Propolis Extracts

Two Distinct Propolis Extracts, Bio 30 from NZ and GPE from Brazil, Share a Common Biological Property: Blocking the Oncogenic PAK1 Signaling to Suppress the Growth of NF Tumor Xenografts in Mice.

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ABSTRACT

It has recently been well established by our group and others that the oncogenic kinase PAK1, a Rac/CDC42-dependent Ser/Thr kinase, is essential for the growth of more than 70% of all human cancers and NF (neurofibromatosis)-associated tumors, but not the normal cell growth. Thus, we have developed or identified a series of synthetic chemicals or natural products such as FK228 which selectively block this kinase for the therapy of these PAK1-dependent cancers/tumors, in particular pancreatic cancer, melanoma, MM (multiple myeloma), glioma and NF tumors for which no effective therapeutic has been developed (available on the market) as yet.

Among these anti-PAK1 drugs the antibiotic FK228, a ring peptide, that inactivates PAK1 by inhibiting HDAC (histone deacetylase) directly, is the most potent (IC50 around 5 pM for cell growth), but still in clinical trials (phase 2) for mainly CTCL (cutaneous T-cell lymphoma). Thus, we have recently started identifying a few natural anti-PAK1 products which are available on the market so that patients suffering from these PAK1-dependent illnesses could have an immediate benefit without a time-consuming clinical trial.

Here we present that the extracts from at least two vastly distinct propolis sources, CAPE (caffeic acid phenethyl ester)-based extract of NZ (New Zealand) propolis (Bio 30) and ARC (artepillin C)-based extract of Brazilian green propolis (GPE), share a very unique common property that inactivates selectively the oncogenic PAK1 signaling by their major anti-cancer ingredients, CAPE and ARC, respectively. Moreover, we demonstrate for the first time that both extracts are able to suppress almost completely the growth of NF tumor xenografts in nude (immuno-deficient) mice.

Thus, we are currently conducting the first trial of both Bio 30 and GPE for NF and a few other PAK1-dependent cancers. Bio 30 is the most effective and inexpensive propolis product on the market, and causes an allergic skin rash to only 1% of the population, whereas GPE is the much more expensive propolis product, but causes no allergy so far.

Key words: PAK1, Bio 30, Brazilian green propolis, CAPE, artepillin C, Neurofibromatosis,

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INTRODUCTION

PAK1 and Cancers

Discovery of many different oncogenes and tumor suppressor genes during last three decades has completely changed both our understanding of cancers and approach to cancer therapy. For most of these gene products were found signal transducers that regulate cell proliferation positively or negatively. Many oncogene products are abnormally activated GTPases (GTP-dependent proteins) such as RAS, or protein kinases such as SRC. Similarly, many tumor suppressor gene products are transcription factors such as p53 and RB, and inhibitors of GTPases or kinases, such as NF1 and NF2/ merlin, respectively. Thus, it became possible to generate specific chemical inhibitors of these oncogenic signal transducers or mimetics (agonists) of these anti-oncogenic signals, which would be useful for the systemic treatment of a variety of malignant or benign tumors.

We shall call such a new generation of anti-cancer drugs "signal (blocking) therapeutics", to distinguish them from a series of the conventional anti-cancer drugs which target mainly DNA or microtubules (MT), thereby handling mainly the fast-growing cancers. These DNA/MT poisons such as cisplatin, 5-FU and Taxol have been successful for the treatment of a variety of cancers, but failed to suppress the growth of pancreatic cancers and slowly-growing tumors such as NF (neurofibromatosis). Furthermore, the DNA/MT poisons cause inevitably a series of side effects such as hair loss and immuno-suppression. These side effects are due to damaging the fast-growing normal cells such as hair and bone marrow cells.

Among the first signal therapeutics, so far Gleevec (STI-571) appears to be most successful. Gleevec is a specific inhibitor of the Tyr-kinases BCL-ABL, PDGF receptor (PDGFR) and Kit (1). It is an ATP antagonist which binds the ATP-binding pocket of these kinases. Since CML (Chronic Myeloid Leukemia) and GIST (Gastrointestinal Stromal Tumors) are caused by abnormal activation of BCL-ABL and Kit, respectively, Gleevec (400-600 mg daily) has been used for the treatment of these rare cancers, which represent far less than 1% of human cancers (1). However, the remaining vast majority of cancers and NF cannot be treated by Gleevec, simply because either BCL-ABL, PDGFR or Kit is not essential for the growth of these cancers/tumors.

