Research Journal of Biological Sciences 3 (9): 1008-1017, 2008
ISSN: 1815-8846

Cancer Intralesion Chemotherapy with Solasodine Rhamnosyl Glycosides

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

Abstract: Solasodine rhamnosyl glycosides (SRGs) are a new class of chemotherapeutic agents for the treatment of cancer. SRGs in a cream formulation Curaderm^{BEC5} is now available for the treatment of skin cancers. Phase 2 clinical trials with intravenous (i.v.) administration of SRGs on patients with internal cancers are currently being done. The specificity and mode of action of SRGs are vastly different than those of other traditional anti-mitotic anticancer drugs. These differences have now led to SRGs intralesion chemotherapy, a very effective, new safer, anticancer modality, resulting in rapid regression of solid tumours using 1% of the dose that is required when compared with SRGs i.v. administration. In this feasibility pilot study large external tumours of animals and man were injected intralesionally with SRGs. Results demonstrate that SRGs when injected intralesionally successfully dispose of large tumours without any clinical adverse effects and that apoptosis is the cause of cancer cell death.

Key words: Cancer, solasodine rhamnosyl glycosides, BEC, apoptosis, intralesion

INTRODUCTION

Cancer is the second highest cause of death in the world and in some countries like Taiwan, cancer is the highest cause of death. The major forms of cancer treatments are surgery, radiotherapy, chemotherapy singly or in combination with various antineoplastic agents and combination of surgery, radiotherapy and chemotherapy.

With surgery and radiotherapy the tumour must be precisely targeted and these procedures are nonspecific. Traditional anti-mitotic chemotherapy is the use of drugs to treat cancer by killing or slowing the growth of cancer cells. Anti-mitotic chemotherapy mainly affects fast-growing cells, like cancer cells, other fast-growing non cancer cells are also affected.

Anti-mitotic chemotherapy is usually given by i.v., intraarterially, orally, intramuscularly, intracavitary or topically in a cream form. For internal cancer, no matter which way the chemotherapy is given, it travels in the blood to reach cancer cells in the body. Anti-mitotic chemotherapy can be time-consuming and requires hospitalization and several course treatments.

All existing anti-mitotic chemotherapeutic agents appear to affect fast growing cells by killing such cells when they are dividing (proliferating cells). When such cells are not dividing (non-proliferating cells) the existing anti-mitotic chemotherapeutic agents have very limited effects upon these cells. Consequently, the time course of treating cancer cells with existing anti-mitotic chemotherapeutic agents is long and repetitive treatments are required. In addition, certain cancers have developed drug-resistance which seriously complicates anti-mitotic chemotherapy. The side effects of anti-mitotic chemotherapy are well known.

The widely used anti-mitotic chemotherapeutic agents work by directly affecting nuclear components. Their efficacy relies on cancer cells dividing more rapidly than normal cells and accordingly, at the cell division stages a larger kill is obtained in the more rapidly dividing cells. This is examplified clinically when patients are being treated by traditional anti-mitotic chemotherapy, as shown by extreme precautions taken with bone marrow cells and the killing of cells that cause hair to grow resulting in hair loss and other severe toxic reactions such as nausea, infection as a result of bone marrow suppression, severe skin reactions and ulceration during chemotherapy. Both bone marrow cells and hair growing cells are fast-growing cells. Indeed the therapeutic indices (LD_{50}/ED_{50}) of anti-mitotic chemotherapeutic agents are usually a measure of the rapidity of cell division of normal cells relative to cancer cells. The higher the ratio (therapeutic index) the safer the drug. With traditional anti-mitotic chemotherapy there is no specificity of these drugs to recognize and interact with cancer cells relative to normal cells.

It is clear that although, the above existing therapies are benefiting humans and other animals, there is tremendous room for improvement for cancer treatment. This is exemplified by the fact that cancer remains the 2nd biggest killer in the world and that more recently adjuvant therapies (such as surgery being used along with anti-mitotic chemotherapy) are increasingly being explored for the treatment of cancer.

Due to the ineffectiveness of current state of the art anti-mitotic cancer therapy, novel treatments must be encouraged. More recently, research has been conducted to find biochemical agents that target cancer cells. Such research generally concentrates on identifying a cell surface receptor that is overexpressed in cancerous cell and targeting a specific pathway such as the apoptotic pathway. Such therapies are generally highly specific to a particular cancer. The reason for this is that in different cancers, the receptors and pathways that are deregulated or otherwise compromised are cell specific. The targeted-therapy revolution has arrived, but the principles and limitations of anti-mitotic chemotherapy discovered by the early researchers still apply. Our group was the first to report that the glycoalkaloids solamargine and solasonine have antineoplastic activities (Cham *et al*., 1987; Cham and Meares, 1987; Cham and Daunter, 1990). These SRGs are extracted from various edible and nonedible *Solanum* plant sp. (Cham and Wilson, 1987; Badami *et al*., 2003; Esteves-Souza *et al*., 2002). A multitude of other investigators have since, confirmed and expanded on the original reports (Freedman *et al*., 2005; Kuo *et al*., 2000; Kuo and Lin, 1999; Lee *et al*., 2004; Liang *et al*., 2004; Liang *et al*., 2007; Liu *et al*., 2004; Millward *et al*., 2006; Nakamura *et al*., 1996; Ono *et al*., 2003; Ono *et al*., 2006; Roddick *et al*., 1990; Vijayan *et al*., 2002). SRGs induce apoptosis in cancer cells by up-regulating the expressions of external death receptors, such as tumour necrosis factor receptor 1 (TNFR-I), Fas receptor, TNFR-I associated death domain and Fas-associated death domain. SRGs also enhance the intrinsic ratio of Bax to Bcl-2 by up regulating Bax and down-regulating Bcl-2 and Bcl-xL expressions. These effects result in activation of Caspase -8, -9 and -3 in cancer cells, indicating that SRGs trigger extrinsic and intrinsic apoptotic pathways in cancer cells (Cham, 1988, 1991, 1993, 1994; Shiu *et al*., 2007; Shiu *et al*., 2008; Paquet *et al*., 2005). A combination of SRGs with cisplatin has resulted in the effective killing of cisplatin-resistant cancer cells, particularly lung cancer cells (Liang *et al*., 2004; Liang *et al*., 2007) and breast cancer cells (Shiu *et al*., 2007; Shiu *et al*., 2008).

