

Chapter 17

PROSPECTS OF BRASSINOSTEROIDS IN MEDICINAL APPLICATIONS

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Abstract: Steroids are an imperative group of hormones which play a key role in the transmission of signals that mediate growth and physiological responses in most pluricellular organisms. Brassinosteroids (BRs), a class of plant-specific steroid hormones, control many of the developmental and physiological processes like their animal counterparts, including regulation of gene expression, cell division and expansion, differentiation, programmed cell death, and homeostasis. Recent studies have indicated that these hormones have antiviral, antifungal, antiproliferative, antibacterial, neuroprotective and immunomodulatory properties in animal system. BRs analogues have been reported to have antiviral activity against herpes simplex virus type 1 (HSV-1), arenaviruses as well as against replication of vesicular stomatitis virus (VSV) in Vero cells. Also, antiherpetic activities both in a human conjunctive cell line (IOBA-NHC) and murine herpetic stromal keratitis (HSK) experimental models have been reported. In human cells, anticancer structure-activity relationship of natural BRs revealed their high cytotoxic activity. Since, BRs and their analogues are reported to inhibit cell growth in cancer cell lines, they may be considered as promising phytohormones for potential anticancer drugs. The use of pollens in folk medicine also indicates scope of steroids of plant pollens in medicines. An attempt has been made in this paper to document the information available on the prospects of BRs in therapeutics.

Key words: Antiviral, anticancer, brassinosteroids, medicines

1. INTRODUCTION

Steroids are vital for both plants and animals because they act as hormones. A number of sterols and steroids are produced in plants (Geuns, 1978). Brassinosteroids (BRs) are a group of naturally occurring polyhydroxy steroidal hormones with significant growth promoting activity (Clouse, 1996; Rao *et al.*, 2002). Grove *et al.* (1979) isolated Brassinolide (BL) from 40 kg of bee-collected rape (*Brassica napus* L.) pollen with growth promoting activities. In 1982, another steroidal substance namely, castasterone was isolated from insect galls of chestnut (*Castanea crenata*) (Yokota *et al.*, 1982; Mandava, 1988). Since there are a number of BRs that have been reported in plants and it was assumed that they are ubiquitous occurrence in plant kingdom and are characterized from 44 plant species including angiosperms, gymnosperms, pteridophyte and an alga (Clouse and Sasse, 1998; Fujioka *et al.*, 1998; Yokota *et al.*, 1998; Rao *et al.*, 2002). Till date, there are 70 BRs that have been isolated from 27 families of higher plants and three families of lower plants (Bajguz and Tretyn, 2003). BRs have a common 5 α cholestane skeleton, but their functional variations are due to the different orientations of functionalities on the basic skeleton (Fujioka and Sakurai, 1997). Classification of these steroids is done on the basis of alkyl-substitution pattern of the side chain (Yokota *et al.*, 1997).

BRs promote cell elongation, cell division, differentiation, disease resistance, stress tolerance and senescence in plants (Kauschmann *et al.*, 1996) (Clouse 2002; Mussig *et al.*, 2002). In plants BRs also confer resistance against biotic and abiotic stresses (Khripach *et al.*, 2000; Ikekawa and Zhao, 1991). They provide resistance against wide spectrum of environmental stresses produced by low and high temperature (Dhaubhadel *et al.*, 1999), drought (Li and Van Staden, 1998), salt (Sasse *et al.*, 1995), infection and pesticides (Sasse, 1999) and heavy metals (Bajguz, 2000; Janeczko *et al.*, 2005; Bhardwaj *et al.*, 2007). The focus of attention during the past few decades was on the physiological properties of brassinosteroids (Krishna, 2003). BRs have multiple involvement in the regulation of plant physiological activities such as cell expansion, cell division, alteration of membrane properties, vegetative growth, reproductive biology, senescence, seed germination and stress management (Ozdemir *et al.*, 2004; Khripach *et al.*, 2000; Bhardwaj *et al.*, 2006). The plethora of stresses against which the brassinosteroids act are thermal, drought, heavy metals, infection, pesticides, salt and even viruses (Dhaubhadel *et al.*, 1999, 2002; Janeczko *et al.*, 2009; Wachsmann *et al.*, 2000, 2002, 2004a, 2004b; Krishna, 2003; Haubrick and Assmann, 2006).

