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REVIEW ARTICLE / DERLEME

Mistletoe in the treatment of malignant melanoma

Malign melanomun tedavisinde ökse otunun yeri

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ABSTRACT

Malignant melanoma is a malignant neoplasia drives from melanocytes. Malignant melanoma, the most causing death, is seen in the third place at skin cancer. Malignant melanoma shows intrinsic resistance to chemotherapeutic agents and variability in the course of the disease which are distinct features separating from other solid tumors. These features prevent the development and standardization of non-surgical treatment models of malignant melanoma. Although there is a large number of chemotherapeutic agents used in the treatment of metastatic malignant melanoma, it hasn't been demonstrated the survival advantage of adjuvant treatment with chemotherapeutic agents. Because of the different clinical course of malignant melanoma, the disease is thought to be closely associated with immune system. Therefore, immunomodulatory therapy models were developed. Mistletoe stimulates the immune system by increasing the number and activity of dendritic cells, thus it has been shown to effect on tumor growth and metastasis of malignant melanoma patient. Outlined in this review are the recent developments in the understanding the role of mistletoe as a complementary therapy for malignant melanoma. *J Clin Exp Invest 2014; 5 (1): 145-152*

Key words: Malignant melanoma, mistletoe, *Viscum album*

ÖZET

Melanoma melanositlerden köken alan malign tümördür. Deri kanserleri içinde 3. sıklıkta görülen melanoma en fazla ölüme neden olan kanser tipidir. Malign melanomu, diğer solid tümörlerden ayıran belirgin özellikleri, kemoterapötik ajanlara gösterdiği intrinsik direnç ve hastalık seyrinin değişkenliğidir. Bu özellikler, malign melanomda cerrahi dışı tedavi modellerinin geliştirilmesini ve standart hale getirilmesini engellemektedir. Metastatik malign melanom tedavisinde kullanılan çok sayıda kemoterapötik ajan olmasına rağmen bu ajanlar ile yapılan adjuvan tedavi çalışmalarında sağ kalım avantajı gösterilememiştir. Malign melanomun farklı klinik seyir göstermesi nedeniyle, hastalığın immun sistem ile yakından ilişkili olabileceği düşünülmektedir. Bu nedenle immunomodulatör tedavi modelleri geliştirilmiştir. Ökse otunun dendritik hücrelerin sayısını ve aktivitesini artırmak suretiyle immün sistemi uyardığı ve böylece malign melanomlu hastada tümör büyümesi ve metastazı üzerine etkili olduğu gösterilmiştir. Derlemede malign melanomun komplementer tedavisinde ökseotunun rolünün anlaşılmasına yönelik güncel gelişmeler özetlenmiştir.

Anahtar kelimeler: Malign melanom, ökse otu, viscum album

INTRODUCTION

Malignant melanoma is the malignant transformation of melanocytes, the pigment producing cells found in the skin, eye, inner ear, and leptomeninges, of which the skin is the most common site for melanoma development [1]. Superficial spreading, nodular, and acral lentiginous, of which nodular melanomas have the worst prognosis are three histologic types of malignant melanoma [2]. Malignant melanoma is the most serious type of skin cancer with the fastest increasing incidence and survival of patients with distant metastases is generally less than one year [3]. The treatment of early-stage malignant melanoma is possible with wide surgical excision and regional lymph node curettage [4]. Despite intensive research, no curative treatment exists for metastatic malignant melanoma [5]. Conventional chemotherapy or combination of radio and chemotherapy have yet no broadly successful therapies and have not led to any considerable prolongation of survival [6]. As scientific medicine have been disappointingly ineffective to offer for melanoma patients with the threat of metastatic disease, the most of the patients turn to supplementary therapy such as aqueous mistletoe (*Viscum album*, VA) extracts

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[7]. The mechanism underlying the anti-tumoral activity of mistletoe preparations has been poorly understood. Moreover, the proposed mechanisms include induction of apoptosis of tumor cells and lymphocytes, inhibition of angiogenesis and stimulation of the cellular compartment of the immune system, raising the number and the activity of natural killer (NK) cells, dendritic cells (DCs) and granulocytes [8-11]. This review investigates the role of the mistletoe preparations on the reduction of melanoma growth and number of metastases in experimental models [8,12-17] with the enhancement of DC infiltration and apoptosis induction in the melanoma cells. In addition, a case report presents in literature, emphasized on a complete remission of malignant melanoma with mistletoe treatment [18]. These findings suggest that mistletoe brings new clinical perspectives as a complementary therapy for malignant melanoma.

