Bufalin for an innovative therapeutic approach against cancer

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ABSTRACT

Bufalin is an endogenous cardiotonic steroid, first discovered in toad venom but also found in the plasma of healthy humans, with anti-tumour activities in different cancer types. The current review is focused on its mechanisms of action and highlights its very large spectrum of effects both in vitro and in vivo. All leads to the conclusion that bufalin mediates its effects by affecting all the hallmarks of cancer and seems restricted to cancer cells avoiding side effects. Bufalin decreases cancer cell proliferation by acting on the cell cycle and inducing different mechanisms of cell death including apoptosis, necroptosis, autophagy and senescence. Bufalin also moderates metastasis formation by blocking migration and invasion as well as angiogenesis and by inducing a phenotype switch towards differentiation and decreasing cancer cell stemness. Regarding its various mechanisms of action in cancer cells, bufalin blocks overactivated signalling pathways and modifies cell metabolism. Moreover, bufalin gained lately a huge interest in the field of drug resistance by both reversing various drug resistance mechanisms and affecting the immune microenvironment. Together, these data support bufalin as a quite promising new anti-cancer drug candidate.

1. Introduction

Bufalin is a cardiotonic steroid composed of a steroid backbone structure bound to a lactone cycle (Fig. 1) [1]. As other cardiotonic steroids such as digoxin and ouabain, its main target is the Na+/K+-ATPase pump. Even if it exerts its major and most prominent effects by perturbing the osmotic balance in cells, more recent studies have indicated that non-toxic doses of these molecules affected signal transduction [2]. First focused in the cardiovascular context, the study of cardiotonic steroids has gained interest in other research areas and their inhibitory activity on cancer development has been reported in the literature [3,4]. More specifically, bufalin is a bufadienolide which can be found in many plant or animal species, but their main sources are skin and parotid gland secretions of venomous toads. In plants and animals, bufadienolides are present mainly as a defence mechanism against predators [5]. Bufalin is also a key active ingredient of the traditional Chinese medicine HuaChansu [6]. It possesses potential therapeutic effects in various medical conditions such as heart failure, infection or inflammatory diseases [7-9] by harbouring cardiotonic, anti-inflammatory, and diuretic properties, but it also has anti-cancer activities [6,9]. Even if bufadienolides were first and mainly identified in toads, some research highlighted their presence in humans. The presence of endogenous bufadienolides in humans has been known for many years mainly with marinobufagenin (MBG) which is produced in humans mainly during preeclampsia [10]. Their biosynthesis takes place in the adrenal gland and cholesterol is the major substrate [5]. Bufalin-like immunoreactive substances have been observed in human serum for several years [11], but the presence of bufalin in human serum was validated for the first time in 2020 by HPLC-MS/MS [12]. This study reported bufalin concentrations of about 5.7 nM in the serum of healthy individuals and of five times decreased concentrations in the serum of patients with hepatocellular carcinoma, further highlighting its role as a potentially protective molecule against cancer development.

1.1. Clinical trials on HuaChansu

The administration of purified bufalin has never been performed in humans yet but there are studies in the literature reporting the results of clinical trials assessing the effect of HuaChansu, a traditional Chinese medicine, whose main active constituent is bufalin, on cancer patients. HuaChansu is a sterilized hot water extract of dried toad skin which can be injected.

