaeanum, also known as S. sodomaeum or S. hermannii) retarded the progression of ocular squamous cell carcinoma in Hereford cattle.

MATERIALS AND METHODS

Solasodine: Studies on the anecdotal information that the Devil's Apple plant had carcinostatic properties against ocular squamous cell carcinoma in Hereford cattle led to the extraction of alkaloids from the fruit of the Devil's Apple. Analysis of the extracted alkaloids revealed that the fruit of the Devil's Apple contained solasodine in the "free" form and in conjugated forms (Cham *et al.*, 1987).

Solasodine is a steroidal alkaloid based on a C27 cholestane skeleton. This compound is essentially a nitrogen analogue of steroidal saponins. In nature these alkaloids defend plants against predators. The aglycone solasodine has a chemical structure very similar to diosgenin and has replaced diosgenin as an important source of raw material for the synthesis of steroid drugs. Solasodine, a nitrogen analogue of diosgenin is reported to be the sole source of cortisone and progesterone. Solasodine occurs in numerous species of the Solanum genus.

Solasodine conjugates have antispermatozoal properties due to their antimitochondrial activities (Daunter and Cham, 1990; Cham, 1996). These observations have since been confirmed as shown by antiandrogenic sequelae resulting in male infertility in dogs by solasodine administration (Gupta and Dixit, 2002).

Anticancer studies with solasodine were uneventful. Solasodine even at relatively high concentrations did not show evidence of antineoplastic activity (Daunter and Cham, 1990; Cham *et al*., 1990; Cham, 1991, 1993; Lee *et al*., 2004; Esteves-Souza *et al*., 2002; Gharzi and Lees, 1990; Cheng *et al*., 1998; Solbec).

Other biological activities of Solanum alkaloids are increasingly being investigated in an attempt to obtain therapeutic application in clinical medicine. It appears that the aglycone solasodine has much less biological activity when compared with its glycosidic forms. It was first reported in 1987 that the sugar moieties of solasodine glycosides were essential to exert biological activity (Cham *et al*., 1987; Daunter and Cham, 1990; Cham *et al*., 1990; Cham, 1991, 1993). Since then much interest has been focused on the solasodine glycosides instead of the alkaloid solasodine and it has now been confirmed by many studies that, in general, biological activity of these glycoalkaloids is dependent on the presence of sugars, in particular rhamnose, for activity (Daunter and Cham, 1990; Cham *et al*., 1987, 1990; Cham, 1991, 1993, 1994; Lee *et al*., 2004; Esteves-Souza *et al*., 2002; Gharzi and Lees, 1990; Cheng *et al*., 1998; Solbec; Chataing *et al*., 1998; Levey and Sipollini, 1998; Yoshikawa *et al*., 2007).

BEC: Follow-up studies on anecdotal information that the Devil's Apple plant had carcinostatic properties and the failure to show that the aglycone solasodine extracted from the fruit of the Devil's Apple plant had anticancer properties prompted further studies with emphasis on the conjugated forms of solasodine.

In 1987 it was reported that the fruit of the Devil's Apple plant contained a mixture of glycoalkaloids, solasodine glycosides (Cham *et al*., 1987). The mixture of the solasodine glycosides was found to be constant when extracted from the fruits, leaves and stems of the Devil's Apple plant and is now known as BEC (Daunter and Cham, 1990; Cham *et al*.,1987, 1990; Cham, 1991, 1993, 1994, 1998; Solbec).

BEC is a standard mixture of triglycosides solasonine (22R, 25R)-Spiro-5-en-3,-yl O--L-rhamnopyranosyl-(1->2gal)-O-,-D-glucopyranosyl-(1->3gal)-,-D-Galactopyranose (33%), solamargine (22R, 25R)-Spiro-5-en-3,-yl O-,-L-rhamnopyranosyl-(1->2glu)-O-,-L-rhamnopyranosyl-(1->4glu)-,-D-glucopyranose (33%) and their corresponding di-and monoglycosides (34%). All the glycosides contain the same aglycone, solasodine.

Solasonine contains one molecule of rhamnose and solamargine contains two such molecules (Fig. 1). The majority of the solasodine diglycosides also contains rhamnose. It is important to realize that rhamnose is a plant sugar and is not usually found in mammalian species.

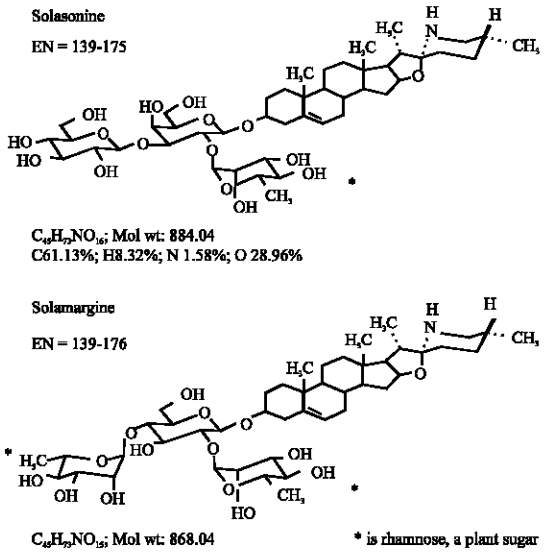

Fig. 1: Structures of solasonine and solamargine

Toxicity of BEC: Acute toxicity studies in mice established that for single intraperitoneal (i.p.) administration of BEC the LD. is 30 mg kg. and in rats the i.p. LD. is 41 mg kg.. The LD. for a single dose by gastric intubation in mice is 550 mg kg.. Multiple i.p. dose studies manifested that the LD. for mice by 14 daily single i.p. injections is 10 mg kg.. The LD. for rats by 8 i.p. administration over 8 days with one injection day. is 20 mg kg. (Cham *et al*., 1987).

