Review

Brusatol: A potential anti-tumor quassinoid from *Brucea javanica*

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1. Introduction

*Brucea javanica* (L.) Merr. (Simaroubaceae), is mainly distributed from South-East Asia to northern Australia. In China, it is mainly found in Fujian, Taiwan, Guangdong, Guangxi, Hainan and Yunnan. Generally, its fruits and leaves can be used as traditional Chinese medicine with cold in property, bitter in prescription, belonging to liver and large intestine channels (Fig. 1). And as traditional Chinese medicine, it is called “Yadanzi” in Chinese, which has heat-clearing and detoxicating and preventing malaria effects. According to the theory of traditional Chinese medicine, tumor initiation is related to pathogenic toxin in vivo. This process can be explained as prolonged retention of phlegm and obstruction of damp leads to their accumulation and stasis in the body, and then it transforms into fire toxin and internal heat accumulation, eventually it forms a lump by degrees (Zhou & Jiang, 2015). Therefore, the heat-clearing and detoxicating effect of “Yadanzi” will contribute to the inhibition of lump or tumor formation. In modern pharmacology, plenty of compounds have been isolated from *B. javanica*, such as alkaloids, triterpenoids and flavonoids. Among them, triterpene lactones are a research hotspot, known as “quassinoids” (Du, Cheng, Zhao, Huang, & Song, 2017; Lahrita, Moriai, Iwata, Itoh, & Kato, 2019). They have extensive bioactivities such as anti-cancer, -malarial, -microbial, and -inflammatory effects. (Li et al., 2019).

The structure of brusatol is shown in Fig. 2. It includes an \( \alpha,\beta \)-unsaturated cyclo-hexa-none ring (A), two cyclo-hexane rings (B and C), a six-membered lactone ring (D) and and tetra-hydrofuran ring (E) which is known as a quassinoid compound. And it is principally obtained from the seeds and fruits of *B. javanica*. (Li et al., 2019). It was reported that the approximate quantitation of brusatol is 0.3% in fruits of *B. javanica*, suggesting that it has a high content in botanical which ensures the stability of the source and benefits to further development (Zhou et al., 2011). Since brusatol was first isolated and identified in 1968, extensive researches on it have been subsequently done (Keng et al., 2002). And it has been shown to have a good anti-tumor activity. The patents on brusatol showed it could be used as the chemotherapeutic drug synergist, one of the compositions for the treatment of breast cancer and active multiple myeloma and the drug for resisting tumor angiogenesis (John, James, Muriel, & Lawrence, 2003; Ren, Zhang, Wang, & Yang, 2011; Yang, Tian, Huang, Chen, & Zhang, 2020; Zhu, Lu, Wang, Zhao, & Wang, 2017). Moreover, investigating its pharmacophore found that an enone carbonyl oxygen, an enolic oxygen at C-2, C-11 \( \beta \)-hydroxy group, a C-8 to C-13 or C-8 to C-11 epoxymethano bridge contributes to the activity of the compound (Fukamiya et al., 2005; Hitotsuyanagi, Kim, Hasuda, Yamauchi, & Takeya, 2006; Luyengi, Suh, Fong, Pezzuto, & Kinghorn, 1996).

Compared with other potential anti-cancer compounds, brusatol shows its uniqueness and superiority. As a quassinoid compound, brusatol specifically inhibits Nrf2 signaling pathway, which sensitizes a broad spectrum of cancer cells (Cai, Liu, Han, & Yang, 2019). Meanwhile, it can also enhance radio-sensitivity and reduce chemoresistance. The combination of brusatol and first-line chemotherapeutic agents has preferable effects than using them alone.

With the development of natural products, more and more attention has been paid to the compounds from the plants. In recent years, brusatol has been a hot topic which has good biological activities, and many scholars have been trying to modify its structure and dosage form to improve its effect. We consider that comprehensive information on brusatol may be of concern to readers. Therefore, we provide this review that summarizes the antitumor activities and potential mechanisms of brusatol and discusses some contents related to pharmacokinetic.

2. Antitumor mechanisms of brusatol

2.1. Colorectal cancer

Colorectal cancer is the third most frequently diagnosed cancer in males worldwide while the second in females. The incidence and mortality have been steadily declining for the past years. However, tumors often exhibit resistance in the process of treatment due to intratumor heterogeneity and clonal evolution (Osumi, Shinozaki, Yamaguchi, & Zembutsu, 2019; Torre et al., 2015).