According to Khripach *et al.* (2000) the metabolism of BRs in mammals has not yet been investigated. It may be speculated, however, that a normal catabolism of the steroidal skeleton will take place. Being normal constituents of practically all plants, BRs have been, and are, consumed by mammals, and so additional harmful effects are not likely from their use in agriculture. This assumption is an important prerequisite for considering BRs as ecologically safe, non-toxic chemicals for agriculture. However, conformation of their safety can be obtained from toxicological studies. A study on animal system reveals their role as antiviral, antifungal, antiproliferative, antibacterial, neuroprotective and immunomodulatory properties in animal systems. BRs analogues have been reported to have antiviral activity against herpes simplex virus type 1 (HSV-1), arenaviruses as well as against replication of vesicular stomatitis virus (VSV) in Vero cells. Antiherpetic activities both in a human conjunctive cell line (IOBA-NHC) and murine herpetic stromal keratitis (HSK) experimental models have been reported. In human cells, anticancer structure-activity relationship of natural BRs revealed their high cytotoxic activity. Since, both BRs and analogues are reported to inhibit cell growth in cancer cell lines thus BRs may be considered as promising phytohormones for potential anticancer/antiviral/antibacterial drugs. In agriculture several abiotic stresses have been reported to occur simultaneously, rather than a particular stress condition, which are most lethal to crops but the co-occurrence of different stresses is rarely addressed by molecular biologists (Mittler, 2006).

1.1 Brassinosteroids and abiotic stress

BRs have the ability to protect the plants from various environmental stresses such as drought, extreme temperatures, heavy metals, herbicidal injuries and salinity (Sasse, 1999; Bajguz and Hayat, 2008). 24-Epibrassinolide (EBL) treatments increased drought tolerance in *Arabidopsis thaliana* and *Brassica napus* seedlings by changing the expression of drought responsive genes (Kagale *et al.*, 2007). In our earlier studies, we have reported that EBL and 28-Homobrassinolide (HBL) treatments (presowing) improved the shoot emergence and plant biomass production in *Brassica juncea* seedlings and plants under heavy metal stress (Cu, Zn, Mn, Co and Ni). EBL and HBL have also been found to reduce the heavy metal uptake and accumulation in *B. juncea* seedlings and plants (Sharma and Bhardwaj, 2007a,b; Sharma *et al.*, 2007, 2008; Bhardwaj *et al.*, 2007, 2008). HBL protected chickpea (*Cicer arietinum*) from Cd toxicity by enhancing the activities of antioxidant enzymes (Superoxide dismutase, Catalase and Guaiacol peroxidase), proline content, nitrate reductase and carbonic anhydrase and also by increasing the plant growth, leghemoglobin content, nodule number, nitrogen and carbohydrate

contents in the nodules and leaf chlorophyll content, which were decreased proportionately with the increasing concentrations of Cd (Hasan *et al.*, 2008). BRs have been reported to be effective in reducing damage caused by pesticides (simazine, butachlor, or pretilachor) in rice (Sasse, 2003).

The phytotoxic effect on cucumber leaves of nine pesticides including three herbicides (paraquat, fluazifop-p-butyl and haloxyfop), three fungicides (flusilazole, cuproxat and cyazofamid) and three insecticides (imidacloprid, chlorpyrifos and abamectin) has been examined (Xia *et al.*, 2006). BRs have been reported to overcome the salt stress by regulating the activities of key antioxidative enzymes and levels of malondialdehyde (MDA) and proline. Our earlier studies highlight that HBL ameliorated the salinity stress in *Zea mays* seedlings and plants by increasing the activities of antioxidative enzymes and by reducing the level of lipid peroxidation (Arora *et al.*, 2008). Jager *et al.* (2008) studied the endogenous level of BR and Abscicic acid (ABA) in wild type (WT), BR-deficient mutant (*lkb*) and BR-perceptive mutant (*lka*) pea plants exposed to water stress.

1.2 Brassinosteroids and biotic stress

BRs stirred inner potentials of plants that are supportive not only in better survival in stress conditions, but also in receding biotic stress caused by pathogens such as viruses, fungi and bacteria (Nakashita *et al.*, 2003; Wachsman *et al.*, 2004a; Swaczynová *et al.*, 2006; Romanutti *et al.*, 2007; Ohri *et al.*, 2008). The potential of BRs to enhance plant resistance against fungal pathogen infection has been documented in several studies (Khripach *et al.*, 2000). Vasyukova *et al.* (1994) reported that the treatment of potato plants with BRs, essentially reduced the incidence of *Phytophthora* infection. BRs and their synthetic derivatives were reported to be good inhibitors of herpes simplex virus type 1 (HSV-1) and arena virus replication in cell culture (Wachsman *et al.*, 2004a, 2004b). Further, Michelini *et al.* (2004) reported the *in vitro* and *in vivo* antiherpetic activity of three new synthetic BRs analogues {(22S,23S)-3 β -bromo-5 α ,22,23-trihydroxystigmastan-6-one, (22S,23S)-5 α -fluoro-3 β -22, 23-trihydroxystigmastan-6-one, (22S,23S)-3 β -5 α , 22,23-trihydroxystigmastan-6-one. Effects of BRs on insect development, particularly on molting, were reviewed by Zullo and Adam (2002). The treatment of brassinosteroids to root knot nematodes (*M. incognita*) also stimulated their antioxidative defence system (Ohri *et al.*, 2007). Further Ohri *et al.* (2008) studied the influence of EBL on the development of *Meloidogyne incognita* and observed that egg hatching and juvenile emergence in root knot nematode was stimulated by BRs treatments.