Which signal transducer is essential for the majority of those remaining cancers/tumors? A systematic study by our group and a few others has recently established that the Ser/Thr-kinase PAK1 is essential for more than 70% of cancers (including major cancers such as breast and prostate cancers, and pancreatic cancer) and even slowly-growing tumors such as NF (2-7). These tumors are highly addicted to this kinase for their anchorage-independent growth, but not for the normal (anchorage-dependent) cell growth. So we call them PAK1-dependent tumors (PDTs). PAK1 is a Rac/CDC42-dependent kinase that phosphorylates several effectors such as the kinase Raf, LIM kinase 1, BAD and the kinase aurora (8-9). Thus, PAK1 is involved in several different features of tumorigenesis, including cell division, anti-apoptosis (cell survival), metastasis/ invasion, and angiogenesis (see Fig. 1).

So recently we have started exploring anti-PAK1 drugs among natural products available on the market, hopefully being able to give an immediate benefit to those who are currently suffering from these formidable cancers/NF (PDTs). Here we present a few examples of propolis extracts which selectively block the oncogenic PAK1 signaling.

Propolis

Propolis, a honeybee product, was first identified as an anti-cancer remedy in a late 1980s when Dezider Grunberger's group at Columbia University found that CAPE (caffeic acid phenethyl ester) is the major anti-cancer ingredient in a propolis sample (10). CAPE is a derivative of CA (caffeic acid) that down-regulates the GTPase Rac, a direct activator of PAK1 (11). Thus, it would inactivate PAK1 eventually. Interestingly, NZ (New Zealand) propolis reportedly showed the highest CAPE content (6-7% of extract) among a variety of propolis samples around the world, whereas Brazilian propolis samples (either green or red) contain no CAPE. Nevertheless the latter are also known to have an anti-cancer property.

The major source of CAPE in propolis is known to be young buds of poplar trees. Thus, most of propolis samples harvested in the Temperate Zone including Europe and East Asia (China, Korea and Japan) are expected to be relatively rich in CAPE. Like many other anti-PAK1 drugs, CAPE has been shown to block both angiogenesis and metastasis, in addition to anchorage-independent growth of cancer cells (12-13).

NF (neurofibromatosis)

The major reason why we recently started working on NZ propolis was to develop the first effective NF therapeutics inexpensively available on the market. For no NF therapeutics was available on the market until then. NF (neurofibromatosis) is a rare genetic disease which is often associated with either benign or malignant tumors developing in brain and along spinal cord and also in skins. There are around 2 millions people on this planet who suffer from this NF. Unlike many other cancers/ tumors which develop in the later stages of our life, NF tumors start to develop in a very early stage of our life, in around 6 months after the birth or even earlier. The conditions of NF patients get gradually worsen with time, losing eye-sight or hearing and eventually facing partial or total paralysis, and even the premature death if their NF tumors become malignant.

There are two types of NF: Type 1 (NF1) and type 2 (NF2). NF1 is caused by dysfunction (loss-of-function mutation) of a tumor suppressor (NF1 gene product) which is a RAS GAP of 2818 amino acids (14). NF2 is caused by dysfunction of another tumor suppressor, a NF2 gene product of 595 amino acids called merlin, which was found recently to inhibit the kinase PAK1 directly (3). NF1 tumors include benign neurofibromas, plexiform and the malignant MPNST (malignant peripheral nerve sheath tumor), representing around 90% of NF tumors. NF2 tumors include mainly two tumors, meningiomas and Schwannomas, representing the remaining 10% of NF tumors. Since the total number of NF patients are less than 1% of the total cancer patients, the progress in R & D of NF therapeutics has been far behind the those of cancer therapeutics. Nevertheless, several years ago the development of an NF1 tumor was found to depend on the key kinase PAK1 (15), like RAS-induced cancers such as pancreatic, colon and lung cancers, because NF1-deficiency leads to abnormal activation of normal RAS, which in turn activates PAK1 constitutively (see Fig. 2). More recently, we found that NF2 tumors also require the same kinase for their growth, mainly because NF2-deficiency causes the abnormal activation of PAK1 (3). Subsequently we confirmed that FK228, the most potent anti-PAK1 drugs, is effective for the treatment of a human NF tumor (MPNST) xenograft in mice (4). Unfortunately, however, it turned out that FK228 would not be available for NF patients until the on-going clinical trials for cancer patients are completed, and this drug

would enter the market with the FDA approval. Thus, an alternative NF therapeutics has been explored among the inexpensive and safe healthcare food supplements freely available on the market.