In colorectal cancer, there is substantial evidence to support that brusatol inhibits colorectal cell lines effectively by inhibition of cell viability and proliferation. When the concentration of brusatol is more than 15 nmol/L in treating HCT116 cells, it can reduce cell viability significantly (Lu et al., 2016). In CT26 cells, it also has an excellent effect and its IC\(_{50}\) value is 373 nmol/L (Oh, Kim, Kim, Lee, & Park, 2017). Some studies also showed that PKO, SW480 and COLO205 cell lines are suppressed by brusatol (Evans et al., 2018). In addition, brusatol decreases xenograft and orthotopic tumors growth with 2 mg/kg injection dose in a mouse model in vivo (Evans et al., 2018; Oh et al., 2017). It is noteworthy that Cisplatin and Irinotecan have been used to cure colorectal cancer in a long time, and the combining effect of brusatol and Irinotecan or Cisplatin has been verified to improve the therapeutic effect (Chen et al., 2018; Evans et al., 2018).

The relevant mechanism is described as follows (Fig. 3). HIF-1 (hypoxia-inducible factor-1) is a crucial signaling pathway which is linked to angiogenesis, metastasis, tumor growth, chemoresistance and radio-resistance. Brusatol restrains the expression of c-myc and then decreases mitochondrial ROS and ROS-mediated transition of ferrous iron to its ferric state under hypoxia. And then it promotes HIF-1\( \alpha \) degradation by activating PHD to induce colon cancer cells death (Oh et al., 2017). Besides, the results explicated
that brusatol suppresses glucose uptake via inhibiting the transactivation function of HIF-1 and downregulating the expression of the well-known HIF-1 target genes, such as VEGF, GLUT1, HK2 and LDHA under hypoxia. Its inhibition of HIF-1 signaling pathway is also correlate with HIF-1α degradation and mitochondrial ROS levels (Lu et al., 2016).

2.2. Leukemia

Leukemia is one of the common cancers, statistically, the incidence and mortality of leukemia are 14.6% and 7.4% in more developed areas while 7.6% and 6.3% in less developed areas (Torre et al., 2015).

Related studies displayed that brusatol has strong antiproliferative and cytotoxic effects with low IC50 values in leukemia cell lines. For instance, NB4, BV173, SUPB13 cell lines show that their IC50 values are 0.03, 0.01, 0.04 μmol/L. Furthermore, HL-60, K562, Kasumi-1 and Reh cell lines are less sensitive to brusatol-induced cytotoxicity, however, their proliferation is inhibited primarily by the G1-phase arrest. And it is worth noting that brusatol induces their terminal differentiation effectively. Especially, HL-60 cell line possesses chemopreventive potential supported by the inhibition of 7,12-dimethylbenz[a]anthracene-induced preneoplastic lesion formation in mouse mammary organ culture. In addition, NB4, U937, BV173, SUPB13, RS4;11, Daudi and DHL-6 cell lines which are extremely sensitive to brusatol, are responsive to brusatol with little induction of differentiation. And it was observed the apoptotic peaks with NB4 and BV173, an arrest in G1 phase with SUPB13, and an arrest in S phase with U937 and RS4;11 cell lines (Cuendet, Gills, & Pezzuto, 2004; Liu et al., 2011; Luyengi et al., 1996; Mata-Greenwood et al., 2002, 2001). Moreover, brusatol also shows potent antileukemic activity against P-388 lymphocytic leukemia in mice in vivo with the dose of 0.125 mg/kg (Lee, Hayashi, Okano, Nozaki, & Ju-Ichi, 1984). Additionally, brusatol restrains nucleic acid metabolism though inhibiting DNA polymerase, RNA polymerase, thymidylate synthetase, dihydrofolate reductase, phosphoribosyl pyrophosphate aminotransferase, and cathepsin protease activities and elevates cyclic AMP levels in P-388 cells (Hall et al., 1979). It also has an inhibitory effect on cell respiration via significantly inhibiting P-388 cell hexokinase, phosphofructokinase, malic dehydrogenase, and succinic dehydrogenase and increasing the concentration of reduced mitochondrial electron-transport cofactors (Elgebaly et al., 1979). Besides, inhibiting DNA and protein synthesis are one of the bioactivities of brusatol. Some studies validated that brusatol decreases the activity of peptidyl transferase, and it blocks the elongation step of protein synthesis in P-388 and L-1210 cell lines. This effect also exhibited in some cancerous tissues from the mice. The free 80 s ribosome is the binding site of brusatol in P-388 cells (Hall, Liou, Chaney, & Willingham, 1983; Hall, Liou, Okano, & Lee, 1982; Liou, Hall, Okano, Lee, & Chaney, 1982; Willingham, Tobias Considine, Chaney, Lee, & Hall, 1984).