2. BIOLOGICAL ACTIVITIES OF BRASSINOSTEROIDS IN VARIOUS TEST SYSTEMS

The potential implications of BRs as a future medicine is acclaimed not only on their antiviral, antibacterial, antifungal, ecdysteroidal, antigenotoxic properties, but also attributed to their anticancerous and antiproliferative activities. As a result, it may become possible to employ these phytohormones in traditional medicines for treating cancer, fungal, bacterial and viral infections etc. (Nakashita *et al.*, 2003; Wachsman *et al.*, 2004a; Swaczynová *et al.*, 2006; Malíková *et al.*, 2008; Romanutti *et al.*, 2007; Ohri *et al.*, 2008;). Although these possible medicinal properties of BRs have been reported recently, but the detailed and systematic investigations in these directions may lead to their use in therapeutics in the forthcoming years.

2.1 Ecdysteroidal activity of brassinosteroids

Ecdysteroids exert widespread effects on insect growth and development. These include roles in morphogenesis, proliferation, programmed cell death, cuticle synthesis, oogenesis and developmental timing (Robertson, 1936; Riddiford, 1993). It is intriguing that some aspects of these pathways share features in common with the wide range of developmental and physiological responses to BRs in plants, which also include promotion of cell division, expansion, and programmed cell death and modulation of reproductive development (Tummel and Chory, 2002). For example, both BL and ecdysteroids are required for cell shape, changes associated with maturation—although they exert this effect in different ways. As described above, BL induces the expression of a range of cell wall-associated proteins that are implicated in cell expansion, providing molecular basis for understanding the role of BRs in directing cell elongation and plant growth. Similarly, ecdysteroids trigger the morphogenesis of adult structures during metamorphosis through coordinated changes in cell shape manifested at the level of the actin cytoskeleton (Von Kalm *et al.*, 1995). It is interesting to speculate that these two responses reflect the basic architectural differences that define plant and animal cells. Thus, the presence of a rigid cell wall in plants demands changes at the level of cell wall-associated proteins to control changes in overall cell shape. Similarly, the integrity of an insect cell is defined by an internal cytoskeleton, which is the target for ecdysteroid triggered changes in cell shape.

Another similarity in steroid responses between plants and insects is programmed cell death. Ecdysteroids trigger the massive death of larval tissues during the early stages of metamorphosis, ridding the animal of these obsolete tissues to make way for their adult counterparts (Robertson, 1936).

This response has been extensively studied in *Drosophila* and shown to occur by autophagy with hallmark features of apoptosis, including DNA fragmentation and caspase activation (Jiang *et al.*, 1997; Jochova *et al.*, 1997; Lee and Baehrecke, 2001). There is evidence that BRs induce programmed cell death during xylogenesis. The specialized xylem vessels that conduct water through plants are made up of individual dead cells called tracheary elements (Roberts and McCann 2000). The BR-deficient *Arabidopsis* mutants *cpd* and *dwf7* have abnormal xylem, implicating the hormone in xylogenesis, although these phenotypes have not been examined in detail (Szekeres *et al.*, 1996; Choe *et al.*, 1999). In addition, Clouse and Zurek (1991) observed that exogenously supplied BL promotes both tracheary element differentiation and cell division in cultured tuber explants of Jerusalem artichoke. Using a zinnia system (*Zinnia elegans* L. cv Canary Bird) in which single mesophyll cells can differentiate directly into tracheary elements, it was observed that exogenously supplied uniconazole (an inhibitor of both gibberellin and BR biosynthesis) prevents uncommitted cells from differentiating into tracheary elements and that BL but not gibberellin overcomes this inhibition (Iwasaki and Shibaoka, 1991). Moreover, BRs appear to act specifically during the final stage of xylogenesis, which involves secondary wall formation and cell death. During this time, the levels of BRs rise dramatically (Yamamoto *et al.*, 2001). The role of BRs in apoptosis and programmed cell death make them potential candidates in the management of lifecycles and different developmental stages of insects and nematodes which spoils the agricultural crops, thereby, helping in sustainable agriculture.

2.2 Antifungal activity of brassinosteroids

Exogenous application of BRs stimulated inner potentials of plants that is helpful not only in better survival in stressful conditions and quality improvement, but also in diminishing disease damage. The potential of BRs to enhance plant resistance against fungal pathogen infection was documented in several studies (Khripach *et al.*, 2000). Vasyukova *et al.* (1994) carried out investigations on the interaction between *Phytophthora infestans* and potato tubers. The treatment of potato plants with BRs, essentially reduced the incidence of *Phytophthora* infection. The increase in resistance in BRs treated potato tubers was associated with enhancement of ABA and ethylene levels and the presence of phenolic and terpenoid substances. BR-induced disease resistance was also noted in barley and cucumber plants. Spraying barley plants at tillering phase with EBL decreased an extent of leaf disease induced by *Helminthosporium teres* Sacc. and increased grain yield even at a dose of 5 mg ha⁻¹ (Pshenichnaya *et al.*, 1997; Volynets *et al.*, 1997a,b). The protective effects of EBL in cucumber against fungi were studied by Churikova

and Vladimirova (1997). The increased activities of peroxidase and polyphenoloxidase enzymes, which are involved in the metabolism of polyphenols, was suggested as a factor contributing to BR induced disease resistance in cucumber plants. The antifungal potential of BRs indicates their prospects for antifungal formulations to plants against several fungal diseases, but the study needs to be carried for other fungal infections in plants.