Among such alternatives we found that a water-miscible CAPE-rich extract of New Zealand (NZ) propolis called Bio 30 and ARC (artepillin C)-based extract of Brazilian green propolis (GPE) are the most effective NF therapeutics, using human NF1 and NF2 tumor xenografts in mice (16-17).

RESULTS

NZ Propolis (CAPE-based)

Bio 30 (alcohol-free liquid) was found to contain not only CAPE but also several other anti-cancer ingredients such as galangin, chrysin, apigenin and CA (caffeic acid) (16). The IC50 of CAPE alone for the growth of NF1-deficient MPNST and NF2-deficient Schwannoma cells is 25 and 36 mM, respectively. However, the IC50 of Bio 30 for Schwannoma cells turned out to be 1.5 mg/ml. Since the CAPE content of Bio 30 is around 12 mg/g, its contribution to 1.5 mg/ml is only 0.018 mg/ml, which is around 0.06 mM, meaning that the anti-mitotic activity of CAPE is potentiated by around 600 times with several other anti-cancer ingredients in this extract (16). Furthermore, this propolis extract contains a lot of lipids which solubilize the water-insoluble CAPE. Although CAPE has been shown to have an anti-cancer activity, its bioavailability is very poor (mainly due to its poor water-solubility) in vivo, and therefore it has never been in clinical trials. Thus, Bio 30 potentiates not only the anti-mitotic ability per se but also the bio-availability of CAPE.

To determine its in vivo effect, we have treated nude mice bearing either human MPNST or Schwannoma xenografts with Bio 30 (100 mg/kg), i.p., twice a week. Over 100 days the slow growth of MPNST was suppressed by 90%, while the fast-growing Schwannoma was almost completely regressed over 30 days (see Fig. 3) (16). Furthermore, when Bio 30 was supplemented with an extra CAPE (5 mg/kg), in 4 out of 6 mice, MPNST was completely regressed, and also its metastasis was significantly delayed and reduced to around 15% of the control.

Thus, Bio 30 has been proved to be the first effective NF therapeutics available on the market. Furthermore, 5 ml of Bio 30 required for daily treatment of each adult NF patient (weighing around 50 kg) costs only a dollar. Thus, it would be economically quite feasible even for a life-long treatment of both NF1 and NF2 tumors. So far Bio 30 shows no side effect at this dose, except that only 1 % of population is known to be allergic to the CAPE-based propolis such as Bio 30.

Using a similar xenograft system, we have confirmed that Bio 30 is effective to suppress the growth of at least human pancreatic and breast cancers as well as gliomas (Messerli, S. et al, unpublished data).

Brazilian Propolis (ARC-based)

Generally speaking, propolis contains several distinct anti-cancer ingredients: not only CA and CAPE, but also artepillin C, chrysin, and propolins. In the case of Brazilian green

propolis, it has no detectable CAPE, but instead a phenolic acid called artepillin C (ARC), whose content is 8%, appears to be the primary anti-cancer ingredient in this propolis (17).

Interestingly, like CAPE and other anti-PAK1 drugs, ARC up-regulates p21, but not p27, strongly suggesting that ARC inactivates the kinase PAK1, but not the kinase AKT (17). Here, we confirm the above notion, and further demonstrate that both ARC alone and ARC-rich extract of Brazilian green propolis (GPE) can suppress almost completely the growth of NF2 tumor xenograft in mice (see Fig. 4) (17). However, unlike the case of Bio 30, the remaining ingredients in GPE do not appear to boost significantly the anti-cancer/NF activity of ARC alone (17). So far the only notable advantage of GPE over Bio 30 is that GPE does not cause any allergic reaction, though it is significantly less potent than Bio 30. Thus, GPE would be recommended to those who are allergic to CAPE-based propolis extracts such as Bio 30.