In leukemic cell lines (such as HL-60), brusatol induces cell differentiation though activation of NF-κB. As shown in this research, brusatol activates NF-κB pathway via p50/p65 heterodimer and gathers phosphorylation of IκBα, which leads to NF-κB translocation from the cytoplasm to the nucleus eventually and induces cell differentiation. The cell differentiation in diverse cell lines above is involved in subsequent expression of various markers of differentiation, such as CD11b, CD13, CD14, and the differentiation effect is irreversible treated by brusatol after 48 h (Cuendet et al., 2004). Furthermore, the expression of c-myc is regulated by brusatol at the post-transcriptional level, which plays a central role in terminal differentiation (Mata-Greenwood et al., 2002).

Cytarabine (Ara-C), Daunorubicin (Dnr) and Arsenic trioxide (ATO) are regarded as the drugs to treat acute myeloid leukemia, but they have produced chemoresistance gradually (Sun et al., 2016). THP1 and U937 cell lines are resistant to Ara-C, Dnr and ATO with high IC50 values. Likewise, it is known that Nrf2 is a key target of chemoresistance (Tao et al., 2014). Hence, brusatol, as an inhibitor of Nrf2, is combined with chemotherapeutic agents to improve the sensitivity of acute myeloid leukemia cells. And at high concentration, brusatol has minimal effects in bringing about early apoptosis in acute myeloid leukemia cells (Karathedath et al., 2017) (Fig. 4).

2.3. Liver cancer

Liver cancer is more common in men than in women with high incidence and mortality. It is estimated that China alone accounting for about 50% of the new liver cancer cases and deaths worldwide. Hepatocellular carcinoma (HCC) is the most common form of liver cancer whose incidence is increasing, and closely associated
with advanced liver disease (Malek, Schmidt, Huber, Manns, & Gretten, 2014; Torre et al., 2015).

Brusatol is capable of inhibiting the growth of hepatocellular carcinoma cells, and its IC50 values were found to be 0.69 μmol/L (Hep3B), 0.34 μmol/L (Huh-7), 12.49 μmol/L (LM3) and 18.04 nmol/L (Bel-7404) (Ye et al., 2018). Likewise, Huh6, Huh7.5, HepG2 and Hepa-1c1c7 cell lines are also suppressed by brusatol at a certain level. What is more, the nude mouse model was injected with brusatol (2 mg/kg), and the burden of Bel7404 xenograft tumor was relieved (Murakami et al., 2018; Olayanju et al., 2015). Some researchers suggested that brusatol triggers apoptosis via endogenous apoptotic pathway in liver cancer cells. Furthermore, cellular autophagy is linked to apoptosis though treating autophagy inhibitor chloroquine (CQ). Besides, brusatol makes a dent in the capacity of cell invasion and migration in hepatocellular carcinoma cells. EMT, as a key process in the metastasis of liver cancer, is also weakened by brusatol (Ye et al., 2018). What is worth mentioning is that combination of brusatol and Sorafenib can yield a better result in tumor treatment (Murakami et al., 2018).

Combating liver cancer with brusatol mainly involves the following signaling pathways. Phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway is inhibited by brusatol, which leads to cell apoptosis and autophagy (Ye et al., 2018). In Hepa-1c1c7 hepatoma cells, related researches showed that brusatol exerts improving chemoresistance though inhibiting Nrf2 pathway, however, the inhibition is independent of its repressor Keap1, the pro-tumoral and autophagic protein degradation systems and signaling pathways that are known to modulate Nrf2 activity, such as AKT1/2, JNK1/2, which is beyond expectation. And it may involve a new form of regulation (Olayanju et al., 2015) (Fig. 5).