A pilot study was performed by the Fudan University Cancer Hospital (Shanghai, China) and the M.D. Anderson Cancer Center (Houston,
Texas, USA) to investigate its adverse effects and its effectiveness against hepatocellular carcinoma, NSCLC and pancreatic cancer. HuaChansu was i.v. administered for 14 days followed by 7 days off. No dose limiting toxicities were calculated as no significant adverse event were observed at doses 8 times higher than what is used in traditional Chinese medicine. Indeed, 73 % of patients had no toxicities greater than grade 1. Moreover, as HuaChansu contains cardiotonic steroids, cardiac function was assessed by electrocardiogram, but no alteration of the cardiac function was observed. The toxicities were haematological (leukopenia and thrombocytopenia), gastrointestinal (loss of appetite, constipation and diarrhea), mucocutaneous (dental ulcers and rashes), cardiovascular (premature ventricular contraction and hypertension). On the 15 patients enrolled, 6 (40 %) had stable disease (median duration = 6.0 months; range 3.5–11.1 months) and one patient with hepatocellular carcinoma had 20 % regression (duration = 11 months) [13]. These encouraging results highlighted that HuaChansu was well tolerated and capable of disease stabilization, and have led to another phase II clinical trial by the same groups to study the effects of the combination of gemcitabine with HuaChansu in pancreatic cancer (NCT00837239) [14]. This trial compared the effect of gemcitabine plus HuaChansu versus gemcitabine plus placebo in locally advanced or metastatic pancreatic adenocarcinoma. A total of 80 patients were enrolled and no toxicity was observed with the combination. However, no benefit of the combination on progression-free survival (PFS), objective radiographical response rate (ORR) nor time to progression was observed [15]. Three hypotheses are proposed to explain the loss of effectiveness of such combination: 1) a too low dose of HuaChansu, as no toxic effects were observed; 2) a too short exposure to see a positive effect, this short exposure time is in line with the characteristics of advanced pancreatic cancer which evolves very quickly; 3) the resistance of pancreatic cancer to many treatments, as many clinical trials have tested the combination of gemcitabine with other molecules without significative benefit [6,13].

Nevertheless, HuaChansu is approved by the Chinese FDA for use at oncology clinics in China and various clinical studies have recently demonstrated its significant anti-cancer properties in patients [6].

In hepatocellular carcinoma, a clinical study (379 patients) compared the effect of HuaChansu versus transarterial chemoembolization (TACE) in preventing recurrence in post-resection patients with small tumours. Compared to TACE, HuaChansu prolonged recurrence-free survival and decreased recurrence rate at 1, 2 and 3 years. The adverse events were mild [16]. Another study (60 patients) compared the effect of HuaChansu injection combined

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<th>Apoptotic mediators affected by bufalin.</th>
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<tr>
<td>Increase</td>
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<td>cleaved caspase 3</td>
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<td>Bad</td>
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with stereotactic body gamma knife radiosurgery versus gamma knife alone. The results indicated clear benefits of the combination with improved 1-year survival, overall survival and disease-free progression as well as quality of life with reduced side effects and analgesic use in the combination cohort [17]. Finally, a study using HuaChansu alone (100 patients) reported a lower progression rate, an increased survival rate and improved liver function as well as patient quality of life [18].

- In non-small-cell lung cancer, a metaanalysis (32 studies, 2753 patients) investigated the combination of HuaChansu with platinum-based chemotherapy. The results indicated that the combination improved the overall response rate and the 1- and 2-years survival rates as well as the quality of life by alleviating chemotherapy-induced adverse effects (neutropenia, thrombocytopenia, nausea, vomiting, anaemia, liver injury, renal injury, and alopecia) [19]. Another study (64 patients) investigated HuaChansu versus chemotherapy as maintenance therapy after response to chemotherapy. The results reported similar overall survival but improved quality of life and 1-year survival rate [20]. A third study (64 patients on maintenance therapy after a 1st line chemotherapy), more focused on immunity, reported decreased serum concentration of CTLA-4 in patients treated with HuaChansu compared with chemotherapy [21].

- In gastric cancer, a metaanalysis (14 studies, 976 patients) investigated the combination of HuaChansu with chemotherapy versus chemotherapy alone and highlighted improved response rate and quality of life with the combination [22].

- In gallbladder carcinoma, a clinical trial (25 patients) assessed the efficacy and safety of gemcitabine-oxaliplatin combined with HuaChansu. The combined treatment was well tolerated with moderate myelosuppression as the main toxicity. In this study, 23 patients were evaluated, 8 (34.8 %) had partial response while 7 (30.4 %) had stable disease [23].

Altogether, these clinical trials reported a benefit to patients from the use of HuaChansu in combination with chemotherapy in many cancers.

1.2. In vitro and animal studies

Nowadays, the vast majority of experiments conducted with bufalin were in vitro studies mainly using cancer cell lines and, in some cases, using primary cultures when specified. In addition, a few in vivo studies were performed and reported below when available.