Macroscopic postmortem examination following administration of BEC revealed no well-defined symptoms directly attributable to toxic effects of the glycoalkaloids; nevertheless, these solasodine glycosides are known to be cholinesterase inhibitors. Symptoms of acute toxicity are depressed nervous system activity, followed by failure of heart and respiratory system (Cham, 1988). Lethal toxicity studies with solamargine, one of the main components of BEC, revealed that the single dose i.p. LD. in rats is 42 mg kg.. It is interesting to note that the LD. for solamargine is identical to that of BEC Furthermore, no appreciable toxic effects are observed at doses below 35 mg kg. body weight as indicated by blood parameters, enzyme levels and histological sections of kidney, liver and cardiac muscle (Chami *et al*., 2003). The toxicological data obtained for BEC was similar to those obtained for solanine, the potato glycoalkaloid (Tschesche and Wulff, 1973).

BEC PRECLINICAL

Ex vivo studies have demonstrated that BEC is effective against a wide variety of human cancers in a

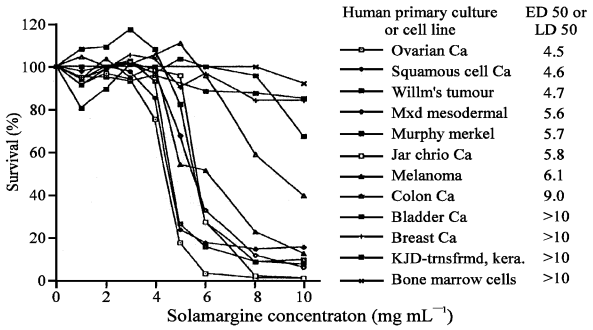

Fig. 2: Effect of solamargine on various primary cell lines and cell cultures

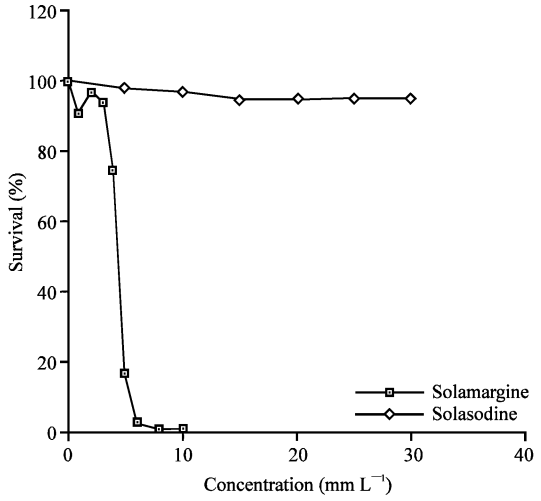

Fig. 3: High concentrations of solasodine (the alkaloid without bound sugars) did not have any significant effect on cancer cells, whereas, low concentrations of solamargine (the glycoalkaloid with bound sugars, in BEC) killed all the cancer cells. It is quite clear that the sugar part when attached to the alkaloid, plays an important role in killing cancer cells

dose-dependent manner and that BEC selectively kills cancer cells without harming normal cells (Fig. 2), (Daunter and Cham, 1990; Cham, 1994).

Consequently further research confirmed and elaborated the original cell culture observation of BEC (Solbec). It has now been well established that a variety of cell types respond differently to BEC. Normal cells are not affected by BEC whereas cancer cells are destroyed by BEC.

Cell culture studies show clearly that, in order for antineoplastic activity to be observed, the compound must be in a glycosidic form. Figure 3 shows that the alkaloid solasodine is not effective in killing cancer cells, even at high concentrations. Whereas, solasodine glycosides are very specific in eliminating cancer cells. The sugar moiety without the solasodine does not exhibit anticancer properties (Daunter and Cham, 1990; Cham *et al*., 1990). These observations have been confirmed with other cancers such as Ehrlich carcinoma and human K562 leukemia where it was shown that solasonine and a mixture of glycoalkaloids containing solasonine inhibited the growth of these cancers in a dose-dependent manner. The aglycone solasodine has low antineoplastic activity thus confirming that the role of the sugar moiety is very important in the cytotoxic activity of solasonine (Esteves-Souza *et al*., 2002). Other *in vitro* cytotoxic and antitumour studies of total glycoalkaloids from S. pseudocapsicum determined the potential for these glycoalkaloids as a possible therapeutic application (Vijayan *et al*., 2002).

Studies of glycoalkaloids and their metabolites on the inhibition of the growth of human colon (HT29) liver (HepG2) cancer cells and normal human liver HeLa (Chang) cells determined that the glycoalkaloids solamargine and solasonine but not their aglycone solasodine was effective against the cancer cells without harming normal cells and that these glycoalkaloids had superior anticancer effects when compared with doxorubicin and camptothecin. Importantly, -solanine and -chaconine (both contain the aglycone solanidine) which have the same sugar moieties as solasonine and solamargine (both contain the aglycone solasodine) respectively did not differentiate between cancer cells and normal cells (Lee *et al*., 2004) confirming previous observations (Cham, 1996). This indicates that both the glycosidic moiety and solasodine but not solanidine are important for substantial specific antineoplastic activity. Nevertheless, the toxicity of -solanine and BEC in mice are similar (Cham *et al*., 1987).