2.4. Lung cancer

Lung cancer is the leading cause of cancer death in males and females. In addition to known factors such as tobacco, air pollution is also taken into account which may cause lung cancer (Hamra et al., 2014; Torre et al., 2015).

In A549 cells, brusatol has a satisfactory result with IC50 value less than 0.06 μmol/L (Liu et al., 2011; Su et al., 2013). Also, brusatol decreases A549 cells viability with 20% at 20 nmol/L and 45% at 40 nmol/L, and induces apoptosis independent of caspase-3 due to the inhibition of Nrf2 activity (Zhou, Li, Ni, Ding, & Zhong, 2016). Additionally, several studies described the results of inhibiting tumors in vivo with the dose of 2 mg/kg. It was illustrated that at the time of tumors being not initiated, treatment with brusatol cannot prevent initiation of chemically induced lung cancer, whereas post-treatment with brusatol suppresses the progression of chemically and genetically induced lung cancer effectively (Tao, Rojo de la Vega, Chapman, Ooi, & Zhang, 2018).

With the use of clinical drugs, lung cancer has become less sensitive and even resistant to these drugs for the past decades. Multiple studies suggested that brusatol enhances the efficacy of chemotheraphy and radio-sensitivity owing to the inhibition of Nrf2 (Sun et al., 2016). Co-treatment brusatol and cisplatin induces apoptosis, inhibits proliferation and suppresses tumor growth in A549 xenografts depending on blocking Nrf2 pathway. It was substantiated that brusatol promotes ubiquitination and degradation of Nrf2 but is not connected to Keap1 in A549 cells. (Ren et al., 2011). Likewise, A549 cells possess serious radioresistance due to the activation of Nrf2 after exposure to irradiation. And it was reported that radiation induces the compensatory increase of Nrf2. Brusatol promotes to downregulate the expression of Nrf2 and accumulate ROS, and then results in DNA damage to reinforce radio-sensitivity. Brusatol combined with irradiation inhibits proliferation of lung cancer cell lines and obtains a better effect of radiotherapy (Sun et al., 2016). Oncogenic KARS mutations activate Nrf2 signaling pathway to promote drug resistance. And the result showed that combinatorial therapy with brusatol and cisplatin overcomes KRAS tumor resistance to cisplatin (Tao et al., 2014). Likewise, some researchers established HCC827GRKU cells which are resistant to gefitinib, and cross-resistance to epidermal growth factor receptor tyrosine kinase inhibitors, Afinatin and Osimertinib. By proving experimentation, brusatol suppresses tumorigenicity, migration and invasion which is correlated with Nrf2 inhibition (Park et al., 2018).

It was also found that brusatol can be used as a good protein synthesis inhibitor. Brusatol induces cytotoxicity or overcomes chemical resistance by inhibiting protein translation. Its mechanism is that brusatol downregulating expression levels of majority of detected proteins, and the short half-life proteins, such as Nrf2, has the most obvious effect in A549 cells. Also, brusatol inhibits cap-dependent and cap-independent translation, but it is independent of Keap1/Nrf2 activity, which is similar to other translation inhibitors such as silvestrol in A549 cells. (Harder et al., 2017; Vartanian et al., 2016).

2.5. Pancreatic cancer

Pancreatic cancer is one of the fatal malignancies which is common in men and has an aggressive course. Unfortunately, it is difficult to detect owing to asymptomatic characteristic (Goral, 2015). Brusatol has an antitumor activity with IC50 values of 0.36 μmol/L on PANC-1 and 0.10 μmol/L on SW1990 pancreatic cancer cell lines (Zhao, Lau, Leung, Che, & Lin, 2011). Brusatol is clarified to suppress growth and induce apoptosis in PANC-1 and PATU-8898 cell lines. And brusatol inhibits the cell proliferation in time- and concentration-dependent manner. Afterwards, the induction of apoptosis is attached to the endogenous apoptotic pathway. Thereafter, it suggests that brusatol activates JNK and p38 MAPK pathways while inhibits NF-κB and STAT3 pathways (Xiang et al., 2017). Pancreatic cancer, as it is known, is high resistant to chemotherapeutic agents. Compared to the first-line chemotherapeutic agents gemcitabine (GEM) and 5-fluorouracil (5-Fu), brusatol was validated to possess the similar effects. As a matter of fact, the effect of the combined treatment exceeds that of single-agent brusatol. More detailed mechanisms were substantiated that brusatol causes cell cycle arrest at G2/M phase and