2.3 Antigenotoxic activity of brassinosteroids

EBL found to show the antigenotoxic properties and this was evaluated through *Allium cepa* chromosomal aberration bioassay. Howell *et al.* (2007) has studied the effect of EBL on the mitotic index and growth of onion (*Allium cepa*) root tips. Low doses of EBL (0.005 ppm) nearly doubled the mean root length and the number of mitosis over that of controls. Intermediate doses of EBL (0.05 ppm) also produced mean root lengths and number of mitosis that were significantly greater than those of the controls. But the highest dose of EBL (0.5 ppm) produced mean root lengths and number of mitoses that were less than control values. Sondhi *et al.* (2008) isolated and characterized the EBL from leaves of *Aegle marmelos* Correa. (Rutaceae) which was further evaluated for the antigenotoxicity against maleic hydrazide (MH) induced genotoxicity in *Allium cepa* chromosomal aberration assay. It was observed that the percentage of chromosomal aberrations induced by maleic hydrazide (0.01%) declined significantly with 24-epibrassinolide treatment. EBL (10^{-7} M) proved to be the most effective concentration with 91.8% inhibition.

The acute toxicity data obtained at the Sanitary-Hygienic Institute of Belarus for 24-epibrassinolide are: LD 50 (orally and dermally) in rats (male/female) is more than 2000 mg per litre. Dermal toxicity in rats (male/female) is more than 2000 mg per litre. The formulation, Epin (0.025% solution of 24-epibrassinolide), in mice and rats (orally and dermally) has an LD of more than 5000 mg per litre. Repeated experiments confirmed the value of LD 50 for 24-epibrassinolide orally in mice and showed a value for Epin which was higher than 15,000 mg per litre (white rats, orally or intranasally). In concentrations of 0.2%, 24-epibrassinolide did not irritate mucous membranes of rabbits' eyes; this compound, or a solution of Epin, did not irritate the skin. The Ames test for mutagenic activity carried out at the Scientific Research Center of Toxicologic and Hygienic Regulation of Biopreparations of Russia, with or without metabolic activation, was negative (*Salmonella typhimurium* TA 1534, TA 1537, TA 1950, TA 98, TA 100). In micro-nuclear or chromosome aberration tests (mice CBAB1/6) neither 24-epibrassinolide nor Epin caused spontaneous mutations. Complex biological testing on *Tetrahymena pyriformis* carried out at the Sanitary-Hygienic Institute

of Belarus has confirmed the genetic safety of 24-epibrassinolide and the absence of mutagenic activity over seven generations. In acute, subacute, and chronic experiments, 24-epibrassinolide showed low toxicity and very little cumulative effect. In prolonged experiments, 24-epibrassinolide showed no toxicity but a pronounced adaptogenic effect (increasing adaptive ability of the population).

2.4 Antiviral and antibacterial activities of brassinosteroids

One of the important aspects of the protective action of BRs in plants is related to their ability to stimulate resistance to viruses (Rodkin *et al.*, 1997; Bobrick *et al.*, 1998). It has been reported that BR treatment reduced virus infection in the starting plant material, various stages of plant development, and the first and second tuber generations of potato. The plants obtained from BR treated sowing material increased the crop yield by 56% and significantly reduced virus infection. The tobacco plants when given treatment of BRs against tobacco mosaic virus (TMV), the bacterial pathogens *Pseudomonas syringae*, and the fungal pathogen *Oidium* species, showed lowered infection and better growth. Similarly in rice, the infection caused by *Magnaporthe grisea* and *Xanthomonas oryzae* which caused rice blast and bacterial blight respectively, was significantly reduced by BR treatments (Nakashita *et al.*, 2003). Potato cuttings cultured in a medium containing brassinolide, 24-epibrassinolide or 28-homobrassinolide were more resistant to viral infection through all stages of development (Khripach *et al.*, 2000).