Multidrug resistance is a common problem in the treatment of cancer. The glycoalkaloids in BEC, in particular solamargine, have been investigated as a combination therapy with prevalent chemotherapy agents in the treatment of lung cancers.

Expression of TNF receptors are lost in advanced lung cancers and human A549 lung adenocarcinoma cells are resistant to the cytotoxic effects of TNF- and cisplatin. Solamargine elevated the expressions of TNF-R1 and -R2 and overcame the resistance of A549 cells to TNF- and -. Combinational treatment of solamargine and cisplatin synergistically enhanced apoptosis. Solamargine sensitizes A549 cells through TNFRs and expresses anticancer potential against TNFs-and cisplatin-resistance lung cancer cells (Liang *et al*., 2004; Liu *et al*., 2004).

Dose response curves of solamargine, vinblastine and cisplatin with ovarian cancer cells and fibroblasts have established that the absolute concentration of vinblastine for killing cancer cells is 6 times and for cisplatin 40 times higher than solamargine. Moreover, the therapeutic index LD normal cells/ED cancer cells is much higher for solamargine than cisplatin and vinblastine. The therapeutic index is a measure of selectivity in killing cancer cells relative to normal cells. The higher the ratio the more specific the compound is to attacking and killing the cancer cells relative to the normal cells. Cisplatin is not specific for killing cancer cells, whereas vinblastine kills more normal cells than cancer cells and solamargine kills more cancer cells than normal cells (Cham, 1993). More recent studies have confirmed that BEC is more effective than other antineoplastic agents such as doxorubicin and camptothecin and when the antineoplastic activity of BEC glycoalkaloids were compared with a wide variety of the best of the standard therapies, BEC was shown to be at least twice as effective. The antiproliferative activities of BEC against human promyelocytic leukemia (HL-60) cells were superior to cisplatin (Ono *et al*., 2006). Furthermore, BEC when tested in combination with other standard chemotherapeutic agents showed a potentiation of the drugs tested (Solbec).

BEC CLINICAL

In vivo studies with terminal tumours in mice, rats and large animals give compelling evidence of the antineoplastic nature of BEC. Single BEC dose studies on mice with the terminal tumour Sarcoma 180 activity was dependent on the number of doses of BEC. Two doses of BEC at 8 mg kg resulted in 42% complete regression of the tumour whereas three and four doses obtained over 92% total regression (Fig. 4). BEC therapy in these animals

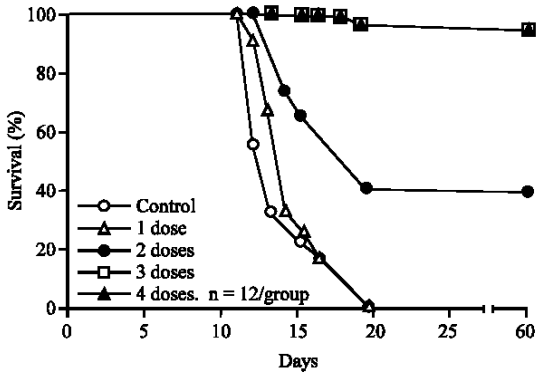

Fig. 4: Survival of mice with Sarcoma 180 treated with varying doses of 8 mg BEC kg

Table 1: LD_{50}, ED_{50} of BEC in μML^{-1} and therapeutic indices of normal cells, cancer cells in cell culture and whole animals. The observed parameters are within the same range whether in cell culture or in whole animals

	LD_{50}	ED_{50}	Therapeutic index LD_{50}/ED_{50}
Cell culture			
Bone marrow cells	34	--	--
Melanoma	--	6.7	5.1
Ovarian cancer	--	5.0	6.8
Colon cancer	--	10.2	3.3
Whole animal-single dose			
Control mice	34	--	--
Mice with Sarcoma 180	--	10	3.4

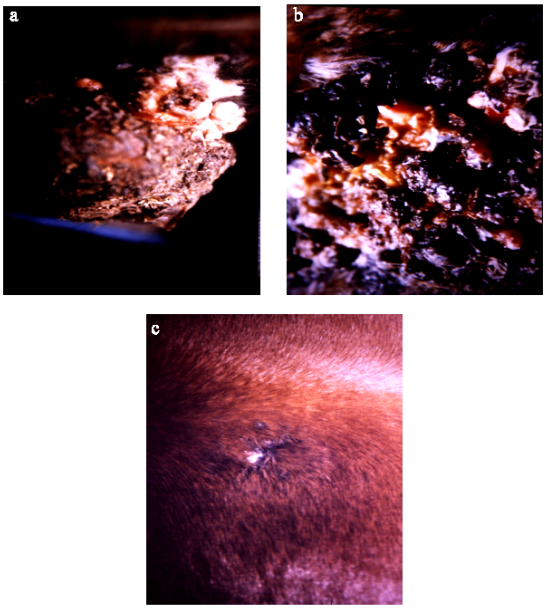

Fig. 5: A sarcoid of approximately 500 grams on the chest of a horse before injection (a); after two injections of BEC, showing the rapid degradation of the cancer (b); and the site where the cancer was after completion of BEC therapy (c)

with Sarcoma 180 not only extended their life span significantly, the tumour was completely ablated and the treated mice had normal life spans (approximately 3 years) when compared with healthy untreated control mice (Cham *et al*., 1987).

An interesting observation is that the LD, ED and consequently the therapeutic indices of BEC are similar whether cell culture or whole animal studies are used as models (Table 1).