It is now known that SRGs as anticancer agents are far more effective than taxol, cisplatin, gemcitabine, camptothecin, vinblastine, methotrexate, 5-fluorouracil, epirubicin and cyclophosphamide (Kuo *et al*., 1999; Http:/www.solbec.com.au). SRGs have vastly different modes-of-actions as chemotherapeutic agents when compared with traditional anti-mitotic anticancer agents (Daunter and Cham, 1990; Esteves-Souza *et al*., 2002; Freedman *et al*., 2005; Kuo *et al*., 2000; Kuo *et al*., 1999; Lee *et al*., 2004; Liang *et al*., 2004; Liang *et al*., 2007; Liu *et al*., 2004; Millward *et al*., 2006; Nakamura *et al*., 1996; Ono *et al*., 2003; Ono *et al*., 2006; Roddick *et al*., 1990; Vijayan *et al*., 2002; Cham, 1988, 1991, 1993, 1994; Shiu *et al*., 2007; Shiu *et al*., 2008; Http:/www.solbec.com.au; Cheng *et al*., 1998).

Firstly, the rhamnose part of the SRGs are recognized by and bind to, specific receptors that are present on cancer cells but not (or at least in much lesser quantities) on normal cells. These receptors have been given the term Endogenous Endocytic Lectins (EELs) (Daunter and Cham, 1990) and have now been identified as rhamnose binding glycoproteins (Http:/www.solbec.com.au). Once SRGs bind to the EELs, the SRGs-EELs complex is internalized in the cancer cell by receptor-mediated endocytosis through endosomes, ultimately being localized in the lysosomes. Two subsequent events follow, anti-mitochondrial activities are observed and the lysosomes are ruptured by the solasodine part of the SRGs. Lysosomal rupture may be dependent, in part, on mitochondrial disruption. Overexpressing Bcl-xL, an antiapoptotic protein known to preserve mitochondrial functions, also impedes lysosomal and mitochondrial disruption, indicating signalling between the 2 organelles. The contents of the lysosomes, consisting of many hydrolytic enzymes that can digest fats, proteins, nucleotides and carbohydrates are spilt into the cytoplasm of the affected cell leading to sudden death by apoptosis of the cancer cells (Cham, 2007a, 2007b, 2007c).

Hence, the 2 phenomena resulting in specific killing of cancer cells are:

- Recognition and binding of the rhamnose part of SRGs by specific EELs on cancer cells but not normal cells elicit specificity, a rarity that has long eluded cancer therapy.
- Tumouricidal effects of the solasodine part of SRGs on affected cancer cells by anti-mitochondrial action and rupturing of lysosomes, triggering the mechanism of apoptosis.

Unlike established anticancer, anti-mitotic drugs, SRGs are not anti-mitotic their action. That is, they do not merely interfere with the cell division process. Rather the cell itself is killed through the interaction. Importantly, the mechanism of action of SRGs incorporates cell lysis through disruption of the membrane of the lysosome thus releasing the contents of the lysosome within the cell, which then kill the cell by apoptosis. Unlike other specific anticancer treatments such as matrix metalloproteinase inhibitors, SRGs are toxic to cancer cells and the tumours are rapidly eradicated as opposed to merely being constrained. Unlike anti-angiogenics

approaches, cell death from SRGs is rapid and not dependent upon starving the cancer cell.

Apart from the obvious advantage of providing a different line of attack against cancer thus dealing with multi-drug resistance, it is now accepted that SRGs act preferentially upon cells transformed to cancer.

Importantly, traditional anti-mitotic antineoplastics are mostly only effective at treating cancer when it is at proliferating stages of cancer growth (when the cancer cells are dividing), whereas SRGs are effective at both proliferating and resting (non-proliferating) cancer cells.

Although, SRGs possess these advantageous antineoplastic properties, there are certain challenges that SRGs must overcome before proper therapeutic efficacy for internal cancers can be achieved. With most, if not all traditional anti-mitotic antineoplastics, i.v. is the route of administration for the treatment of internal cancer. Indeed, clinical trials with a mixture of SRGs by i.v. administration are currently underway (Millward *et al*., 2006). Administration by i.v. poses a number of serious limitations such as toxicity to body organs, bioavailability of the drug (pharmacokinetics such as biological half-life, protein binding, etc). Because of the dilution effect of the drug within the vascular system, a relatively high dose of the drug has to be administered for it to have a therapeutic effect on the cancer cells and this high dose, also depending on the pharmacokinetics and pharmacodynamics, may pose a serious limitation because of toxicity and side effects to the body.

The specificity and tumouricidal effects of SRGs result in immediate death of cancer cells but not normal cells (Cham, 2007a, 2007b, 2007c). During apoptosis, dead cell fragments are rapidly absorbed through phagocytosis by macrophages.

Toxicity of SRGs: In general, glycoalkaloid poisoning is associated with the central nervous system such as rapid and weak pulse, rapid and shallow breathing, delirium and coma caused by the inhibition of acetyl cholinesterase activity. SRGs are found in the leaves and fruits of many *Solanum* plants ranging from the toxic belladonna to the edible eggplant. *Ex vivo* studies have shown that SRGs selectively kill a wide variety of cancer cells without harming normal cells. Furthermore, studies have shown that it is possible to kill cancer cells but not normal cells within a specified organ. For example SRGs at given concentrations kill liver cancer ($HepG_2$) cells but not normal human liver (Chang) cells. Similarly, leukemic cells but not bone marrow cells and melanoma cells but not melanocytes are effectively eliminated by SRGs treatment (Lee *et al*., 2004; Cham, 1991, 1993, 1994, 2007a, b, c).