BRs and their synthetic derivatives are reported to be good inhibitors of herpes simplex virus Type 1 (HSV-1) and arena virus replication in cell culture. The arena virus was susceptible to the compounds throughout its replicative cycle, and the HSV-1 was likely affected at a late step in multiplication (Wachsman *et al.*, 2000). Twenty-seven BRs derivatives/ analogues when tested against measles virus were found to possess antiviral activity. The selectivity index (SI) values of BRs were higher than those obtained with the reference drug ribavirin (Wachsman *et al.*, 2002). Furthermore, BRs and their synthetic derivatives were reported to be good inhibitors of herpes simplex virus type 1 (HSV-1) and arena virus replication in cell culture. Wachsman *et al.* (2004b) described synthetic methods to obtain BRs analogues and report the scope of antiviral activity of these compounds against RNA and DNA viruses. Some of the compounds showed selectivity indexes (SI) 10- to 18-fold higher than ribavirin, a broad spectrum antiviral compound, when tested against Junin virus (JV) (*Arenaviridae*); a good antiviral activity against measles virus (MV) (*Paramixoviridae*), with SI values also higher than ribavirin used as reference drug, and a similar or lower activity

Table 1. Cytotoxic and antiviral activities of brassinosteroid derivatives against measles virus (source: Wachsmann *et al.*, 2004b)

S. No.	Brassinosteroids derivatives (IUPAC name)	CC50* (mM)	EC50** (mM)
1	(22R,23R)-2 α ,3 α , 22,23-Tetrahydroxy-5 α -stigmastan-6-one	62	42
2	(22S,23S)-2 α ,3 α , 22,23-Tetrahydroxy-5 α -stigmastan-6-one	259	65
3	(22E)-2 α ,3 α -Dihydroxystigmast-22-en-6-one	158	40
4	(22R,23R)-2 α ,3 α ,22,23-Tetrahydroxy- β -Homo-7-oxa-stigmastan-6-one	427	8
5	(22R,23R)-2 α ,3 α ,5 α ,22,23-Pentahydroxystigmastan-6-one	262	131
6	(22S,23S)-2 α ,3 α ,5 α ,22,23-Pentahydroxystigmastan-6-one	364	58
7	(22R,23R)-3 β -Acetoxy-22,23-dihydroxy-5 α -stigmastan-6-one	238	40
8	(22S,23S)-3 β -Acetoxy-22,23-dihydroxy-5 α -stigmastan-6-one	226	30
9	(22R,23R)-3 β -Acetoxy-5 α ,22,23-trihydroxystigmastan-6-one	230	21
10	(22S,23S)-3 β -Acetoxy-5 α ,22,23-trihydroxystigmastan-6-one	461	23
11	(22R,23R)-3 β -Bromo-22,23-dihydroxy-5 α -stigmastan-6-one	114	76
12	(22S,23S)-3 β -Bromo-22,23-dihydroxy-5 α -stigmastan-6-one	152	38
13	(22R,23R)-3 β -Bromo-5 α ,22,23-trihydroxystigmastan-6-one	36	13
14	(22S,23S)-3 β -Bromo-5 α ,22,23-trihydroxystigmastan-6-one	185	4
15	(22E)-3 β -Bromo-5 α -hydroxystigmast-22-en-6-one	139	5
16	(22R,23R)-3 β , 5 α ,22,23-Tetrahydroxystigmastan-6-one	819	30
17	(22S,23S)-3 β , 5 α ,22,23-Tetrahydroxystigmastan-6-one	1044	26
18	(22E)-3 β ,5 α -Dihydroxystigmast-22-en-6-one	901	585
19	(22R,23R)-3 β -Fluoro-22,23-dihydroxystigmastan-6-one	215	7
20	(22S,23S)-3 β -Fluoro-22,23-dihydroxystigmastan-6-one	43	1
21	(22S,23S)-3 α -Fluoro-22,23-dihydroxystigmastan-6-one	301	38
22	(22R,23R)-3 β -Fluoro-5 α ,22,23-trihydroxystigmastan-6-one	42	16
23	(22S,23S)-3 β -Fluoro-5 α ,22,23-trihydroxystigmastan-6-one	250	6
24	(22R,23R)-5 α -Fluoro-3 β ,22,23-trihydroxystigmastan-6-one	100	75
25	(22S,23S)-5 α -Fluoro-3 β ,22,23-trihydroxystigmastan-6-one	160	3
26	(22E)-3 β -Fluoro-5 α -chlorostigmast-22-en-6-one	858	32
27	(22E)-5 α -Fluorostigmast-2,22-dien-6-one	935	701
28	(22E)-3 β -Hydroxystigmast-5,22-diene (stigmasterol)	479	117
29	Ribavirin {1- β -D-ribofuranosyl-1-2-4 triazole-3-carboxamide}	840	52

*Compound concentration required to reduce cell viability by 50% after 24 h incubation at 37°C using stationary-phase cells. ** Compound concentration required to inhibit virus yield (plaque forming units) by 50%. [IUPAC, International Union of Pure and Applied Chemistry.] (Source: Wachsmann *et al.*, 2004b)

against herpes simplex type 1 and 2 (HSV-1 and HSV-2) (*Herpesviridae*) when compared to foscarnet or acyclovir, respectively. Structure activity relationship studies (SAR) were analyzed, in order to detect which stereochemistry, type and position of functional groups were needed to develop a selective class of virus inhibitors. A variety of chemical structures are currently known as inhibitors of pathogenic virus replication. Nucleoside synthetic analogues are the most successful ones, in clinical use, against infections caused by most perilous viruses disseminated among human population.