Epidemiology

Malignant melanoma is an aggressive skin disease with high incidence mortality. According to a World Health Organization estimate, there are 132,000 new cases of melanoma per year worldwide [19]. The American Cancer Society (ACS) estimates 68,130 new cases of melanoma in the United States in 2010 with 8,700 deaths, mostly male deaths, constituting a serious public health issue [20]. Malignant melanoma has become a cancer with a major socioeconomic impact because of a high mortality rate of metastatic disease and a relatively high incidence among adolescents and young adults [21]. In addition to its incidence and propensity to affect young adults, melanoma is a major health problem with high metastatic potential toward the skin, lung, brain and the gastro-intestinal tract, aggressive clinical behavior and notable resistance to currently available chemotherapeutic and immunological treatments [22]. The higher incidence of melanoma is also closely associated with the some host factors important risk factors for melanoma such as skin type, presence of dysplastic nevi, degree of pigmentation, susceptibility to ultraviole radiation and episodes of sunburn, immunosuppression, certain melanoma susceptibility genes and family history of the disease [23]. The molecular mechanisms underlying the melanoma development remain unclear, even though there is advances in the knowledge of risk factors for melanoma [22].

The treatment of malignant melanoma

The therapeutic management of malignant melanoma is quite challenging issue. It requires careful clinical examination, skin biopsies and precise immuno-histological analysis. After tumor staging, medical or surgical intervention is made. Survival has been found to be strongly correlated with thickness and ulceration of primary tumour [24]. For patients with thicker than 4 mm melanoma, 5-year survival rate is approximately 30-50% (17). Therefore, early diagnosis is the most critical step in the disease management. For metastatic disease the prognosis is poor, with a 5-year life expectancy of <10% and a median survival of 6-8,5 months. Chemotherapy for advanced disease remains unsatisfactory [25]. Since melanoma is one of the most resistant malignancy to medical therapy among the solid tumours, therefore early diagnosis and surgical removal of the primary tumor is virtually the only curative approach currently available [26]. In 2011, for advanced malignant melanoma treatment, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved new drugs, ipilimumab and vemurafenib [27]. However, time is needed to conclude whether these drugs can be replaced with dacarbazine (DTIC), a drug used for over 30 years in the therapy of metastatic melanoma, even if the response rate was only 10-15%, so the number of living is less than 6 years [26]. When all survival rates were evaluated, it was found that there was no difference between the single use or combination therapies, including CVD (sisplatin, vinblastin, DTIC) and PVB (sisplatin, vinblastin, bleomisin). The response rates of these combination therapies at phase II studies is 20-40%, the tumor response rate is <5% and <2% for log-term. As a result, the adjuvant treatment with chemotherapeutic agents seems to be not effective for survival [28].

Because malignant melanoma shows a different clinical course, it is thought to be closely associated with the immune system. Therefore, immunomodulatory therapy models, interferon alpha (INF-α) and interleukin-2 (IL-2), were developed [29]. The respond rate of these therapies is 10-20 % and long time remission is seen in only 3-5 % of the cases. Also the findings of several randomized controlled trials (RTCs) conducted on the use of INF-α are conflicting in terms of therapeutic efficiency [30-32]. Most importantly, so far there has not been demonstrated any overall survival (OS) benefit, even after adjustment for quality of life incorporating patient's values for the toxic effects of INF-α treatment and melanoma recurrence [33]. Therefore, routine use of INF-α accompanied by clinically relevant toxic effects and represents a substantial economic burden for the health-care system is fostering a continues debate among oncologists [34-36].