Direct intralesion injections of BEC into accessible large tumours in animals are very effective. When administrations of 100 mg BEC kg estimated tumour weight is injected directly into the tumour at multiple sites of the tumour, a rapid response is obtained. Figure 5-7 illustrate horses with cancers that were treated with BEC by direct injection of BEC into the tumour (in preparation).

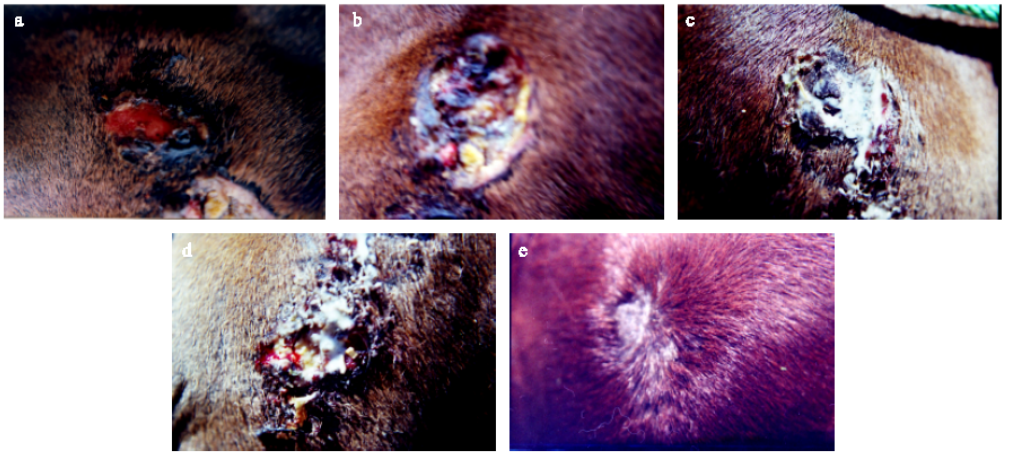

Fig. 6: A melanoma on the chest of a horse before BEC injection (a); during BEC therapy (b-d); and the site where the melanoma was after completion of BEC therapy (e)

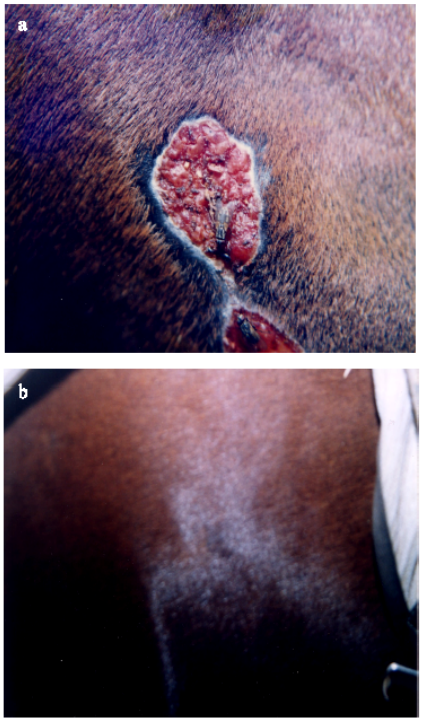

Fig. 7: Two SCCs joined by a bridge on the neck of a horse before BEC injection (a) and after treatment was completed (b). When the treated area was completely healed it was indistinguishable from the horse's normal skin

MODE OF ACTION OF BEC

Specific recognition and binding of BEC by tumour cells: Cells contain endogenous lectins on their plasma membrane that act as receptors for certain sugars, usually in conjugated forms. Once bound to these receptors the sugar conjugate molecule is then internalized ultimately ending up in the lysosome. It was predicated that BEC exerted its antineoplastic activities through interaction of BEC with cancer cells by a biochemical process not previously described. This postulate was based on, firstly; the plant sugar rhamnose is conjugated to solasodine in BEC. Rhamnose is rarely found in mammalian cells (other than in the skin tissue of rabbits) so it is unlikely that normal mammalian cells would have the receptor for this molecule. Secondly, cancer cells may have mutated (oncogenes) such that these cells may have receptors for molecules that do no occur naturally in mammalian cells (Daunter and Cham, 1990).

The specificity of BEC to interact with tumour cells relative to normal cells and the involvement of rhamnose as a key molecule, were shown by the following observations.

Solasodine glycosides but not solasodine have antineoplastic activity. In cell culture work it was shown that the aglycone solasodine (alkaloid without the sugar) did not inhibit uptake of tritiated thymidine of equivalent concentrations of solamargine (alkaloid solasodine conjugated with two rhamnose and one glucose molecules). High concentrations of solasodine did not have any significant effect on ovarian cancer cells (Fig. 3). Thus, the sugar part of the solamargine played an important role in killing cancer cells. It was further shown that the sugar parts (rhamnose and glucose) of the solamargine without the solasodine molecule did not have any effects on the ovarian cancer cells even if the concentrations of these sugars were more than 100 times higher than is found on the solamargine molecule (Daunter and Cham, 1990; Cham *et al*., 1990).

- Rhamnose inhibits the anticancer efficacy of BEC

Mice inoculated with Sarcoma 180 cells resulted in 100% mortality. Four administrations of BEC resulted in complete inhibition of Sarcoma 180 activity and all the animals survived. Injection of increasing concentrations of rhamnose resulted in decreased antineoplastic activity of BEC. This indicated that rhamnose, although at high concentrations, inhibited the efficacy of BEC. These observations suggested that the binding of BEC on tumour cells may be mediated through rhamnose, which forms part of solasonine, solamargine and diglycosides of solasodine in BEC (Cham *et al*., 1990; Cheng *et al*., 1998).