Several other molecules have been designed to interrupt specifically human immunodeficiency virus (HIV) replication by inhibiting viral proteases or, in the case of influenza virus, to mimic the substrate for sialidase enzymatic activity. Unfortunately, viruses respond to antiviral treatment with a rapid selection of drug resistant mutant particles, compelling virologists to search for new active compounds. Animal viruses tested for their susceptibility to BRs analogues comprised two RNA monocistronic viral families, *Paramyxoviridae* and *Arenaviridae*, and one DNA virus family *Herpesviridae*, all of them are important human pathogens. Preliminary studies by Wachsmann *et al.* (2004b) with brassinolide have shown significant *in vitro* antiviral activity against various RNA and DNA viruses (Tables 1 and 2).

Table 2. Antiviral Activity of Natural Brassinolide against several RNA and DNA Viruses (Source: Wachsmann *et al.*, 2004b)

S.No.	Viruses	Brassinolide (% inhibition)*
1.	Poliovirus type I	96
2.	VSV Indiana strain	100
3.	HSV-1 F strain (tk+)	96
4.	Tacaribe TRLV 11573 strain	55
5.	Junin IV 4454 strain	74

*Vero cells (African green monkey kidney cells) were grown as monolayers in minimum essential medium (MEM) supplemented with 5% inactivated calf serum and 50 g/ml gentamycin. Maintenance medium (MM) consisted of MEM + 2% calf inactivated serum. The monolayers were infected at a moi (multiplicity of infection) of 1 with the different viruses. After 1 h adsorption at 37°C, the cultures were covered with MM or with MM containing brassinolide at a concentration of 1 µM. After 24 h of incubation at 37°C, supernatants were harvested and titrated by a plaque assay. The inhibition of virus replication was calculated by comparison with virus titres obtained in cultures without the presence of the brassinolide. (Source: Wachsmann *et al.*, 2004b)

Michelini *et al.* (2004) have reported the *in vitro* and *in vivo* antiherpetic activity of three new synthetic BRs analogues. Chemical synthesis of three

new synthetic BRs analogues like (22S,23S)-3 β -bromo-5 α ,22,23-trihydroxystigmastan-6-one, (22S, 23S)- 5 α -fluoro-3 β -22,23-trihydroxystigmastan-6-one, (22S, 23S)-3 β -5 α ,22,23-trihydroxystigmastan-6-one and their antiherpetic activity both in human conjunctive cell lines (IOBA-NHC) and in the murine herpetic stromal keratitis (HSK) experimental model were tested. All compounds prevented HSV-1 multiplication in NHC cells in a dose dependent manner when added after infection with no cytotoxicity. Significantly, *in vitro* studies showed that EBL is capable of reducing or even arresting the growth of the HIV in cultured infected cells. Khripach *et al.* (2005) reported that EBL may be used in the prevention and cure of HIV infection and related conditions (AIDS related complex), both symptomatic and asymptomatic, or when exposure to HIV virus was suspected. Further, Talarico *et al.* (2006) investigated that the replication of herpes simplex virus (HSV) Type 1 in Vero cells is inhibited in the presence of (22S,23S)-3 β -bromo-5 α ,22,23-trihydroxystigmastan-6-one (6b), a synthetic brassinosteroid derivative. Since a late step of virus multiplication is hindered by 6b, a study was performed on drug-drug combination with acyclovir (ACV) and foscarnet (FOS). It was determined that 6b would act synergistically with low concentrations of ACV and moderate concentrations of FOS against HSV. The best drug combination tested in this study resulted in an increase of 29.3 and 47.2% in antiviral activity for ACV (0.036 μ M) and FOS (37.5 μ M) in the presence of 14.8 and 6.9 μ M of 6b, respectively.

Vesicular stomatitis virus (VSV), a member of the Rhabdoviridae family, is an enveloped single-strand RNA virus that causes an economically important disease in cattle, horses and swine. Romanutti *et al.* (2007) reported the antiviral effects of a synthetic BR ((22S, 23S)-3 β -bromo-5 α ,22,23-trihydroxystigmastan-6-one) against the replication of vesicular stomatitis virus (VSV) *in vero* cells. Synthetic BR affected the late event of the virus growth cycle and inhibited virus protein synthesis and viral mature particle formation. Time-related experiments showed that 6b mainly affects a late event of the virus growth cycle. Virus adsorption, internalization and early RNA synthesis are not the target of the inhibitory action. Results obtained indicate that the antiviral compound adversely affects virus protein synthesis and viral mature particle formation. Synergistic *in vitro* Interactions between (22S,23S)-3 β -Bromo-5 α ,22,23-Trihydroxystigmastan-6-one and Acyclovir or Foscarnet against Herpes simplex Virus Type 1 were studied.