In vivo animal toxicity studies in mice revealed that for single intraperitoneal (i.p.) doses of the SRGs (BEC) the LD_{50} is 30 mg kg^{-1} and in rats the i.p. LD_{50} is 41 mg kg^{-1}. The LD_{50} for a single dose by gastric intubation in mice is 550 mg kg^{-1}. Multiple i.p. dose studies indicated that the LD_{60} for mice by 14 daily single i.p. administrations is 10 mg kg^{-1}. The LD_{50} for rats by 8 i.p. administrations over 8 days with one injection per day is 20 mg kg^{-1}. In rats no appreciable toxic effects were observed at doses less than 35 mg kg^{-1} as indicated by blood parameters, enzyme levels and histological sections of kidney, liver and cardiac muscle. No effects on the cardiovascular system such as changes in heart rate were observed (Cham, 1988; Chami *et al*., 2003).

Human toxicity studies of SRGs at various concentrations up to 50% in cream formulations produced no changes in vital signs, plasma biochemical parameters, blood haematological parameters or urinalytical parameters. Adverse effects were local skin irritation, pain at the site of cream application, erythema and burning sensation for short duration. These local adverse effects were mainly due to the excipients salicylic acid and urea in the cream formulations (Cham, 1994; Cham and Daunter, 1990; Cham *et al*., 1990; Cham *et al*., 1991; Cham *et al*., 1992; Punjabi *et al*., 2000; Punjabi et al., 2008).

SRGs in a formulation called Coramsine, a 1:1 mixture of solasonine and solamargine when administered i.v. in phase I clinical studies in man, produces dose-limiting hepatotoxicity at doses above 1.0 mg/kg/day over 2 h or 1.5 mg/kg/day over 4 h. Doses of 2.25 mg/kg/day over 24 h exceed the Maximum Tolerated Dose (MTD). T½ for solasonine is 5.57±1.27 h and for solamargine this is 8.4±2.00 h. The clearance for solasonine is 5.6±1.6 and for solamargine the clearance is 3.0±0.7 L/h (Millward *et al*., 2006).

Carcinogenicity and mutagenicity of SRGs: Traditional anti-mitotic anticancer drugs lack specificity, as they enter the cells mainly by diffusion. Due to their DNA reactivity anticancer drugs can cause a second tumour and different than the one originally treated, several years after a curative treatment. SRGs rupture lysosomes and also affect the mitochondria in cancer cells. This new cancer therapy lacks the mutagenic and carcinogenic potential of currently used anti-mitotic chemotherapeutic agents. To confirm this, mice were treated with SRGs and the treated mice together with their offsprings of 6 generations were followed for over one year. No carcinogenicity or mutagenicity was observed (Cham, 1988, 1993, 1994). This was also shown by the Ames Test (Http:/www.solbec.com.au). These observations were confirmed using the Ames Test (Http:/www.solbec.com.au).

Because of the unique mode of action of SRGs, the objective of this pilot study, was to evaluate the efficacy and safety of intralesion injection of SRGs into large tumours in which the progress could be visually monitored clinically with the intention to establish whether this chemotherapeutic approach would be suitable and extendable to treat smaller deepseated internal tumours that are not visually observable clinically. This procedure differs from other antineoplastic treatment procedures in that relatively high doses of SRGs can be applied directly to the tumour resulting in rapid death of tumour cells. The overall body load of the drug is far less than i.v. administration resulting in diminished toxicity to the body.

MATERIALS AND METHODS

Patient selection: Two horses (horses 1 and 2) with chondrosarcomas, one horse (horse 3) with multiple squamous cell carcinoma lesions on his penis and a human patient with a large intracranial squamous cell carcinoma were selected for the pilot study. The horse with the multiple Squamous Cell Carcinoma (SCC) lesions was under general anaesthetic at each of its 3 treatment periods. The other 2 horses with chondrosarcomas and the human male patient were not under anaesthetic.

Treatments administered: The study medication was BEC which is a standard mixture of SRGs, solamargine (33%), solasonine (33%) and their corresponding di- and monoglycosides (34%). All the glycosides contain the same aglycone, solasodine (Cham, 1988). A sterile solution of 10% BEC, $^{w}/_{w}$, in dimethylsulfoxide, was used in this study.

The weights of the chondrosarcoma lesions in the 2 horses were approximated. For horse 1 the weight of its tumour was estimated at 400 g. and for horse 2 the estimated tumour weight was 500 g. Horse 3 had multiple SCC lesions on the penis, the estimated weight of these lesions ranged from 5-100 g. The SCC on the head of the human was estimated to be 500 g.

The doses injected intralesionally were 100 mg BEC per 1 kg tumour weight. Thus for a tumour weight of 500 g the dose was 0.5 mL of the 10% BEC solution and for an estimated tumour weight of 5 g the dose was 0.005 mL of the 10% BEC solution.

Horse 1, 2 and the human were injected intralesionally on 2 separate occasions. The second injection occurred approximately 48 h after the first injection. Each injection was done intralesionally on multiple sites of the tumour.

Horse 3 underwent general anaesthesia prior to multiple injections in each tumour. In this case the horse was treated once a week for 3 weeks.

Apoptosis induced by SRGs: To observe the morphological changes of chromatin of cells after SRGs (in this case BEC), ovarian cancer cells were treated with BEC (6 μg mL^{-1} for 0-1.25 h), then stained with haematoxylin and observed by light microscopy (x500).

Effects of vinblastine on ovarian cancer cells: Ovarian cancer cells were treated with vinblastine (10 μMol L^{-1} for 3 h) and monitored by light microscopy (x500).

RESULTS AND DISCUSSION

Horses: The chondrosarcomas in the 2 horses responded rapidly to SRGs therapy. Only 2 injections, 48 h apart, were necessary to obtain complete eradication of these large tumours.

Figure 1 illustrates that a chondrosarcoma of approximately 400 g is rapidly eliminated by SRGs chemotherapy. Figure 1a shows the tumour before treatment, Fig. 1b shows that the tumour is breaking down 5 days after the first injection of SRGs and Fig. 1c illustrates the site where the cancer was 12 weeks after SRGs therapy. There was no recurrence for at least 5 years after treatment.