- Mice with advanced cancers tolerated toxic doses of BEC

Mice which were at their terminal stages after inoculation with Sarcoma 180, tolerated and became symptom-free of cancer by single dose administration of BEC at concentrations of BEC three times the LD. for normal mice. After evaluation of a number of possibilities that could have accounted for these observations it was concluded that the Sarcoma 180 cells which were in great abundance in the mice just prior to their terminal stages recognized and bound BEC by means of specific receptors, reducing the bioavailability of BEC to normal cells, which in turn reduced the toxicity of BEC. These *in vivo* observations are in agreement with *ex vivo* studies (Daunter and Cham, 1990).

These results provide evidence that BEC selectively destroys tumour cells relative to normal cells and the mode of entry of BEC into tumour cells appears to be mediated by the sugar moiety rhamnose of the solasodine glycosides (Cham *et al*., 1990). These observations have now been confirmed by many recent studies (Esteves-Souza *et al*., 2002; Cheng *et al*., 1998; Solbec; Chataing *et al*., 1998; Chami *et al*., 2003; Vijayan *et al*., 2002; Hall *et al*., 2006; Verpoorte, 1998; Badami *et al*., 2003; Nakamura *et al*., 1996; Roddick *et al*., 1990) and indeed this receptor has now been identified and characterized as a rhamnose binding protein on cancer cells (Solbec).

This rhamnose binding protein constitutes the receptor on cancer cells an is now known as Endogenous Endocytic Lectin (EEL) receptors (Daunter and Cham, 1990). It has been determined that these EELs are more prevalent on tumour cells than on normal cells (Daunter and Cham, 1990; Cham *et al*., 1987, 1990; Cham, 1988, 1991, 1993, 1994).

Specific tumourocidal effect of BEC: After it was established that binding occurred preferentially on tumour cells but not normal cells, it remained to be determined how and why the cancer cells were destroyed after binding to BEC.

Morphological examination of cancer cell death induced by the binding of the cancer cells to BEC manifested that the formed complex of receptor-BEC resulted in endocytosis of the complex with concomitant events that led to the tumourocidal activity.

It was considered that receptor-mediated endocytosis was the mode of entry of BEC into cancer cells (Daunter and Cham, 1990; Cham *et al*., 1987, 1990; Cham, 1988, 1991, 1993, 1994). It is generally accepted that the "coated pit endocytosis" process is involved with receptor-mediated endocytosis. The coated pit is a specialized region of the plasma membrane that is coated with clathrin which aids the stability and transport processes. The coated pit forms a coated vesicle and then loses its clathrin coat. It then joins other coated pits to form a receptorsome or endosome. Gradual transformation of endosomes results in the formation of lysosomes. The Golgi Apparatus and Rough Endoplasmic Reticulum are also involved in the formation of lysosomes and their hydrolytic enzymes contents.

Original morphological observations by phase contrast microscopy of cancer cells when treated with BEC revealed intense damage in all cancer cell lines but not in normal cells (Daunter and Cham, 1990; Cham *et al*., 1987, 1990; Cham, 1988,1991, 1993, 1994).

Once the BEC-receptor is inside the cell by the receptor-mediated endocytosis process, the complex is eventually taken up by the lysosomes. The lysosome then hydrolyses BEC and the alkaloid solasodine is generated. Solasodine in turn causes the lysosome to rupture. The contents of the lysosome is spilt into the cell. The contents of the lysosome consist of an acid milieu and contain many hydrolytic enzymes that digest fats, proteins, nucleotides and carbohydrates. These enzymes due to their sudden abundance in the cytosol then break down and digest the contents of the living cell which lead to sudden death of the affected cells. Indeed it has been reported that BEC causes the cytoplasm of the cancer cells to undergo dissolution, the nuclei contract and become dark staining, nuclei then enlarge, the chromatin clumps and finally the nuclei disintegrate resulting in cellular debris (Daunter and Chem, 1990). These phenomena have been observed in cell culture, whole animals and in situ skin cancer where they were shown to occur when neoplastic cells were treated with BEC (Daunter and Cham, 1990; Cham *et al*., 1990, 1987).