A number of biologically active steroids bearing unusual side chains, isolated from marine sponges, have been studied for their antiviral activity. Orthoesterols A, B and C have been reported to be active against feline leukemia virus (FeLV), mouse influenza virus and mouse coronavirus. Weinbersterols A and B also isolated from a sponge exhibited *in vitro* activity against FeLV with IC₅₀ (inhibitory concentration) values of 40 and

52 µg/ml, respectively and also showed activity against HIV with IC₅₀ value of 10 µg/ml. The effect of brassinolide on the replication of several viruses, initially tested at a concentration of 1 µM (Table 1), showed that this compound displays a broad spectrum of antiviral activity, with higher inhibition values than that reported for other natural steroidal molecules. Some synthetic BRs were tested against herpes simplex virus type 1 (HSV-1) by Michelini *et al.* (2008) which induced an ocular chronic immunoinflammatory syndrome named herpetic stromal keratitis that might lead to vision impairment and blindness in mice.

2.5 Antiproliferative and anticancer activities of brassinosteroid

Franěk *et al.* (2003) observed that EBL at subnanomolar concentrations modulated growth and production characteristics of a mouse hybridoma. A mouse hybridoma was cultured either in standard serum-free medium, or in medium diluted to 30%, in which the cells underwent nutritional stress. Steady-state parameters of semicontinuous cultures conducted at EBL concentrations from 10⁻¹⁶ to 10⁻⁹ mol l⁻¹ were evaluated. Typical effects of the EBL found both in standard and in diluted media were increased in the value of mitochondrial membrane potential, drop of intracellular antibody level, increase in the fraction of the cells in the G₀/G₁ phase, and decrease in the fraction of the cells in the S phase. Viable cell density was significantly higher as compared to control at EBL concentrations ranging from 10⁻¹³ and 10⁻¹² mol l⁻¹. So EBL might induce perturbations in the cell division mechanism, in mitochondria performance, and in secreted protein synthesis in a mammalian cell line.

Swaczynová *et al.* (2006) studied the anticancer properties of BRs. Natural types of BRs affected the viability, proliferation, differentiation, apoptosis and expression of some cell cycle related proteins in cancer cell lines. Cytotoxic activity of BRs were tested *in vitro* by Calcein AM assay. IC₅₀ values were estimated for human breast adenocarcinoma cell lines (MCF-7–estrogen-sensitive, MDA-MB-468–estrogen-insensitive), human acute lymphoblastic leukemia cell line (CEM) and human myeloma cell line (RPMI 8226). TUNEL, DNA ladder assay and immunoblotting techniques were used for the analysis of changes of cell viability, proliferation, differentiation and apoptosis. 28-Homocastasterone inhibited the viability of cancer cell lines and significantly reduced or induced the expression of *p21*, *p27*, *p53*, cyclins, proteins of Bcl-2 family and ER- α . The antiproliferative properties could be used for the development of new brassinosteroid-derived generation of anticancer drugs.

There have been few reports on the effects of BRs on cell division of mammalian cells. Up to now the inhibitory effects of BRs on mammalian cell division are unknown. To determine basic anticancer structure–activity relationships of natural BRs on human cells, several normal and cancer cell lines have been used. Several of the tested BRs were found to have high cytotoxic activity. Hence, in this regard Malíková *et al.* (2007) tested the effects of the most promising and readily available BR analogues with interesting anticancer properties, 28-homocastasterone and 24-epibrassinolide, on the viability, proliferation, and cycling of hormone-sensitive/insensitive (MCF-7/MDA-MB-468) breast and (LNCaP/DU-145) prostate cancer cell lines to determine whether the discovered cytotoxic activity of BRs could be, at least partially, related to brassinosteroid-nuclear receptor interactions. Both BRs inhibited cell growth in a dose dependent manner in the cancer cell lines. Flow cytometry analysis showed that BR treatment arrested, MDA-MB-468, LNCaP and MCF-7 cells in G1 phase of the cell cycle and induced apoptosis in MDA-MB-468, LNCaP, and slightly in the DU-145 cells. These results proved that natural BRs, at micromolar concentrations, can inhibit the growth, at micromolar concentrations, of several human cancer cell lines without affecting the growth of normal cells. Therefore, these plant hormones are promising leads for potential anticancer drugs. Compounds 6b [(22S,23S)-3 β -bromo-5 α ,22,23-trihydroxystigmastan-6-one], 1d [(22R,23R)-2 α ,3 α ,22,23-tetrahydroxy-Homo-7-oxa-stigmastan-6-one], 8a [(22R,23R)-3 β -fluoro-22,23-dihydroxystigmastan-6-one], 9b [(22S,23S)-3 β -fluoro-5 α ,22,23-trihydroxystigmastan-6-one] and 10b [(22S,23S)-5 α -fluoro-3 β ,22,23-trihydroxystigmastan-6-one], with selectivity indexes (SI) of 40, 57, 31, 37 and 53, are the derivatives with good antiviral activity against MV. These SI values are higher than those obtained with ribavirin (used as reference drug). A comparative analysis of 50% cytotoxic concentration (CC50) values, using confluent non-growing cells, gives an indication of structure–activity relationship. According to their degree of cytotoxicity the compounds were divided in three groups: low, intermediate and high cytotoxicity. By observing the chemical structures of compounds belonging to the first group we can see that less cytotoxic activities are related to the presence of a 3 β -hydroxy group on C-3 (ring A) and a double bond between C-22 and C-23 (side chain). The replacement of a 5 α -hydroxy group by a 5 α -fluoro group enhances cytotoxicity. Halogenated brassinosteroid derivatives in C-3 position are more cytotoxic than those with an acetoxy group in the same position. For compounds 1d, 6b, 10b and ribavirin, cytotoxicity measurements were also done with replicating cells; CC50 values were low, but they still competed favourably with ribavirin against MV.