2. Bufalin induces apoptosis in cancer cells

Bufalin causes DNA fragmentation as observed by TUNEL assay in in vivo models of human prostate, breast, pancreatic, hepatocellular, and colorectal cancer exposed to bufalin [24–28].

2.1. Caspase-induced apoptosis

Bufalin promotes the expression, cleavage, and activity of caspases in various cancers (Table 1). Also, a large scale analysis of genes in lung cancer cells exposed to bufalin indicated that CASP9 gene was upregulated by 5.5 fold [29]. Moreover, the use of pan-caspase inhibitors in colorectal cancer rescued cells from apoptotic death [30]. A similar result was obtained in oral squamous cell carcinoma with the use of caspase 3 or 9 inhibitors [31], as well as in hepatocellular carcinoma exposed to either caspase 3, 8, 9 or 10 inhibitors [32].

Three different mechanisms underly caspase activation by bufalin: 1) in colorectal cancer, caspase 3 activation was linked to Bax and Bak since the knockout of either one prevented bufalin-stimulated increase in cleaved caspase 3 [28]; 2) in lung cancer, bufalin-promoted caspase 3 activation could be prevented by N-acetylcysteine (NAC) thus indicating its stimulation of oxidative stress [33]; 3) in hepatocellular carcinoma, a crucial role of bufalin in inducing Fas-mediated caspase 10-dependent apoptosis pathway was highlighted [32]. Furthermore, in prostate cancer, bufalin increased Fas levels [18], while the use of siRNA to knock down Fas expression led to a restored cell viability. Increased levels of Fas and Fas-ligand were documented also in lung cancer [35]. Of note, in bladder carcinoma, bufalin treatment led to the down-regulation of cellular Fas-associated death domain-like interleukin-1β-converting enzyme inhibitory protein and X-linked inhibitor of apoptosis protein [36], while an increased expression of APAF, which links cytochrome c to activated caspase 9, has also been reported in many cancers (Table 1).

Bufalin also affects Parp protein cleavage, Parp being a molecular indicator of caspase-mediated cell death. (Table 1). In breast cancer, Parp inhibition prevented bufalin-induced cell death [37]. The cleavage of Parp in bufalin-treated cells is linked to caspases activation. Indeed, the cleaved Parp induction in colon cancer following bufalin exposure was reversed with pan-caspase inhibitors [30]. A study on bladder cancer indicated that the degradation of Parp polymers was concomitant with the proteolytic activation of caspases 3, 8 and 9 [38]. Parp cleavage was also induced by ROS after bufalin treatment as NAC decreases the cleaved Parp accumulation in colorectal cancer [30]. Finally, a study in multiple myeloma indicated that PARP1 over-expression partially suppressed bufalin-induced apoptosis [39].

2.2. Bcl-2 protein family-mediated apoptosis

A decreased Bcl-2 expression had been documented in many cancer types (Table 1). In addition, an increased Bax/Bcl-2 ratio has been also observed in many studies (Table 1). Concerning the protein Bax, its expression was upregulated in colorectal cancer and hepatocellular carcinoma [40,41], while it was redirected from the cytosol to the mitochondria in prostate and lung cancers [33,34]. Bax translocation was associated with ROS as NAC inhibited it [33]. In osteosarcoma, a downregulation of TPTC1 (tumour protein translationally-controlled 1), a Bax antagonist, was also observed [42], whereas an increased expression of the pro-apoptotic proteins Bak, Bad and Bid could also be induced by bufalin (Table 1).

Similarly, the expression of the anti-apoptotic protein Bcl-xl as well as Mcl-1 were decreased in many cancers treated with bufalin. In NSCLC, Mcl-1 overexpression moderated the bufalin-induced apoptosis. Moreover, decreased Mcl-1 levels by bufalin are linked to its proteasomal degradation. Indeed, only the Mcl-1 protein level was decreased while the mRNA rate did not change and the use of the proteasome inhibitor MG132 suppressed the decrease in Mcl-1 protein level. A Mcl-1 ubiquitination was also observed by immunoprecipitation. This mechanism was linked to the activation of GSK3-β by bufalin as GSK3 siRNA blocked the Mcl-1 proteasomal degradation [43]. In gallbladder cancer, Mcl-1 decrease was associated to the inhibition of phosphorylated c-Met by bufalin [44], while in breast cancer, it was associated with the inhibition of the transcriptional factor STAT3 [45].