Similarly, Fig. 2a shows a tumour of approximately 500 g prior to SRGs treatment, necrosis of the treated tumour one week after the first injection (Fig. 2b) and the site where the tumour was 12 weeks after SRGs therapy (Fig. 2c) with no recurrence for at least 5 years after treatment.

Figure 3 illustrates multiple SCCs of varying sizes on the penis of horse 3. This horse was given 3 courses of SRGs injections before complete remissions of all the tumours were achieved. The extent of multiple lesions are seen in Fig. 3a and 3b. Figure 3c shows that the tumours were extended throughout the entire penis. Figure 3d shows the intralesion injections into each individual tumour mass with SRGs. This horse was under general anaesthetic during SRGs injections. Massive haemorrhagic necrosis of the tumour masses occurred during the 3 weeks treatment courses as shown in Fig. 3e and 3f. After the final treatment (3d set of injections) a large tumour separated entirely and fell off while, the horse was waking up from the anaesthetic (Fig. 3g). Figure 3h and 3i show the successfully treated penis with no signs of any tumour 3 months after the treatment of the cancer with SRGs. Figure 3j shows that the horse was in excellent condition after treatment. There was no recurrence of the tumours after 5 years follow-up.

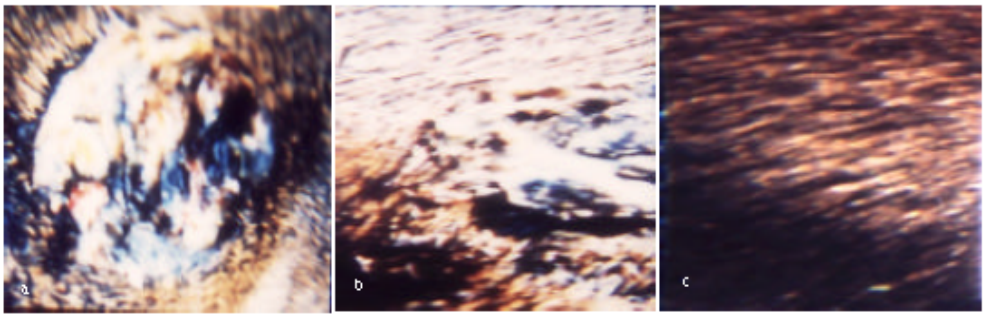

Fig. 1: A sarcoid of approximately 400 g on the chest of a horse before injection (a); after 2 injections of BEC (b) the site where the cancer was after completion of BEC therapy (c). When the treated area was completely healed it was indistinguishable from the horse's normal skin

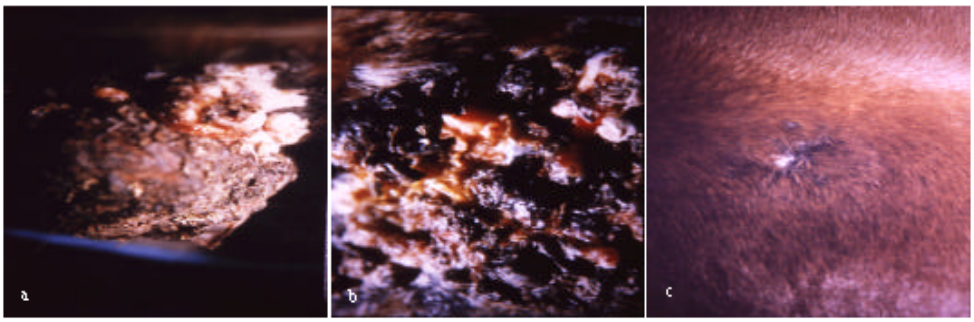

Fig. 2: A sarcoid of approximately 500 g on the chest of a horse before injection (a); after 2 injections of BEC, showing the rapid degradation of the cancer (b) and the site where the cancer was after completion of BEC therapy (c)

Clinically the horses did not appear to be adversely affected by the SRGs treatment other than horse 3 which lost its appetite for several days after each treatment. Whether this was due to the anaesthetic or SRGs is unknown.

Human: Figure 4a, b show the extent of this large SCC tumour before SRGs therapy commenced. The estimated tumour mass was 500 g. Multiple injections of 50 mg BEC (total volume of 0.5 mL of a 10% solution of BEC in DMSO) were injection on 2 occasions with a 2 day interval. Rapid breaking down of the tumour mass occurred and 7 days after the first injection a massive erosion of the tumour was observed (Fig. 4c). A vertical view of the treated lesion shows that a large proportion of the tumour mass was destroyed, to the extent, that the brains were partly exposed (Fig. 4d).

The family of this patient was concerned that the treatment was too severe and decided to discontinue the treatment. Nevertheless, it is quite clear that SRGs therapy was very effective.

The excellent anticancer therapeutic effects of SRGs observed in this pilot study reflect and expand on the cell culture, tissue culture, animal and human work previously reported. It is unlikely that the solvent carrier DMSO had therapeutic effects as this was shown not to be the case in many other reports. Intradermal injection of 50 mg BEC in DMSO in normal skin over a surface of 25 cm^2 did not have any observable effects on the injected area. This demonstrates specificity of SRGs for cancer cells but not normal cells, an observation previously confirmed by cell culture, animal and human studies (Cham, 1993, 2007a, 2007b, 2007c).

Cell culture: Figure 5a and f show that the responses of ovarian cancer cells towards SRGs result in rapid death of the cancer cells. These observations are characteristic of apoptosis which is also known as programmed cell death. The apoptotic cells reveal cell shrinkage, condensation of chromatin and nuclear fragmentation. The shrinkage of cells, chromatin condensation and nuclear fragmentation were increased with the increasing treatment time.