In order to distinguish possible involvement of other subcellular organelles in the death of cancer cells when exposed to BEC, a model was studied that did not contain lysosomes. Many, if not all, mammalian cells contain lysosomes. However, mammalian spermatozoa have no lysosomes, but contain sac-like structures known as acrosomes (Abou-Haila and Tulsiani, 2001). Interaction of spermatozoa with BEC resulted in immediate immobilization of the spermatozoa but, unlike with cancer cells, the spermatozoa remained intact and did not lyse. Further studies showed that BEC also had antimitochondrial properties (Daunter and Cham, 1990; Cham, 1996). Consequently it was surmised that both antimitochondrial and the rupture of lysosomes were involved with the antineoplastic activity of BEC (Daunter and Cham, 1990; Cham *et al*., 1987, 1990; Cham, 1991, 1993, 1988, 1994). The conclusion has since been ratified by a large body of evidence. Amongst other important properties, lysosomes are also important for their role in apoptosis, the programmed death of certain cells. If the enzymes of a single lysosome are released into a cell, there is little change in the cytosol, but a massive enzymatic discharge by many lysosomes can be fatal to the cell. Apoptosis is induced through a lysosomal-mitochondrial pathway that is initiated by lysosomal rupture (Yuan *et al*., 2002). A lysosomal pathway, characterized by rupture of lysosomal membranes is activated by apoptosis. These lysosomal events occur simultaneously with mitochondrial permeabilization and caspase activation (Paquet *et al*., 2005). Caspase activation is known to be involved in apoptosis and in some systems apoptosis can be effectively inhibited by caspase inhibitors. Cellular events such as mitochondrial damage have been implicated in TNF--induced apoptosis and necrosis. Apoptosis and necrosis appear to be controlled by parallel pathways and these two modes of cell death can be interconvertible under certain conditions. ATP depletion, such as in the case of antimitochondrial activity, can convert cell death from an apoptotic morphology to a necrotic morphology, suggesting that intracellular ATP levels may regulate the mode of cell death (Ono *et al*., 2003). The direct interrelationship of BEC in causing specific cancer cell death by apoptosis has also been confirmed. Solamargine (in BEC) but not khasianine and solasodine possessed potent cytotoxicity to human hepatoma cells. Only solamargine could induce "sub-G1", an apoptotic feature, in flowcytometry (Cham, 1996; Cheng *et al*., 1998; Kuo *et al*., 2000; Kuo and Lin, 1999). It was further shown that solamargine induced morphological changes of chromatin condensation, DNA fragmentation and sub-G1 peak in a DNA histogram of A549 cells, indicating cell death by apoptosis. Solamargine elevated the expressions of TNF-R1 and -R2 and overcame the resistance of A549 cells to TNF-.-. It has been proposed that solamargine sensitizes A549 cells through TNFRs and mitochondria-mediated pathways and may have anticancer potential against TNFs-and cisplatin-resistance lung cancer cells (Liang *et al*., 2004; Liu *et al*., 2004).

Systemic cancers: Phase I clinical trials of Coramsine (1:1 mixture of solasonine and solamargine, the main components of BEC) in patients with advanced solid tumours produced dose-limiting hepatotoxicity in intravenously administered doses above 1.0 mg kg:day: over 2 h or 1.5 mg kg:day: over 4 h. Injection of 2.25 mg kg:day: over 24 h exceeded the MTD. Activity was observed against resistant tumours. Pharmacokinetic parameters for solasonine and solamargine were linear across the narrow range of doses studied with elimination T of 5.57±1.27 h (solasonine). 8.40±2.00 h (solamargine) and Clearance of 5.6±1.6 Lh: (solasonine), 3.0±0.7 Lh: (solamargine) (Millward *et al*., 2006).

In Phase II studies Coramsine has been administered systemically to over 40 patients with cancers as diversified as glioblastoma multiform, colon, rectal, bladder, liver, metastasized melanoma to the lungs and other respiratory cancers. Patients treated were in very late stage disease and were non-responsive to other antineoplastic therapy. Nevertheless, Coramsine therapy with these patients resulted in tumour size reduction, tumour marker reduction, extended expected life span, reduction in tumour growth rates, improved quality of life, reduced use of analgesia, reduction of oedema, pain reduction and improved appetite. Oral therapy with Coramsine using enteric coated tablets showed remission in secondary endometrial cancer to the lung, pancreatic cancer and metastatic melanoma (Solbec).

SKIN CANCERS

Phase I clinical trials (62 patients): BEC at various concentrations up to 50% in a cream formulation was shown to be very safe. There were no changes observed in vital signs. Haematological, biochemical and urinalytical parameters did not alter by topical applications of BEC in cream formulations. No BEC or its metabolites could be detected in the blood indicating that no systemic absorption of BEC occurred. Local adverse effects were limited to local skin irritation and erythema. Some patients experienced some pain at the site of cream application for short durations (Cham *et al*., 1987, 1991, 1990; Cham, 1989; Evans *et al*., 1989).

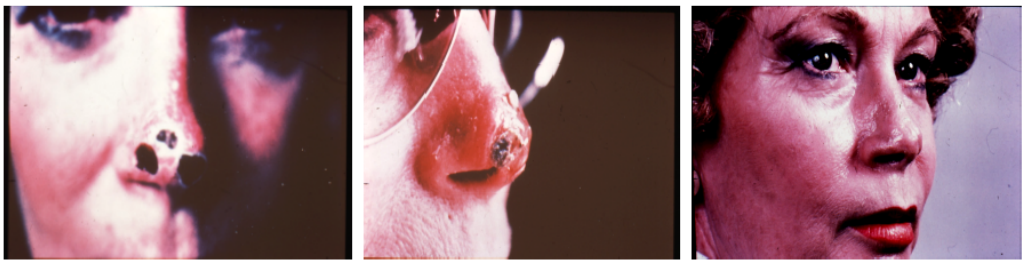

Fig. 8: (a) Illustrates several lesions on the nose before treatment started; (b) shows the lesion 3 weeks after commencement of Curaderm BEC5 treatment. The lesions ulcerated over a wide area giving appearance of one large single lesion. During this period the ulceration progressed to the extent that the soft tissue (cartilage) of the nose was visible; (c) illustrates where the BCC was. The patient was treated with Curaderm BEC5 for 13 weeks. Although true comparison between the photographs in this case is difficult in view of the different exposures, it can be seen that the nose resumed its original shape. A biopsy taken at the conclusion of the treatment indicated histologically that BCC was no longer present. No clinical recurrence was seen 10 years after completion of therapy