2.3. IAP (inhibitor of apoptosis protein family)-mediated apoptosis

After bufalin treatment, decreases in survivin and livin levels were observed in colorectal cancer [12], and cIAP1 level was decreased in oesophageal carcinoma [46]. In bladder cancer, bufalin caused an increase of Bax/Bel-2 ratio leading to a downregulation of IAP family members [38]. By contrast, other studies documented increased levels of the AIF in cancers treated with bufalin (Table 1).
2.4. p53-mediated apoptosis

An increased p53 protein level has been observed in prostate and lung cancers treated with bufalin, where it was associated with an increased p21 protein level [24,47]. A study in glioblastoma reported an increased nuclear translocation of p53 following bufalin exposure. This nuclear p53 accumulation was induced by a decrease of the XPO1 exportin expression. These results were highly supported by the fact that exportin inhibitor leptomycin B, a known potent and selective inhibitor of p53 nuclear export, caused the same nuclear retention of p53 as the one obtained by bufalin. Silencing XPO1 led to an increase in nuclear p53 expression and consequently attenuated bufalin effect [48]. Additional studies highlighted the crucial role of p53 in bufalin-induced apoptosis. Indeed, bufalin induced apoptosis in WT p53 colorectal cancer cells and autophagy in mutant p53 cells, while p53 downregulation restored cell viability [54]. Similar results were obtained in prostate cancer [48] where bufalin induced apoptosis in WT and mutant p53 cells but not in p53-null cells [47].

2.5. Death receptor-induced apoptosis

Following bufalin treatment, death receptor proteins and death receptor-related factors were upregulated in bladder cancer [36,38] while DR4 and DR5 were upregulated in nasopharyngeal carcinoma [54]. In breast cancer, bufalin upregulated DR4 and DR5 and this effect was linked to the activation of ERK, JNK and p38-MAPK and the downregulation of Chl-b [64]. Another study indicated that bufalin promoted the clustering of DR4 and DR5 in aggregated lipid rafts and the use of a cholesterol-sequestering agent reversed such clustering and reduced bufalin-induced apoptosis [65]. In the latter three studies, bufalin also increased TRAIL expression, known to be involved in the induction of apoptosis [54,64,65]. Likewise, in breast cancer, bufalin downregulated Mcl-1 expression and promoted TRAIL-induced apoptosis [45].

2.6. Mitochondria-induced apoptosis

Bufalin modifies mitochondria morphology and biogenesis. A study in glioma using MitoTracker green and Transmission Electron Microscopy reported that bufalin led to a relatively loose mitochondrial network and caused mitochondria to split into many smaller mitochondria which affects their function. [49]. Bufalin also affected the expression of the mitochondrial division/fusion related proteins DRP1 and Mfn2. DRP1 was downregulated in the cytoplasm but upregulated in mitochondria, while the opposite observation was reported for Mfn2. These data indicate that bufalin can disrupt the mitochondrial division/fusion balance to induce apoptosis [49]. Bufalin also decreased the oxygen consumption rate, an indicator of mitochondrial function, in glioblastoma [48]. Finally, bufalin stimulated the release of cytochrome c from the mitochondria to the cytosol and upregulated the proapoptotic nuclease endonuclease G release by mitochondria and its nuclear translocation [49].