![Fig. 5. Major mechanisms of brusatol against liver cancer. Brusatol inhibits the PI3K/Akt/mTOR pathway to induce autophagy, CQ, as an autophagy inhibitor, not only inhibits cell autophagy, but also reverses the Brusatol-induced cell apoptosis. Brusatol also regulates EMT with cancer metastasis inhibition.](image-url)
accentuates apoptosis, abrogates GEM- and 5-Fu-induced activation of NF-κB, suppresses EMT process and reverses brusatol-induced Nrf2 pathway activation with brusatol monotherapy or combination treatment. In addition, it was reported that the results in vivo are in keep with in vitro (Lu et al., 2017; Xiang et al., 2018) (Fig. 6).

2.6. Other cancers

2.6.1. Melanoma

Brusatol with low-dose Ultraviolet A (UVA) is more effective than treat with them alone in melanoma A357 cells. In vivo, brusatol combined with UVA also reduces melanoma-derived tumors burden. Its mechanisms have been certified that combination treatment boosts ROS generation and inhibits A375 cell growth and proliferation by regulating cell cycle related protein to result in G1 phase arrest. Moreover, co-treatment blocks AKT-Nrf2 signaling pathway and triggers cell apoptosis (Wang et al., 2018).

Multiple myeloma: Brusatol triggers the increase of oxidative stress and compromises multiple myeloma cell viability and proliferation. Also, brusatol combined with pyocyanin brings a better result in MM1S and U266 cell lines (Liu, Tuckett, Fennell, Garippa, & Zakrzewski, 2018).

2.6.2. Ovarian cancer

There are related studies in regard to the effect of brusatol on ovarian cancer. Nrf2 induces cisplatin resistance by suppressing the iron export related gene SLC40A1, as a consequence, brusatol along with Desferal (an iron chelating agent) ameliorates cisplatin resistance (Wang et al., 2018).

2.6.3. Endometrial cancer

Tamoxifen, as an agent to cure hormone receptor positive breast cancer, was found it unfortunate to cause side effect. It was reported to induce endometrial cancer. Brusatol selectively inhibits Nrf2 pathway and retards upregulation of SQSTM1 to prevent Tamoxifen-induced endometrial cancer. Additionally, combination of brusatol and metformin overcomes progestin resistance (a main obstacle in endometrial cancer therapy), though downregulating Nrf2/AKR1C1 in endometrial cancer (Fan, Wang, Wei, & Zheng, 2017; Feng et al., 2017).

2.6.4. Breast cancer

Brusatol demonstrates significant inhibitory activities against breast cancer MCF-7 cells and its IC_{50} value is 0.08 μmol/L. Besides, brusatol improves the sensitivity to Taxol as a result of suppressing Nrf2 pathway and enhancing ROS levels in MCF-7 and MDA-MB-231 breast cancer cell lines (Liu et al., 2011; Wu, Harder, Wong, Lang, & Zhang, 2015).

2.6.5. Nasopharyngeal carcinoma

Brusatol is also used to assess the cytotoxicity against KB cells derived from a human epidermoid carcinoma of the nasopharynx in vivo. It was found that brusatol has a positive effect on KB cells by measuring its IC_{50} value 0.20 μmol/L (Anderson, O’Neill, Phillipson, & Warhurst, 1991).

2.6.6. Glioma

It is common to observe mutations in genes encoding isocitrate dehydrogenases (IDHs) 1 and 2 in cancer. In gliomas, the vast majority of patients have IDH1 and IDH2 gene mutations. The evidence suggested that brusatol has inhibitory effect on IDH1-mutated U87, U251 and MGG152 cells, and IC_{50} value of IDH1-mutated U251 cells is about 20 nmol/L. Meanwhile, it was confirmed that brusatol can induce apoptosis and inhibit proliferation of IDH1-mutated cells and tumor xenografts (Liu et al., 2019; Tang et al., 2020).