Figure 6 shows that in the case of vinblastine, a well known and widely used anticancer drug, the divisions of the ovarian cancer cells are slowed down. Arrows indicate cells in the process of cell division which takes longer if vinblastine is present. Normal cells are also affected by

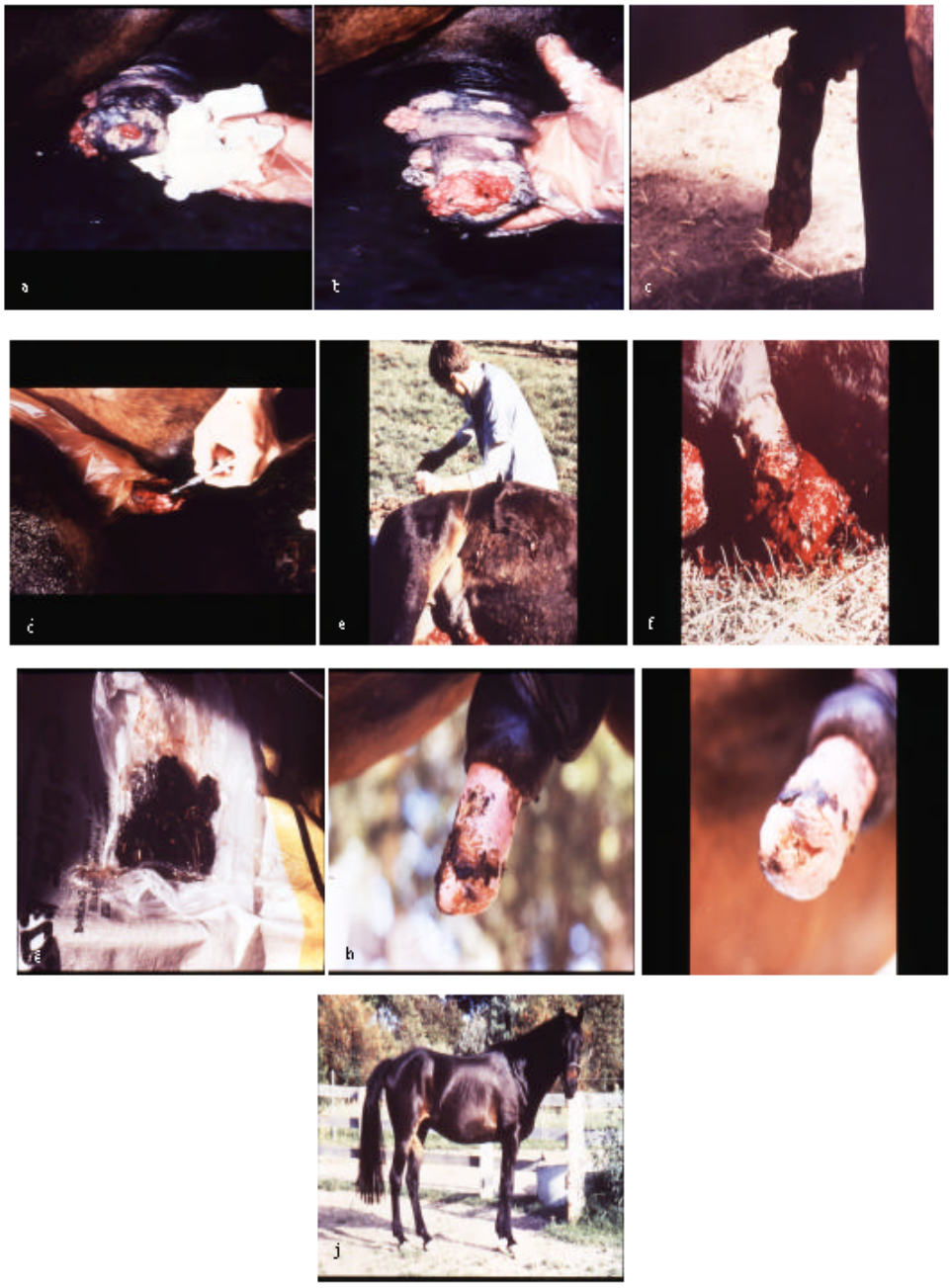

Fig. 3: Multiple large SCCs on the penis of a horse. This horse was given three courses of BEC injections before complete remissions of all the tumours were achieved. The extent of multiple lesions are seen in (a) and (b). (c) shows that the tumours were extended throughout the entire penis. The veterinarian injected each individual tumour mass with BEC (d). The horse needed general anaesthetic during BEC therapy. Massive haemorrhagic necrosis of the tumour masses occurred during the treatment course (e) and (f). After the final treatment (third injection) the tumour separated entirely and fell off while the horse was waking up (g). Successfully treated penis showing no signs of any tumour (h) and (i) 2 years after the initial diagnosis and treatment of the cancer. The horse was in excellent condition after treatment (j)

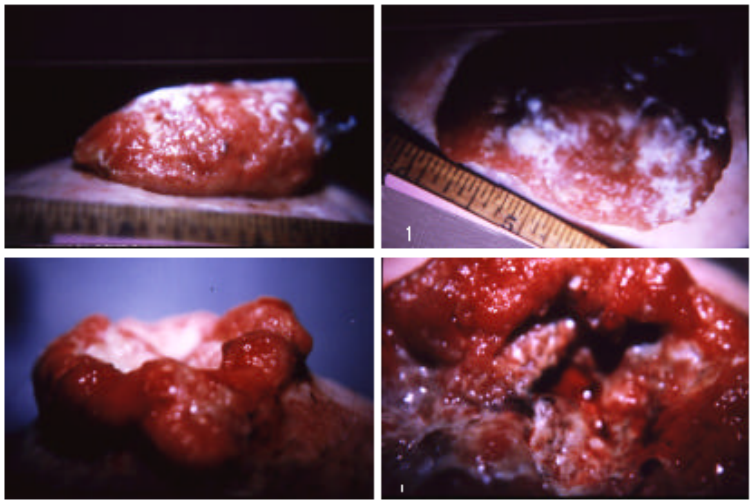

Fig. 4: A large SCC on the head of a male subject (a) and (b). The patient was given 2 injections of BEC which resulted in the tumour collapsing and reducing in size (c) side view, (d) top view