2.7. Stress-induced apoptosis

An increased intracellular Ca\(^{++}\) concentration was also observed after bufalin treatment and was associated with the loss of mitochondrial membrane potential (MMP). In osteosarcoma, the loss of MMP was a consequence of the downregulation of protein phosphatase 2 (PPA2) [42] and was associated with a production of mitochondrial ROS in many cancers including melanoma, colon and lung cancer [30,35,53]. In glioblastoma, this bufalin-induced ROS production led to the disruption of the electron transport chain and NAC inhibited bufalin-induced apoptosis [66]. In lung cancer, the production of ROS by bufalin mediated mitochondrial permeability transition [33]. In glioblastoma, ROS production was associated to bufalin-induced DNA damage. Indeed, an increased fluorescence of phosphorylated H2AX was observed following bufalin treatment and its was attenuated with NAC [48]. A study on pancreatic and oral cancers indicated that bufalin induces apoptosis by downregulating hTERT expression (hTERT protects against mitochondrial damage by binding to mitochondrial DNA and reducing mitochondrial ROS production) related to an increased phosphorylation of JNK and p38-MAPK. Actually, the use of JNK or p38-MAPK inhibitors opposed bufalin-induced hTERT downregulation and hence moderated apoptosis induction by the mitochondrial pathway [67].

Bufalin leads to endoplasmic reticulum (ER)-stress induced apoptosis as indicated by changes in the expression of ER stress markers. In nasopharyngeal carcinoma, bufalin increased GRP78, IRE-1 and IRE-1\(\beta\) (inositol-requiring enzyme 1), ATF-6\(\alpha\), Calpain 1 and GADD153 [54]. In glioma, bufalin exposure led to the upregulation of CHOP (C/EBP homologous protein) and GRP78. The role of ER stress response was confirmed by the attenuated bufalin-induced apoptosis in cells transfected with siCHOP RNA. In this context, ER stress was associated with an increased phosphorylation of ERK and eIF2\(\alpha\) [50]. In gastric cancer, bufalin led to ER stress via the IRE1-JNK pathway and to a significantly increased expression/phosphorylation of target genes of the ER stress pathway including IRE1, CHOP and p-eIF2\(\alpha\).

Modulation of the anti-apoptotic protein Hsp27 level by bufalin also plays a key role in apoptosis induction. Its expression significantly decreased after bufalin exposure in osteosarcoma and pancreatic cancers [42,68]. A comparative proteomics approach reported that the level of the anti-apoptotic protein Hsp27 remarkably decreased after treatment with bufalin. Bufalin downregulates Hsp27 in vitro and in vivo through proapoptotic degradation to induce apoptosis. Ectopic expression of Hsp27 reduced the bufalin-induced apoptosis [42].

2.8. Other mechanisms inducing apoptosis

In glioma, bufalin induces the mitochondrial translocation of Annexin A2 to modulate apoptosis [49]. In prostate cancer, the protein level of AR and its transcriptional target PSA were decreased by bufalin, while the AR coactivators SRC-1 and SRC-3 were suppressed, and these downregulations were not dissociable from apoptosis [47]. In leukemia, Tiam1 was upregulated by bufalin, and this led to the activation of Rac1, Pak and JNK pathway and to apoptosis. Regarding TNF-induced apoptosis, bufalin increased THAP1 in lung cancer [29] and FADD in nasopharyngeal carcinoma [54], suggesting that bufalin could improve the apoptotic effect of TNF.

3. Bufalin induces necroptosis in cancer cells

Necroptosis is another type of cell death distinct from apoptosis that can be induced by DNA damage, oxidative stress, and specific receptors. Necroptosis is mainly mediated by the TNF receptor superfamily. When cancer cells are stimulated to activate TNF receptors, caspase 8 activation is inhibited to block apoptosis. RIP1 and RIP3 are phosphorylated to form a necrotic complex which associates to PGAMS, disintegrates mitochondria and induces necroptosis. Inducing necroptosis could be a way to overcome resistance to apoptosis, for example MDR overexpression in breast cancer [69].

In breast cancer, bufalin upregulated RIP1, p-RIP1, RIP3 and PGAMS [37,69,70]. The necroptosis inhibitor Nec-1 and the ROS scavenger NAC but not pan-caspase inhibitors decreased bufalin-induced necroptosis indicating that bufalin stimulated ROS production to induce necroptosis [69,70]. In another study, bufalin-induced cell death was prevented by RIP1 inhibitor or RIP3 shRNA while RIP3 ectopic expression enhanced bufalin-induced cell death [37]. Moreover, it also increased TNF\(\alpha\) levels and the phosphorylation of the TNF receptor [70].