Dendritic cell-based immunotherapy of malignant melanoma

Dendritic cells (DC) are also an important tool for tumor-antigen-specific immunotherapy of cancer because of their critical role in mounting a specific immune response where their intratumoral and peritumoral density as well as their functional status are correlated with clinical staging of the disease and patient's survival [24-37]. DC originates from hematopoietic stem cells with in the bone marrow. Under phsiological conditions both myeloid and lymphoid precursors differentiate into immature dendritic cells. In peripheral tissues, upon the uptake of antigen, receiving danger signals or in the context of inflammation, DC undergo maturation process and migrate to the secondary lymphoid organs [37]. This maturation process is characterized by increased surface expressing of antigen presenting surface molecules like major histocompatibility complexes (MHC class I interacts with CD8+ cells whereas MHC class II interacts with CD4+ cells) and co-stimulatory molecules such as CD-54, CD-58, CD-83, CD-80 and CD-86 and secrete several pro-inflammatory cytokines (IL-2,IL-4,IL-6, IL-12, TNF-α etc.) [19,38]. (Figure 1) As a result of this maturation process, DC are well equipped to activate naive T cells; CD 8+ T cells differentiate into cytotoxic T cells (CTL) while CD+4 T cells differentiate into T helper-1 (Th1) or T helper-2 cells (Th2) which interact with macrophages and B cells, respectively, thus providing a link between the adaptive and innate immune system in the secondary lymphoid organs [19,37]. A Th1 immune response and also CTL activation are the goal of utilizing DC as a cancer vaccine in order to eliminate tumor [19]. DC are not just regulators of the immune response, but also maintenance of the immune tolerance in the thymus and secondary lymphoid organs. DC present self antigen (Ag) to develop immune cell and induce deletion of autoreactive T cells result in self tolerance depending on the status of the immune system: steady state versus infection. This situation very important for DC-based anti-tumor immunotherapies to control DC differentiation to prevent undesirable effects of vaccination such as tolerance induction by tolerogenic DC [29]. Moreover, immune evasion of tumor cells by down-regulation of surface or intracellular molecules are also limiting factors for the efficiency of the DC-based vaccination in tumor patients. The other limiting factors are the secretion of soluble immunosuppressive cytokines by tumor cells that convert immature into tolerogenic DC and presence of naturally occurring, antigen-specific regulatory T cells. However, DC-based vaccination led to regression of individual metastases but no complete remission [24].

Mistletoe and malignant melanoma

In studies using mistletoe preparations, regression of tumor metastases and complete remission was observed in experimental models with increasing the number and activity of DC (8,12-17). Mistletoe preparations, the most common form of complementary/alternative cancer therapy, are used often the protocols of adjuvant treatment with standard chemotherapy or radiotherapy. This combined treatment increased the cancer patient's quality of life with stimulating immune system [39].

Mistletoe is a semiparasitic plant of the *Loranthacea* family that grows wild on deciduous hardwood trees like the apple, oak, ash, and elm (var *album*), or on coniferous tress like pine and fir (*Pinus* and *Abies* varieties) [40]. Mistletoe has been used in tradional medicine since ancient times, especially used by the Druids and ancient Greeks, and it appears in legend and folklore as a panacea [41]. Extracts from mistletoe have been used, as a sedative, vasodilator, diuretic, analgesic, cardiotonic, anti-spasmolytic, therapeutically aganist various diseases including cancer, atherosclerosis, hypertension, dizziness, chorea, hysteria, periarthritis, spondylitis and arthritis [42-44].

Mistletoe was firstly used as an anticancer agent in 1917 by Steiner and Wegman, founders of anthnoposophic medicine. Moreover, it has became the most commonly used form of adjuvant cancer therapies in Europe from 1970s. Particularly in Germany, Austria and Switzerland, mistletoe preparations are most frequently used in the treatment of cancer patient [45,46]. Commercially available extracts, preperad from *Viscum album* L., are marketed under a variety of brand names, including Iscador (Iscar), Eurixor, Helixor, Isorel (Vysorel), Iscucin, Plenosol (Lektinol) and Abnova-viscum [46,47]. Commercial preparations are widely differ with regard to their chemical compositions that depens on the species of the host tree (apple, elm, oak, pine, poplar and spruce) and the time of year harvested, lectin content the main active ingredient, and methods of preparations, including alcoholic-aqueous extraction and fermentation of aqueous extract with lactic acid bacteria [48].