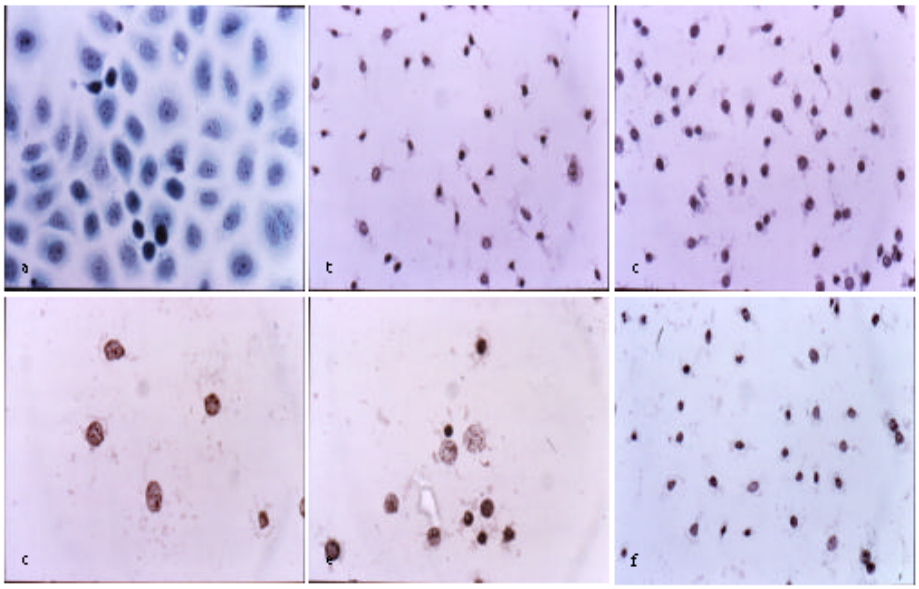

Fig. 5: Untreated ovarian cancer cells, the cells are all viable (a). BEC causes the cytoplasm of the cancer cells to undergo dissolution, the nuclei contract and become dark staining (b), nuclei then enlarge (c), the chromatin (contents of nucleus) clumps (d) and finally the nuclei disintegrate (e). Only cellular debris is left after the interaction of the cancer cells with BEC (f). This cell death is characteristic of apoptosis which is also known as programmed cancer cell death

vinblastine. Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of cell cycle). Microtubules are vital for cell division and thus cell division is impeded by vinblastine. The effectiveness of vinblastine depends on how much faster the cancer cells are dividing compared with normal cells. It is quite clear that the anticancer properties of SRGs are much different than the anticancer properties of vinblastine.

Intralesion injection is far superior to i.v. and intracavitary injection if the lesion being treated is rapidly and specifically degraded by the chemotherapeutic agent.

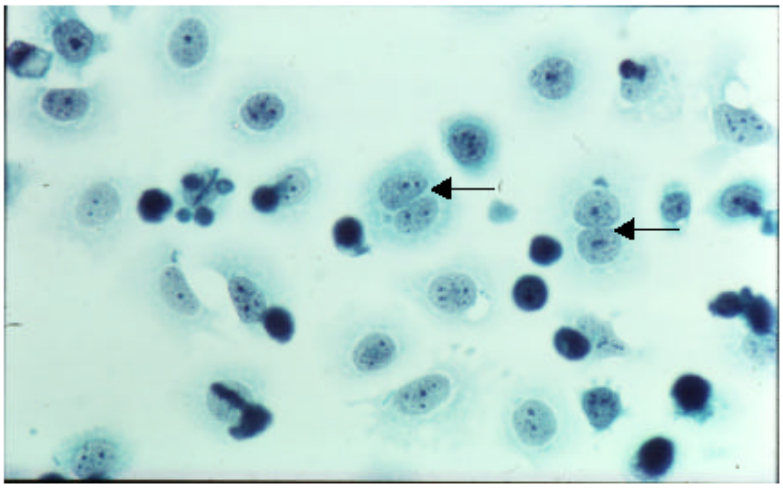

Fig. 6: The same strain of ovarian cancer cells as in Fig. 5a, but now in contact with vinblastine. The cancer cells remain alive, but vinblastine, a well known and widely used anticancer drug slows down the division of the cancer cells. Arrows indicate cells in the process of cell division which takes longer if vinblastine is present. Normal cells are also affected by vinblastine. The effectiveness of vinblastine depends on the turnover of cancer cells relative to normal cells

SRGs kill cancer cells rapidly whether they are dividing or resting in contrast to most traditional anti-mitotic chemotherapeutic agents which only affect cancer cells in a substantial time dependent manner. Cancer cells metabolize SRGs whilst these cells are being killed and thus the availability of the administered doses of SRGs to other organs such as the liver are reduced. The metabolites at high concentrations are non toxic (Cham and Daunter, 1990; Cham, 1991, 1993).

It is clear why i.v. has been the preferred method for traditional anti-mitotic chemotherapy administration because their modes of action are very different to SRGs and are not amenable to intralesion injection.

Some activity against resistant solid tumours has been observed in phase 2a human clinical studies with i.v. administration of SRGs. In order to observe limited activity 1.5 mg SRGs/kg/day over 4 h for 5 days, every 2 weeks, was required. Thus for a patient of approximately 60 kg 1 course treatment required 450 mg SRGs over 5 days and this was repeated every 2 weeks. MTD was 2.25 mg/kg/day and hepatotoxicity was observed at doses above 1 mg/kg/day. For a 60 kg patient the MTD was 135 mg SRGs for a daily dose whereas hepatotoxicity occurred at a dose of 60 mg SRGs. For one course of treatment the SRGs doses were 675 and 300 mg for MTD and hepatotoxicity, respectively (Millward *et al*., 2006; Http:/www.solbec.com.au).

For intralesion injection as described in this communication, the dose is independent of body weight and is in the order of 2 mg SRGs for the treatment of a tumour of 10 g. A full treatment course is in the order of 4 mg SRGs for 10 g of tumour. Four mg of SRGs are present in approximately 20 g of the eggplant or aubergine (*Solanum melongena*) a fruit which is eaten as a vegetable, throughout the world (Bajaj *et al*., 1979; Jones and Fenwick, 1981). Compared with i.v., infusions, direct administration of SRGs into tumours reduced the required body doses by at least 100 times. Furthermore, it is known that high doses of SRGs once consumed by cancer cells are rendered non toxic (Cham and Daunter 1990; Cham, 1991, 1993).