In glioblastoma, bufalin induced necroptosis in cells that escaped from apoptosis. Indeed, when caspase-8 was functionally lost, necroptosis occurred through the formation of a necrosome complex containing RIPK1, RIPK2 and MLKL (mixed lineage kinase domain-like
increased expression of Beclin-1 in colorectal cancer [74] and hepato-

protein). In this context, increases in TNF, TNFR1 and RIPKK were also observed [71].

4. Bufalin induces autophagy in cancer cells

Bufalin has been reported to induce autophagy but it is highly dependent on the cell characteristics and type. Indeed, in cells harbouring wild type p53, bufalin mainly induces apoptosis while it induces autophagy in mutant p53 cells [30]. Depending on the context, autophagy can be viewed as a protective mechanism to escape apoptosis or as an alternative mechanism to kill the cell. When bufalin-induced autophagy acts as a protective mechanism, it could be validated by using an autophagy inhibitor [49,50,72,73]. For example, in hepatocellular carcinoma, autophagy induction was highlighted by the observation of double-membrane vacuoles by transmission electron microscopy, the detection of acidic vesicular organelles using acridine orange staining, and the cleavage of microtubule-associated protein light chain 3 (LC3). In this context, autophagy inhibitor opposed the effect of bufalin on cell viability but enhanced apoptosis [27]. Bufalin induced autophagy by increasing the conversion of LC3-I to LC3-II thus leading to increased levels of LC3-II. This has been observed in colorectal cancer [74], hepatocellular carcinoma [73,75,76], glioma [50] and gastric cancer [49]. This increase in LC3-II rate was associated to an increased expression of Beclin-1 in colorectal cancer [74] and hepatocellular carcinoma [62-65], and a decreased expression of p62 through proteasomal degradation in hepatocellular carcinoma [76] and gastric cancer [49]. Bufalin-induced autophagy can be triggered by ROS and ER-stress which leads to the activation of the IRE1-JNK pathway. This was observed in gastric [49] and colorectal cancer [74] as well as in hepatocellular carcinoma [76]. Another way to trigger autophagy is through the depletion of ATP and the activation of the AMPK-mTOR pathway. Indeed, bufalin-induced ATP depletion leads to the phosphorylation of AMPK which decreases the phosphorylation of mTOR and its downstream targets 4EBP1 and PD[mTOR. The pERK/eIF2α/CHOP pathway also plays a crucial role in this process [50,72,75]. The levels of other autophagy-related proteins such as ATG5 and ATG8 were increased in colorectal cancer [74] and hepatocellular carcinoma [76].

5. Bufalin induces senescence in cancer cells

Studies on multiple myeloma and prostate cancer also highlighted a senescence-like inducing effect of bufalin. It was observed by an increased volume of the cells and by an increase in the number of SA-Gal positive cells [47,77]. In prostate cancer, bufalin induced a selective activation of p53-related senescence. Moreover, bufalin stimulated the expression of genes related to senescence such as CYR61/CCNI and its related family member CTGF/CCN2 as well as the p53 target gene CDKN1A (p21).

6. Bufalin modulates cell cycle and proliferation in cancer cells

Bufalin induced a cell cycle arrest in G2/M phase in many cancer types that were associated with a decreased activity of Cdk1 [78]. In leukemia, the effect of bufalin on cell cycle was similar to that of topoisoasemerase inhibitors with a selective inhibition of the activity of topoisomerase II but not topoisomerase I [79]. Other studies highlighted a G0/G1 phase arrest explained by the decreased expression of p21 and p27, two universal cyclin inhibitors, p27 controlling cell cycle transition from S to G2 phase [80] (Table 2).