The fermented aqueous exracts of mistletoe are biologically and biochemically standardized. One of these preparations is Iscador, which is marketed as Iscador M (from apple trees) contains 250 ng total lectins/ml, Iscador Qu (from oak trees) contains 375 ng total lectins/ml whereas Iscador P (from pine

trees) contains only trace amount of lectins [12]. The antiproliferative effects of Iscador M special, Iscador Qu special and Iscador P were investigated in a panel of 16 human tumor cell lines comprising nine different tumor types, central nervous system, gastric, non-small cell lung, mammary, prostate, renal, uterine cancer cell lines as well as hematological malignancies and melanomas. While Iscador M special and Iscador Qu special showed antitumor activity with a more than 70% growth inhibition in the mammary cell line, Iscador P showed no proliferative activity [12]. Eurixor, an unfermented aqueous exract of mistletoe, harvested from poplar trees, is standardized to contain a spesific amount of ML-I. Helixor, unfermented aqueous exract of mistletoe is marketed as Helixor A (from spruce trees), Helixor M (from apple trees) and Helixor P (from pine trees) [49].

Mistletoe gained attention as a potantial anticancer agent because of their immunomodulatory and cytotoxic properties [9,39,50-53]. Mistletoe extracts stimulate the immune system non-spesifically, by increasing the number and activity of T lymphocytes, B lymphocytes, dendritic cells, natural-killer (NK) cells, neutrophils and activating phagocytic activity of granulocytes with subsequent release of cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12. All of these immune mechanisms, if stimulated, can induce tumor cell lysis [50-53].

From the various components found in most mistletoe extracts, lectins, alkaloids, viscotoxins and polysaccharides, several enzymes, peptides (such as viscumamide), amino acids, thiols, amines, cyclitoles, lipids, phytosterols, triterpines, flavonoids, phenylpropanes and minerals, the most active compounds in cytotoxity and immuno-modulatory effects are lectins and viscotoxins [54-56]. The three mistletoe lectins (MLs) Ml-I, -II, -III are the main therapeutic components of mistletoe exracts [57]. The antitumor effect of lectins is though to be induction of the death of tumor cells via binding of B-chain carrying the carbohydrate-binding site to the target cell surface, which enables the protein to enter the cell, and inhibition of protein synthesis by A-chain removing an adenine residue from the 28S RNA of the 60S subunit of the ribosome due to its ribosome-inactivating properties (Rip type II) [58,59].

Of the three MLs, ML-I is the widely investigated in the immunomodulation and anticancer studies [53,60,61]. Malignant melanoma cells represent an ideal target for ML-I cytotoxic therapy because primary malignant melanomas and their metastases express particularly high numbers of ML-I binding sites [62,63]. A highly significant antiproliferative effect of ML-I via the induction of apoptosis on malignant melanoma cells was demonstrated in vitro experiments [14,64]. One of these processes was observed by Thies A. et al. (2005) that all three MLs inhibited melanoma cell proliferation in a dose-dependent manner starting at very low ML concentrations (0.001-100 ng/ml) with ML-I being the most cytotoxic lectin on ultra sensitive cell line MV3 at 1x10-13 ng ML-I/ml [13]. In addition their obtained in vitro results, they (Thies et al. 2008) established a clinically relevant human melanoma xenograft scid mouse model to analyze the effect of ML-I on melanoma growth and spread [15]. Because of the expressing high number of ML-I binding sides and the being ultra sensitive to ML-I cytotoxicity in vitro (Thies et al. 2005), the human melanoma cell line MV3 was used to model a targeted therapy in vivo to analyse apoptosis rates, the number of infiltrating DCs and vascular counts in primary melanomas (PM) and their spontaneous lung metastases (LM) [15]. Purified ML-I administered at low-dose (30 ng ML-I per kg) daily for 19 days reduced both tumor weight by 35% and the number of LMs by 56 % compared to control mice. They demonstrated a significant direct cytotoxic effect of ML-I on malignant melanoma cells in the PTs and LMs by assessing apoptotic rates of the malignant melanoma cells. ML-I increased apoptosis rates in the melanoma cells of PTs at all doses, however, apoptosis rates didn't increase in parallel to increased ML-I concentrations as one would have expected from their in vitro data (Thies et al, 2005) [15]. The direct cytotoxic effect of ML-I seems to play only a minor role in the antitumorigenic effect in vivo because no significant reduction in tumor weight was noted in the higher ML-I doses, all of these results demonstrate the importance of in vivo models [33]. The number of tumour-infiltrating DCs was examined to evaluate the importance of immunomodulatory effects of ML-I on melanoma growth and spread. Low-dose lectin-I treatment significantly raised the total number of DCs and also protected them against apoptosis in PT [15].