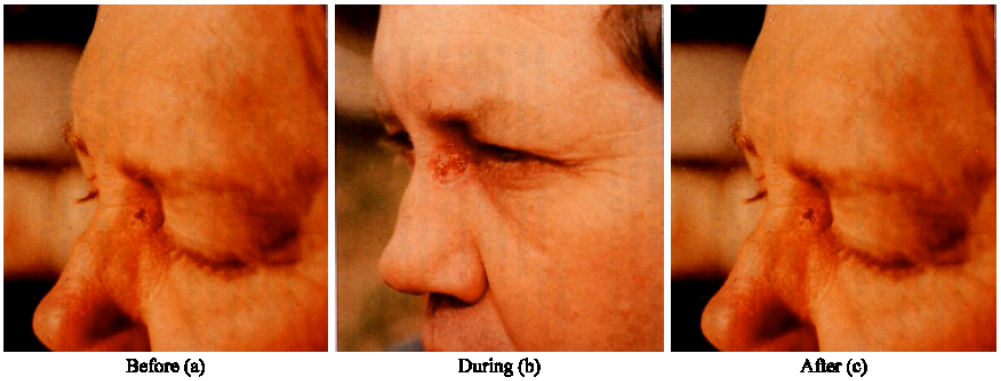

Fig. 9: A BCC close to the eye. This lesion was present for 4 months and was growing rapidly (a); (b) illustrates the lesion 2 weeks after commencement of Curaderm BEC5 treatment. There was a distinct area of ulceration which was larger in area than the clinically distinguishable BCC prior to the Curaderm BEC5 treatment; (c) shows where the BCC was after treatment with Curaderm BEC5. The treatment period in this case was 5 weeks. A biopsy taken at the conclusion of the treatment indicated histologically that BCC was no longer present

Phase II clinical trials (129 patients): In all the clinical trial studies biopsies were taken before and after treatment with BEC formulations. BEC in cream formulations were very effective in treating actinic keratoses, Basal Cell Carcinomas (BCCs) and Squamous Cell Carcinomas (SCCs). It was concluded that very low concentrations of BEC, 50 mg kg^{-1} cream, were optimal for treating non melanoma skin cancers and this formulation was termed Curaderm BEC5. Morphological observations determined that skin cancer cell death caused by Curaderm BEC5 was similar to those obtained in malignant cell culture studies and whole animal studies. Moreover, the concentration of BEC effective in killing the skin cancer cells were in the same concentration range as those obtained in cell culture and animal studies. In addition, as were the cases with cell culture and whole animal studies, BEC selectively destroyed the skin cancers without affecting the normal skin cells. Normal cells replaced the destroyed skin cancer cells during Curaderm BEC5 therapy. The in situ clinical and histological observations with skin cancer also confirmed the cell culture and whole animal studies showing that BEC was killing the cancer cells in their "resting" nonproliferative stages as well as during their "dividing" proliferative stages. These observations are in stark contrast to the other well established antineo-plastic drugs that only kill cancer cells while they are dividing and also kill normal cells when they too are dividing (Cham *et al*., 1987, 1990, 1991, 1992; Cham, 1989, 1994; Cham and Daunter, 1990; Evans *et al*., 1989).

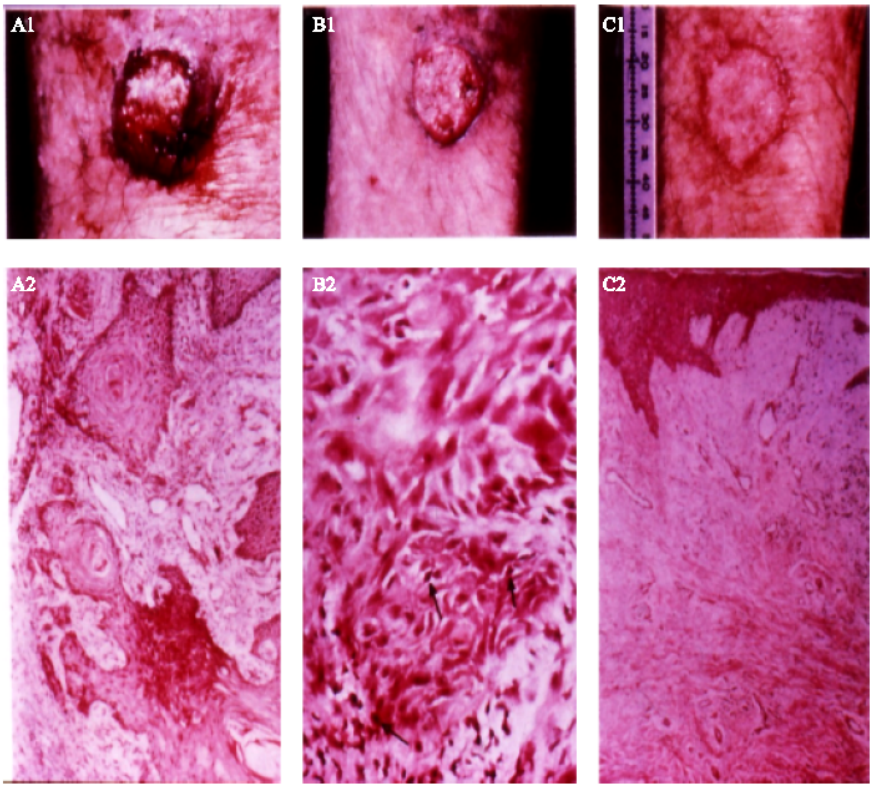

Fig. 10: Clinical and histological (microscopic) diagnosis of an SCC on a leg of a patient before treatment (lane A); during Curaderm BEC5 therapy (lane B); and site of treated SCC after completion of therapy (lane C); 1. Clinical diagnosis; 2. Histological diagnosis. Arrows indicate cancer cells dying during Curaderm BEC5 treatment (lane B 2)