Due to activation of the Nrf2 pathway, IDH1-mutated cells promote the glutathione (GSH) synthesis and scavenging of ROS to enhance cell proliferation. Brusatol blocks the Nrf2 pathway to suppress GSH synthesis, accumulate ROS and eventually results in cell damages. In addition, some researches substantiate that accumulating Amyloid-β (Aβ) in gliomas causes neurotoxicity, brusatol can ameliorate it by Nrf2/HO-1 pathway, which contributes to treat glioma (Liu, Xu, Zhang, & Gao, 2019; Liu et al., 2019; Tang et al., 2020).

2.6.7. Head and neck squamous cell carcinoma

When applied to head and neck squamous cell carcinoma cells, brusatol can decrease cell viability significantly with IC_{50} values 24, 38, 16, 14, 6, 22, 25 and 20 nmol/L on UMSSC47, UDSCC2, JMAR, TU167, LN686, YD-10B, NN-9 and FaDu cell lines, respectively. From the study, brusatol inhibits metastasis, angiogenesis, proliferation and apoptosis effectually. Related molecular mechanisms have been shown that brusatol blocks STAT3 by interfering with its phosphorylation and formation of dimers and its upstream kinase, furthermore, inhibits the ability of STAT3 to bind DNA (Lee et al., 2019) (Fig. 7, Table 1).

3. Pharmacokinetics

Considerable efforts have been devoted to the studies on antitumor activity of brusatol. Hence, a pharmacokinetic and distribution study is essential for purpose of in-depth researches and broad applications.

Groups of researchers investigated that the plasma concentration of brusatol decreases rapidly with the half-life time in 10 min. In organs, brusatol is distributed in liver, kidney, lung and spleen, and its concentration can peak in 15–30 min and decline tardily. Apart from the constant peak concentration in the liver, its concentration peaks in other tissues in a dose-dependent manner. Of concern is that brusatol in lung is 10 folds or higher than that in other organs, which informs that brusatol may target at lung. Unfortunately, brusatol cannot cross the
blood–brain barrier (Guo et al., 2017; Zhang et al., 2016). Subsequently, excretion of brusatol in rats has been investigated, suggesting that average cumulative excretion rate in urine is 5.82% during 24 h, 0.71% in bile during 12 h and the rapid drop in plasma concentration. All the plasma/urine/bile/feces samples were collected in a tube to analysis metabolites. The results substantiated that brusatol has four metabolites, and according to their structures, hydroxylation, hydrolysis and glucuronidation is dominating metabolic pathways in vivo (Guo et al., 2018). In view of the short half-life and distribution of brusatol, multiple administration may be considered, but at the same time, attention should be paid to its potential accumulation in the lung and even toxic effects. We should also take into account the pharmacological activity of its metabolites. But there is no relevant literature on these problems until now, we infer that the metabolites of brusatol have similar biological activity to brusatol due to the good effect but short half-life of brusatol. These problems could be the subject of research in the future (Fig. 8).

### 4. Efficacy enhancement of brusatol

It was demonstrated in a number of studies that brusatol exhibits excellent activities on inhibition of proliferation and improvement of chemoresistance. Paradoxically, the low aqueous solubility and insufficient intracellular delivery of brusatol limits its potential application seriously. Hence, taking advantages of Pluronics as drug carriers and tumor microenvironment-responsive drug release profiles synthesizes redox-sensitive micelles composed of disulfidelinked Pluronic-linoleic acid (F68-SS-LA). Brusatol is loaded in micelles (Bru/SS-M) which has the advantages of high loading efficiency, narrow size distribution, and excellent storage stability. As a result, the fast-micellar destabilization and drug release can enhance cellular uptake and then improve intracellular concentration of brusatol in a high reduction environment. Pharmacological researches testify that Bru/SS-M enhances the effect of antitumor which is associated with the loss of mitochondrial membrane potential, intracellular ROS generation and upregulate of apoptosis rate in Bel-7402 and MCF-7 cell lines (Zhang et al., 2018). Also, for improving the poor aqueous solubility and rapid first-pass metabolism after oral administration of brusatol, a novel brusatol self-microemulsifying drug delivery system (BR-SMEDDS) is developed in order to acquire a better effect, which possesses smaller size, higher negative zeta potential and drug quantity, and excellent stability (Zhou et al., 2017, 2018).