Clinical studies with mistletoe lectins demostrated that mistletoe preparations stimulate the cytokine secretion and monocyte function, the precursors of DCs [65]. The previous *in vitro* studies by Stern et al. indicated that aquous mistletoe extract induced the maturation of DCs with an increased expression of co-stimulatory molecules CD80, CD86 as well as antigen presenting molecules HLA class I and II [66]. Like these studies, Elluru et al. demostrated

that mistletoe QuSpez induced maturation and activation of DCs presented with increased expression of co-stimulatory molecules CD40, CD80 and CD86 and secretion of inflammatory cytokines IL-6 and IL-8 [8]. Duong Van Huyen et al. demonstrated that antitumoral effects of mistletoe extract (Iscador Qu FrF) are mediated by IL-12 dependent pathway, investigated on B16F1 melanoma implanted mice. In IL-12-deficient strain of mice, the inhibition of melanoma growth were abrogated by mistletoe [16]. Antitumor properties of IL-12 include an enhancement of Th1, CD8+CTL cells (19) and NK-cell functions [67]. Preliminary results indicated that mistletoe extract treatment induces an increase in the NK cells activity [8,68]. Furthermore, DCs educated by mistletoe Qu Spez stimulated CD4+T cells and activated melanoma antigen Melan-A/MART-I-specific M77-80 CD8+T cells as sugessed by the increased levels of secretion of TNF-α and IFN-γ in a allogenic mixed lymphocyte reaction [8]. In addition, an increase in CD4 + T and CD8 + T cells were reported by Gardin (2008) in immunological tests carried out before and after mistletoe treatment in four cancer patients, hodgkin disease, breast cancer and multiple myeloma who received seven subcutaneous doses of mistletoe 20 mg, twice weekly [69].

In contrast to the effects of ML-I on primary tumours (PTs), there was not a significant induction of apoptosis in the melanoma cells in the lung metastases (LMs) at any ML-I concentration [15]. The significant reduction of LMs at low-dose ML-I (30 ng ML-I per kg) seemed to be the consequence of successful reduction of the PT mass due to ML-I treatment because correlation analyses revealed a highly significant positive correlation between the weight of the PTs and the number of corresponding LMs in all groups [15]. The reduction of the number of LMs but not of their size underlines the considerably stronger cytotoxic effect on circulating tumour cells, which are more prone to apoptosis induction than cells within the PT [70], while in established metastases cytotoxic effects of ML-I seem to play a minor role [15]. The stimulation of the immune system plays the prominent role in its antimelanoma effect via induction of maturation and activation of tumor infiltrating DCs in the PTs of all treatment groups since DC express high number of ML-I-binding sites [15,17].