DISCUSSION

It would be worth noting that Brazilian red propolis extract (RPE) contains neither CAPE nor ARC, but still is able of suppressing the growth of PDT cells such as pancreatic cancer cell lines (18). This observation strongly suggests that RPE also blocks the oncogenic PAK1 signaling. In other words, wherever honey bees go, they manage to collect anti-PAK1 products from their surroundings to make a powerful anti-cancer propolis sample. A big mystery to be solved in the future would be: how can they recognize anti-PAK1 compounds such as CAPE and ARC in the nature? Our recent study on nematode (C. elegans) strongly suggests that PAK1 normally shortens the life span of this worm, as PAK1 KO (knock out), CAPE or ARC activates the (tumor suppressing) transcription factor "FOXO" which eventually activate a heat-shock gene called Hsp16 (Maruta, H. et al., unpublished observation). Very interestingly, the activation of both FOXO and Hsp16 gene leads to a significant extension (by 50%) of the life span (19). Thus, it is most likely that honey bees must feel comfortable or happy somehow when they suck anti-PAK1 compounds, as they suck sugars (glucose or fructose) to make sweet honey.

Acknowledgment

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Note: Due to the space limit, we present only the two major in vivo data (Figs. 3 and 4) here. For the remaining detailed data, see our recently published papers (16, 17).

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Figures Legends:

Fig. 1. Roles of PAK1 in tumorigenesis

Fig. 2. Oncogenic RAS-PAK1 signaling pathway blocked by NF1 and NF2 gene products. The RAS-induced activation of PAK1 through PI-3 kinase and GTPase Rac/CDC42 is normally blocked by either NF1 gene product, a RAS CAP, or NF2 gene product (merlin), a PAK1 inhibitor, suppressing the tumorigenesis.

Fig. 3. Bio 30 suppresses the growth of NF tumors (Schwannoma) grafted in mice. Nude mice carrying an NF2-deficient tumor were treated with of Bio 30 (100 mg/kg), i.p., twice a week.

Fig. 4. ARC/GPE suppresses the growth of NF tumor grafted in mice. Nude mice carrying the NF2-deficient tumor were treated with either ARC alone (50 mg/kg) or GPE (500 mg/kg), i.p., twice a week.

Fig. 1. Roles of PAK1 in tumorigenesis

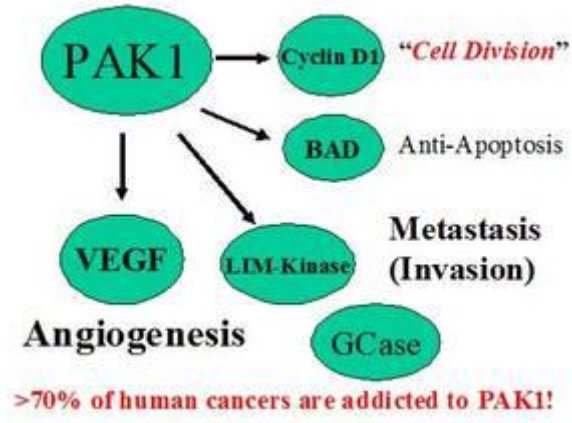
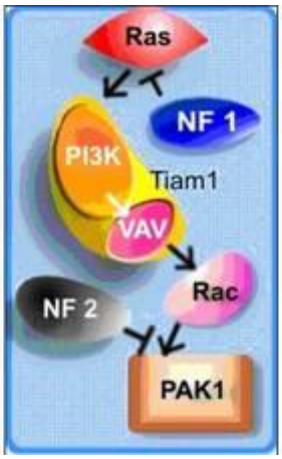


Fig. 2. Oncogenic RAS-PAK1 signaling pathway blocked by NF1 and NF2 gene products



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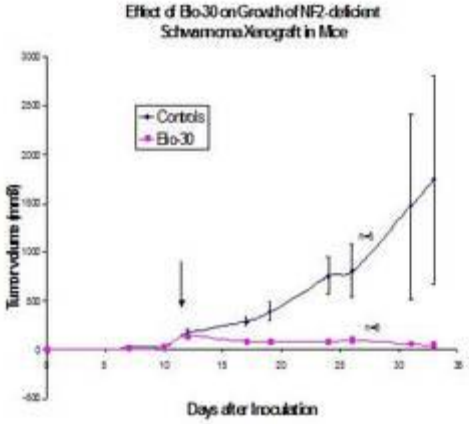

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