| Tumor types              | Effective time (h) | Cytotoxicity/IC50 (μmol·L⁻¹) |
|--------------------------|--------------------|------------------------------|
| Colorectal cancer        |                    |                              |
| CT26                     | 0.26               |                              |
| HCT116                   | 0.09               |                              |
| SW480                    | 0.10               |                              |
| Leukemia                 |                    |                              |
| HL-60                    | 0.10 (96 h)        |                              |
| Kasumi-1                 | 0.14 (96 h)        |                              |
| NB4                      | 0.03 (96 h)        |                              |
| U937                     | 0.02 (96 h)        |                              |
| BV173                    | 0.01 (96 h)        |                              |
| SUPB13                   | 0.04 (96 h)        |                              |
| RS4:11                   | 0.05 (96 h)        |                              |
| Reh                      | 0.19 (96 h)        |                              |
| Daudi                    | 0.01 (96 h)        |                              |
| DHL-6                    | 0.01 (96 h)        |                              |
| Liver cancer             |                    |                              |
| Hep3B                    | 0.60 (48 h)        |                              |
| HuH7                     | 0.34 (48 h)        |                              |
| LM3                      | 12.49 (48 h)       |                              |
| Bel7404                  | 0.02 (48 h)        |                              |
| SMMC721                  | < 0.07             |                              |
| Lung cancer              |                    |                              |
| A-549                    | 0.07               |                              |
| Pancreatic cancer        |                    |                              |
| PANC-1                   | 0.36 (72 h)        |                              |
| SW1990                   | 0.10 (72 h)        |                              |
| Breast cancer            |                    |                              |
| MCF-7                    | 0.08               |                              |
| Nasopharyngeal carcinoma |                    |                              |
| KB                       | 0.20 (48 h)        |                              |
| Head and neck squamous cell carcinoma | |                              |
| UMSCC47                  | 0.02 (24 h)        |                              |
| UDSCC2                   | 0.04 (24 h)        |                              |
| JMAR                     | 0.02 (24 h)        |                              |
| TU167                    | 0.01 (24 h)        |                              |
| LNB86                    | 0.01 (24 h)        |                              |
| YD-10B                   | 0.02 (24 h)        |                              |
| HN-9                     | 0.03 (24 h)        |                              |
| FaDu                     | 0.02 (24 h)        |                              |

* Effective time is not given.
5. Conclusion

Brusatol, as monotherapy or in combination with conventional chemotherapy drugs, has beneficial effects in the treatment of different cancers. And it is a great promising natural compound for clinic treatment. It is worthy to make efforts on the study of enhancing the development of brusatol as a new drug. Effectiveness of brusatol prevents the different development stages of human cancers such as proliferation, migration. Likewise, it was also elucidated to play an antitumor role and ameliorate chemoresistance or radio-resistance by regulating varies signaling pathway such as AKT/mTOR/Nrf2 pathways. Pharmacokinetic studies have demonstrated that brusatol can be distributed in multiple tissues, which is the strong evidence of its potential in treating cancers in vivo. There are also studies on dosage form modification to improve its aqueous solubility and reduce the first-pass effect, ultimately, to improve its efficacy in vivo. In addition, what counts is that the problems of the plasma concentrations declining rapidly and the poor aqueous solubility about brusatol need to be solved. It should not be ignored that brusatol also requires more detailed pharmacokinetic studies to determine whether it can reach effective concentrations in the cancerous sites of human bodies. Dosage or structural modification of brusatol also needs to be studied further to achieve the breakthrough of the above barriers and better efficacy. It is equally important to explore the related mechanisms against cancers incessantly. Then, adequate clinical trials are expected before being applied to cure cancers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 8. Abridged general view of main pharmacokinetics studies. Brusatol was injected intravenously into mice at different doses (1.0 mg/kg; 1.5 mg/kg; 2.0 mg/kg), and the distribution of brusatol in liver, spleen, kidney and lung was analyzed. It was found that the highest concentration of brusatol is in lung and it may target at lung. Brusatol was injected into rats at 1.0 mg/kg to analyze the compound content in the plasma, bile, urine and feces, and the metabolites isolated from them.
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