2.6 Brassinosteroids and cotton leafworm *Spodoptera littoralis* G.

The two important plant hormones 24-epibrassinolide and 24-epicastasterone showed 50% competition for binding at IC₅₀ of 1–3.6 μM with [³H] ponasterone A using cultured imaginal wing discs from last-instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisduval). But, the culture of imaginal wing discs in different concentrations of brassinosteroids, even up to 100 μM , demonstrated no induction of evagination. In contrast, 20E and the non-steroidal agonist RH-5992 competed respectively about 23- and 42-times more effectively with labeled ponasterone A, and their ability (IC₅₀) to induce disc evagination *in vitro* was 158 and 87 nM, respectively. Injections of 10 μg of brassinosteroids in latest moulted last-instar larvae did not cause mortality above controls. Higher mortalities were found when brassinosteroids were injected late in the last instar.

2.7 Brassinosteroids and insects

Schmidt *et al.* (2000) reported that after feeding 24-epi-castasterone to the cockroach *Periplaneta Americana*, an organospecific epimerization of the brassinosteroid to 2, 24-di-epi-castasterone could be detected in female insects. The metabolite being observed only in the ovaries and not in the testes of the insect was identified by GC/MS in comparison with a synthesized authentic sample. Contrary, 24-epi-brassinolide is not metabolized in sexual organs of *Periplaneta Americana*. Effects of BRs on insect development, particularly on molting, were reviewed by Zullo and Adam (2002). EBL or 24-epicastasterone did not affect the evagination of imaginal wing discs, nor was there any effect on intact last instar larvae of the cotton leaf worm, *Spodoptera littoralis*, after oral feeding (Smagghe *et al.*, 2002). Similarly, treatment of root knot nematodes (*Meloidogyne incognita*) with BL revealed much higher percentage of hatching in treated egg masses as compared to control (Ohri *et al.*, 2002). Ohri *et al.* (2004, 2005) further revealed enhanced juvenile emergence of *M. incognita* by brassinosteroids treatments. The treatment of brassinosteroids to root knot nematodes (*M. incognita*) also stimulated their antioxidative defence system (Ohri *et al.*, 2007). Further Ohri *et al.* (2008) studied the influence of EBL on the development of *Meloidogyne incognita*. EBL enhanced the percentage of hatching in treated egg masses as compared to control. EBL treated juveniles induced more gall numbers and larger size of galls in roots of tomato plants.

3. NEUROACTIVITIES

Rupprecht and Holsboer (1999) reported that steroids influence neuronal function by binding to intracellular receptors that can act as transcription factors and regulate gene expression. In addition, some so-called ‘neuroactive steroids’ are potent modulators of an array of ligand-gated ion channels and of distinct G-protein coupled receptors via nongenomic mechanisms, and they can influence sleep and memory. They described how these neuroactive steroids modulate neurotransmitter receptors and address the neuro-psychopharmacological potential that arose from the intracellular crosstalk between genomic and nongenomic steroid effects. Neuroactive steroids could also have a role in the response to stress and the treatment of psychiatric disorders, such as depression, and, as they affect a broad spectrum of behavioral functions through their unique molecular properties, they could constitute a yet unexploited class of drugs.

4. CONCLUSIONS

Steroid hormones are essential for normal growth and development of plants and animals. Brassinosteroids (BRs) an important group of plant steroid hormones being mainly concentrated in plant pollens are reported to be involved in biostimulation in folk medicine. They also form the basis for the production of some anti-inflammatory and metabolism stimulative medicines, which are especially recommended for children and elderly people with chronic infections (Mashkovskii, 1997). Further, they have bright prospects as potential drugs against viruses like measles, herpes and arena viruses; bacteria, fungi and other pathogens. Although it is too early to predict their clinical utility, but there are evidences that BRs are emerging group of compounds as anti-cancer and antiviral drugs. Further illustrative studies to elucidate eventual anti-inflammatory, anti-cancerous and medicinal effects of BRs in animal models would confirm their use as therapeutics in traditional medicines.

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