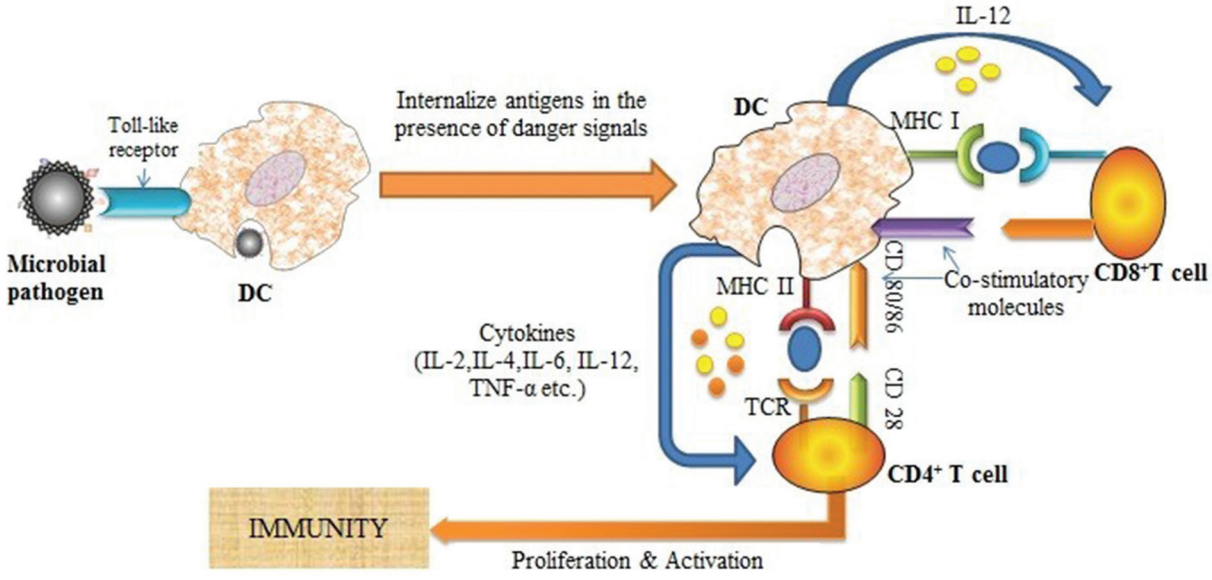


Figure 1. The maturation process of dendritic cells, co-stimulatory molecules an pro-inflammatory cytokines

A CASE REPORT

The complete remission of malignant melanoma with mistletoe treatment

Clinical benefit from adjuvant treatment with a standardized mistletoe extract in patient with malignant melanoma was also demonstrated by Kirsch (2007) [18]. The complete tumor remission was achieved with twice-weekly subcutaneous administration of Iscador®M (Weleda AG, CH-Arlesheim, Switzerland). A 68 years-old male patient with one malignant melanoma at the upper part of the right arm first diagnosed in 1992. In November 1999, another melanoma was surgically removed at the patient's right

shoulder, which the histologic examination revealed nodular melanoma, stage IIA (pT3, pN0, M0). After discovery of the second melanoma and surgical resection, treatment with standardized mistletoe extract (Iscador, M; Weleda AG, CH-Arlesheim, Switzerland) was initiated in November 1999 by the patient's general practitioner. During the course of the mistletoe therapy, axillary removal of 8 lymph nodes became necessary, 3 of which proved to be metastatic. In September 2001, first signs of a defined solitary liver metastasis with a maximum diameter of 2 cm in an area next to segments IV and V were detected during an abdominal ultrasound examination. Moreover, this finding was also confirmed by further sonographic examinations. The solitary liver metastasis was not resected, nor was classical antitumor treatment (chemotherapy or radiotherapy) initiated. The patient continued subcutaneous treatment with Iscador M after dose adaptation to 2 mg twice weekly (0.2 mL of a 10-mg vial) from October 2001 until November 2005. By June 2002, complete remission of the liver metastasis was diagnosed by liver ultrasound examination. No further metastases were discovered in May 2006. The use of low-dose Iscador as the sole postoperative modality for the adjuvant treatment of metastatic melanoma was extremely effective and very well tolerated in this patient. It achieved complete response and absence of all complaints [18]. These findings are in accordance with in vivo studies showing that mistletoe treatment had no negative influence on the vitality, behaviour and physiological responses, appearance, or food and water habits of the animals at any dosage [15].

In conclusion, despite improvements in chemotherapy and immunotherapy, therapeutic quest of the malignant melanoma continues. The early stage malignant melanoma is curable with surgical resection. However, prognosis is poor for patients with advanced melanoma. Malignant melanoma shows intrinsic resistance to chemotherapeutic agents and variability in the course of the disease which are distinct features separating from other solid tumors. These features prevent the development and standardization of non-surgical treatment models of malignant melanoma. These circumtances call for new treatment modalities for melanoma patients; one potential treatment strategy under evaluation is mistletoe, a natural immunomodulator, based treatment. Because of apoptosis induction in the melanoma cells and simultaneously enhancement the number of DCs, mistletoe treatment could have important impact in future melanoma therapy.

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