This pilot study addresses extreme case studies in which the treated tumours are considered to be large. These cases were selected for a variety of reasons, such as, being able to clinically and visually, observe the effects of the therapy and to inject relatively high doses (because of the size of the tumours) and follow possible adverse effects. An impressive observation was the end result of SRGs chemotherapy in that the lesions were completely eliminated without the formation of significant amount of scar tissue. This was clearly shown in the horses where the treated cancer lesions resumed their normal shape and consistency after treatment with SRGs. These observations are in agreement with skin cancer studies where it was shown that the cosmetic end results of treated lesions with Curaderm[BEC]J were outstanding.

Now there is a new class of chemotherapeutic agents, the SRGs, that have specificity and unique mode of action resulting in rapid eradication of tumour cells. These properties of SRGs warrant SRGs intralesion injection studies in humans with smaller internal solid tumours which are soon to commence.

CONCLUSION

SRGs are a new class of chemotherapeutic agents. SRGs are extracted from plant material and express their antineoplastic activities by highly ordered specificity and cancer cell destroying ability due to apoptosis caused by anti-lysosomal and anti-mitochondrial activities. Curaderm^{BEC5}, a topical cream formulation containing SRGs is now available for the treatment of skin cancer without harming surrounding healthy skin cells (Cham, 2007a, 2007b, 2007c; Punjabi *et al*., 2000, 2008; Cerio and Punjabi, 2002). Administration of SRGs by i.v. is currently being evaluated clinically to establish the potential of SRGs for treating internal cancers (Millward *et al*., 2006). The ramifications of the specificity and anticancer mode of action of the SRGs have as yet not been fully recognized and researched. Now for the first time an investigation based on intralesion injection due to the unique properties of SRGs are reported. Although, it is envisaged that the intralesion injections of SRGs may be used to treat much smaller lesions which are not directly visible with the naked eye (internal solid tumours), the pilot studies reported here on non-internal solid tumours, were necessary to obtain the scientific data before embarking on clinical studies on lesions such as breast cancer, prostate cancer and pancreatic cancer using specific means of locating the cancers and administration of SRGs into such cancers. Compared with the i.v. studies the doses for intralesion injections are 100 times less. Such SRGs intralesion clinical studies on internal solid tumours will soon commence.

ACKNOWLEDEGMENT

I thank Dr. David Spall for attending to the human patient and Dr Ian Gawler for attending to the treatment of horse 3. I also thank Aruba Cham and Kai Cham for secretarial research.

REFERENCES

Badami, S., S.A. Manohara Reddy, E.P. Kumar, P. Vijayan and B. Suresh, 2003. Antitumour activity of total alkaloid fraction of *Solanum pseudocapsicum* leaves. Phytother. Res., 17: 1001-1004.

Bajaj, K.L, G. Kaur and M. L. Chada, 1979. Glycoalkaloid content and other chemical constituents of the fruits of some egg plant (*Solanum melongena, L.*) varieties. J. Plant Foods, 3: 163-168.

Cerio, R. and S. Punjabi, 2002. Clinical appraisal of BEC5. Barts and The London NHS.

Cham, B.E., 1988. Monograph on the compound BEC. Drugs of the Future, 13: 714-716.

Cham, B. E., 1991. Solasodine glycosides: A new modality for cancer. In: Proc. 2nd Oceania Symposium, Mark S. Walker. (Ed.) Bioconcepts Publishing, pp: 30-36.

Cham, B.E., 1993. Efficacy and mode of action of solasodine glycosides (BEC) on cancer cells. In: Proc. 4th Oceania Symposium, Mark S. Walker. (Ed.) Bioconcepts Publishing, pp: 41-51.

Cham, B.E., 1994. Solasodine glycosides as anti-cancer agents: Pre-clinical and clinical studies. Asia Pacif. J. Pharmacol, 9: 113-118.

Cham, B.E., 2007a. Solasodine rhamnosyl glycosides specifically bind cancer cell receptors and induce apoptosis and necrosis. Treatment for skin cancer and hope for internal cancers. Res. J. Biol. Sci., 2 (4): 503-514.

Cham, B.E., 2007b. Solasodine rhamnosyl glycosides in a cream formulation is effective for treating large and troublesome skin cancers. Res. J. Biol. Sci., 2 (7): 749-761.

Cham, B.E., 2007c. The eggplant cancer cure. A treatment for skin cancer and new hope for other cancers from nature's pharmacy. Smart Publications, pp: 122.

Cham, B.E. and B. Daunter, 1990. Solasodine glycosides. Selective cytoxicity for cancer cells and inhibition of cytotoxicity by rhamnose in mice with sarcoma 180. Cancer Letters, 55: 221-225.

Cham, B.E. and B. Daunter, 1990. Topical treatment of pre-malignant and malignantskin cancers with Curaderm. Drugs of Today, 26: 55-58.

Cham, B.E., B. Daunter and R. Evans, 1990. Curaderm. Med. J. Aus., 152: 329-330.

Cham, B.E., B. Daunter and R. Evans, 1991. Topical treatment of malignant and premalignant skin cancers by very low concentrations of a standard mixture of solasodine glycosides. Cancer Letters, 59: 183-192.

Cham, B.E., M. Gilliver and L. Wilson, 1987. Antitumour effects of glycoalkaloids isolated from *Solanum sodomaeum* L. Planta Med., 53: 34-36.

Cham, B. E., B. Daunter and R. Evans, 1992. Topical treatment of malignant and premalignant skin cancers by very low concentration of a standard mixture of solasodine glycosides. Clinical Digest Series. Dermatology.

Cham, B.E. and M.M. Meares, 1987. Glycoalkaloids from *Solanum sodomaeum* L. are effective in the treatment of skin cancers in man. Cancer Letters, 36: 111-118.

Cham, B.E. and L. Wilson, 1987. HPLC of glycoalkaloids from *Solanum sodomaeum* L. Planta Med., 53: 59-61.