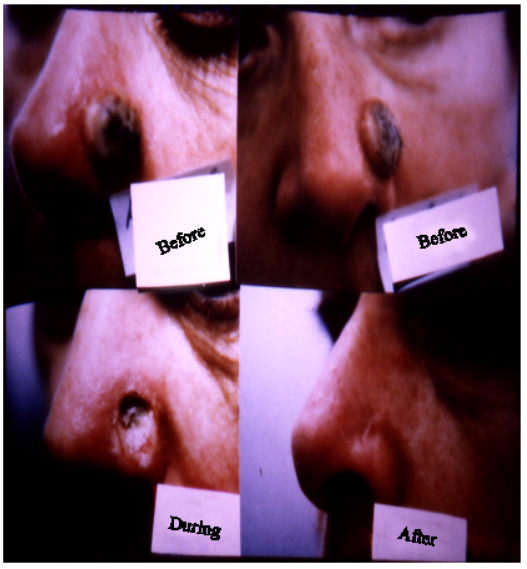

Fig. 11: Squamous Cell Carcinoma (SCC) on the nose of a woman before, during and after Curaderm BEC5 treatment. Curaderm BEC5 was applied six times daily for approximately four and half weeks. Note the depth of the cancer as cartilage was exposed during treatment. At completion, a small scar is all that is left. Surgery would most likely have resulted in removal of a large section of the nose replaced by a prosthesis (plastic nose)

Phase III clinical trials with curaderm BEC5 (232 patients): Single and randomized double-blind placebo controlled studies by independent dermatological hospitals confirmed the efficacy of Curaderm BEC5 for the treatment of keratoses, keratoacanthomas, basal cell carcinoma and squamous cell carcinoma, collectively known as non-melanoma skin cancers. Twice daily application of Curaderm BEC5 under occlusive dressing for 8 weeks resulted in a 78% success rate (Punjabi *et al*., 2000; Cerio and Punjabi, 2002). Treatment regime for 12 weeks resulted in virtually 100% success rate (Cham *et al*., 1992). Success was defined as zero presence of non melanoma skin cancers after histological examination of samples extracted from the lesion site by punch biopsy. In addition, treated patients were followed-up for over 5 and 10 years post treatment and it was established that there were no recurrences of the treated lesions (Cham, 1994; Cham *et al*., 1992). Only local skin irritation and erythema were observed as adverse reactions. The treatment with Curaderm BEC5 was considered to be a safe therapy.

Phase IV clinical trials with curaderm BEC5 (over 50,000 patients): Phase IV studies were important to establish the clinical benefit of Curaderm BEC5 and yielded additional information including risks and optimal use. The clinical benefit was outstanding. These studies were designed to detect any rare or long-term adverse effects over a much larger patient population and timescale than was possible during the initial clinical trials. In the case of treatment with Curaderm BEC5 only 2 adverse effects were documented over a 10 year period with the Health Authorities. Both documented adverse effects were dermatitis at the site of Curaderm BEC5 application. Cessation of Curaderm BEC5 application resulted in remission of the dermatitis. These observations secured Curaderm BEC5 therapy as having exceptional effective and safety profiles (Cham *et al*., 1987, 1990 1991, 1992; Cham, 1989, 1994; Evans *et al*., 1989; Punjabi *et al*., 2000; Walsh, 2000; Cerio and Punjabi, 2002). Figure 8 and 9 show examples of BCC lesions treated with Curaderm BEC5. Figure 10 and 11 show examples of SCC lesions of patients before and after Curaderm BEC5 therapy. Similar cases were followed-up for over 5 years with no sign of recurrences. The cosmetic results of Curaderm BEC therapy were excellent.

CONCLUSION

Compelling evidence that BEC possesses vital antineoplastic activities by expressing an unique mode of action comprising of two separate phenomena in killing cancer cells (Daunter *et al*., 1990; Cham *et al*., 1987, 1990; Cham, 1993) has created much scientific interest. It was shown (Daunter *et al*., 1990; Cham *et al*., 1987, 1990; Cham, 1993) and now widely confirmed (Cham, 1996; Cheng *et al*., 1998; Yuan *et al*., 2002; Paquet *et al*., 2005 Kuo *et al*., 2000; Kuo and Lin, 1999) that cancer cells but not normal cells have specific rhamnose receptors that bind to BEC and its individual components, thus providing the highly desirable specificity of a compound towards cancer cells. The second, as important phenomenon, is what happens after the binding of BEC to the receptor of the cancer cell. It was predicated that EELs were responsible for the internalization of BEC into the cancer cells by the pathway known as receptor-mediated endocytosis. The cancer cell destroying ability was due to antilysosomal and antimitochondrial activities resulting in apoptosis and necrosis.

An ideal skin cancer therapy would be one that when applied topically to an exposed skin cancer it selectively necrotizes the tumour cells or induces them to undergo apoptosis or necrosis without causing damage to the surrounding healthy skin cells. In practice this has long eluded cancer therapy. The anticancer drugs available were neither selective or penetrative. Now for the first time it has been shown with Curaderm BEC5 that it is possible to eliminate skin cancer without harming surrounding healthy skin cells.

It is too early to establish whether BEC or its components will also extend themselves as effective therapies for internal terminal tumours. Much more work is required and is ongoing.

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