Chami, L.A., R. Méndez, B. Chataing, J. O'Callaghan, A. Usubillaga and L. LaCruz, 2003. Toxicological effects of α-solamargine in experimental animals. Phyto Res., 17: 254-258.

Cheng, L.C., T.R. Tsai, J.J. Wang, C.N. Lin and K.W. Kuo, 1998. The rhamnose moiety of solamargine plays a crucial role in triggering cell death by apoptosis. Biochem. Biophys. Res. Commun., 242 (1): 21-25. Curaderm^{BEC5} Global Ltd. http:/www.curadermbec 5global.com

Daunter, B. and B.E. Cham, 1990. Solasodine glycosides. In vitro preferential cytotoxicity for human cancer cells. Cancer Letters, 55: 209-220.

Esteves-Souza, A., T. Sarmento da Silva, C.C.F. Aloes, M. Carvalho, R. Braz-Filho and A. Echevarria, 2002. Cytotoxic activities against Ehrlich carcinoma, human K562 Leukaemia of alkaloids and flavonoids from 2 *Solanum* sp. J. Braz. Chem. Soc., 13 (6): 838-842.

Freedman, M., K.R. Lee, H.J. Kim, I.S. Lee and N. Kozukue, 2005. Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma and stomach cancer cells. J. Agric. Food Chem., 53 (15): 6162-6169.

Jones, P.G. and G.R. Fenwick. 1981. The glycoalkaloid content of some edible Solanaceous fruits and potato products. J. Sci. Food Agric., 32: 419-421.

Kuo, K.W., S.H. Hsu, Y.P. Li, W.L. Lin, L.F. Liu, L.C. Chang, C.C. Lin, C.N. Lin and H.M. Sheu, 2000. Anticancer activity evaluation of the *Solanum glycoalkaloid solamargine*. Triggering apoptosis in human hepatoma cells. Biochem. Pharmacol., 60: 1865-1873.

Kuo, K.W. and C.N. Lin, 1999. Pharmacological composition for treating cancer cells. United states Patent, 6: 214- 803.

Lee, K.R., N. Kozukue, J.S. Han, J.H. Park, E.Y. Chang, E.J. Back, J.S. Chang and M. Friedman, 2004. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J. Agric. Food. Chem., 52 (10): 2832-2839.

Liang, C.H., L.F. Liu, L.Y. Shiu, Y.S. Huang, L.C. Chang and K.W. Kuo, 2004.Action of solamargine on TNFs and cisplatin-resistant human lung cancer cells. Biochem. Biophys. Res. Commun., 322 (3): 751-758.

Liang, C.H., L.Y. Shiu, L.C. Chang, H.M. Sheu and K.W. Kuo, 2007. Solamargine upregulation of Fas, downregulation of HER 2 and enhancement of cytotoxicity using epirubicin in NSCLC cells. Mol. Nutr. Food Res., 51 (8): 999-1005.

Liu, L.F., C.H. Liang, L.Y. Shiu, W.L. Lin, C.C. Lin and K.W. Kuo, 2004. Action of solamargine on human lung cancer cells-enhancement of the susceptibility of cancer cells to TNFs. FEBS. Lett., 577 (1-2): 67-74.

Millward, M., A. Powell, P. Daly, S. Tyson, R. Ferguson and S. Carter, 2006. Results of phase I clinical trials of coramsine in patients with advanced solid tumors. J. Clin. Oncol., 24: 2070.

Nakamura, T., C. Komori, Y. Lee, F. Hashinoto, S. Yahara, T. Nohara and A. Ejina, 1996. Cytotoxic activities of *Solanum* steroidal glycosides. Biol. Biopharm. Bull., 19: 564-566.

Ono, K., S.O. Kin and J. Han, 2003. Susceptibility of lysosomes to rupture is a determinant for plasma membrane disruption in tumor necrosis factor alpha-induced cell death. Mol. Cell. Biol., 23 (2): 665-676.

Ono, M., K. Nishimura, K. Suzuki, T. Fukushima, K. Igoshi, H. Yoshimitsu, T. Ikeda and T. Nohara, 2006. Steroidal glycosides from the underground parts of *Solanum sodomaeum*. Chem. Pharm. Bull., 54 (2): 230-233.

Paquet, C., A.T. Sané, M. Beauchemin and R. Bertrand, 2005. Caspase-and mitochondrial dysfunction dependent mechanisms of lysosomal leakage and cathepsin B activation in DNA damage-induced apoptosis. Leukemia, 19: 784-791.

Punjabi, S., I. Cook, P. Kersey, R. Marks, A. Finlay and G. Sharpe *et al.* 2000. A double blind, multi-centre parallel group study of BEC-5 cream in basal cell carcinoma. Eur. Acad. Dermatol. Venereol., 14 (Suppl): 47-60.

Punjabi, S., L.J. Cook, P. Kersey, R. Marks and R. Cerio, 2008. Solasodine glycoalkaloids: A novel topical therapy for basal cell carcinoma. A double-blind, randomized, placebo-controlled, parallel group, multicentre study. Internat. J. Dermatol., 47: 78-82.

Roddick, J.G., A. L. Rÿrenber and M. Weissenber, 1990. Membrane-disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. Phyto. Chem., 29: 1513-1518.

Shiu, L.Y., L.C. Chang, C.H. Liang, Y.S. Huang, H.M. Sheu and K.W. Kuo, 2007. Solamargine induces apoptosis and sensitizes breast cancer cells to cisplatin. Food Chem. Toxicol., 45 (11): 2155-2164.

Shiu, L.Y., C.H. Liang, Y.S. Huang, H.M. Sheu and K.W. Kuo, 2008. Downregulation of HER2/neu receptor by solamargine enhances anticancer drug-mediated cytotoxicity in breast cancer cells with high-expressing HER2/neu. Cell Biol. Tox., 24 (1): 1-10.

Vijayan, P., S. Vinod Kumar, S. A. Dhanaraj, S. Badami and B. Suresh, 2002. *In vitro* cytotoxicity and anti-tumour properties of the total alkaloid fraction of unripe fruits of *Solanum pseudocapsicum.* Pharmaceut. Biol., 40: 456-460.