More specific mechanisms implicated in cell cycle and proliferation arrests induced by bufalin are described in the literature. In leukemia, bufalin increased the casein kinase 2 (CK2) activity through its nuclear translocation [79], whereas it inhibited the activity of PKA and PKC [79] or it binds to β-tubulin [58] to decrease proliferation. In colorectal
cancer, bufalin induced a mitotic arrest through the downregulation of the polo-like kinase 1 (Plk1) [81] or through the inhibition of Aurora A/B activation [82]. Finally, a gene expression study in lung cancer reported the downregulation of CCPG1 (cell cycle progression protein 1) and of CDCA7L (cell division cycle-associated 7-like protein) [29].

7. Bufalin modulates the activation of specific signalling pathways in cancer cells

Bufalin modifies the expression and phosphorylation of some receptors such as EGFR, VEGFR, c-Met or Axl (Table 3).
Bufalin regulates the PI3K/AKT signaling pathway (Table 3) and in oral carcinomas, AKT overexpression is associated with resistance to bufalin [31]. In colorectal cancer and hepatocellular carcinoma, this effect leads to a decrease in mTOR phosphorylation and an increased GSK-3β activity [28,90–92]. In ovarian carcinoma, bufalin decreases kinase 96k phosphorylation downstream of mTOR [93]. In parallel, MAPK pathway was as well modulated by bufalin. Indeed, bufalin decreased MEKK3 and MKK7 in hepatocellular carcinoma [94]. Accordingly, ERK phosphorylation was also decreased in many cancer types following bufalin exposure (Table 3). However, the activity of bufalin on ERK phosphorylation differs among studies and cancer types. Indeed, bufalin generally moderates ERK phosphorylation but, conversely, it can also induce its phosphorylation in some tumour types. For example, in bladder carcinoma, bufalin promoted p-ERK, while ERK inhibitor opposes bufalin inhibitory effect on migration [95]. An increased ERK phosphorylation was also observed in hepatocellular carcinoma cells exposed to bufalin [96] and in breast cancer where it was associated with induction of apoptosis [64]. In leukemia, a persistent activation of MAPK by bufalin was involved in the induction of apoptosis [97]. Of note, the phosphorylation of the p38-MAPK protein was either increased or decreased according to different studies, however, in those where p38 phosphorylation was increased, the use of a p38 inhibitor decreased bufalin-induced apoptosis [64,67].

Bufalin also activates JNK pathway by increasing JNK phosphorylation in many cancer types and nuclear translocation of its target c-Jun in leukemia [98] (Table 3). Moreover, JNK inhibitor decreased bufalin-induced apoptosis [64,67].

The Hedgehog signaling pathway was also inhibited by bufalin. The expression levels of PTCH2 and Gli proteins were downregulated by bufalin in liver cancer [99]. Additionally, NF-kB expression, phosphorylation and nuclear translocation were decreased by bufalin (Table 3).

Moreover, cell exposure to bufalin promotes the phosphorylation of AMPK leading to the inhibition of its downstream targets mTOR, p70S6K and 4EBP1 in glioma and oesophageal carcinoma [46,50].
Many studies demonstrated that bufalin is a potent inhibitor of the transcriptional coactivators SRC-1 and SRC-3 through proteasomal degradation. This was highlighted in triple-negative breast cancer (TNBC) where SRC-3 is a prognostic marker associated with poor overall survival and progression-free survival [100], but also in other breast cancer subtypes [101,102]. SRC-3 was also emphasized in glioblastoma [103] and in prostate cancer where it is associated with a low level of active androgen receptor (AR) and its downstream target PSA (prostate specific antigen) [47]. A molecular docking model in colorectal cancer indicated that bufalin could directly bind SRC-3 to inhibit its activity [104].

8. Bufalin modulates protein translation

Bufalin affected protein translation by inhibiting mTOR activity, as discussed above, but also acted on the translation of more cancer-specific mRNA related to the eIF4F translation initiation complex. A
Pharmacological Research 184 (2022) 106442

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ically, the main targets of bufalin are the isoforms ATP1A1 or ATP1A3, and it mediates translation by acting on c-MYC translational activity to induce apoptosis [139].

Effects of bufalin in combination with other treatments.

Table 5

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<tr>
<th>Effect on</th>
<th>Cancer type</th>
<th>Bufalin effect</th>
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<tr>
<td>Macrophages</td>
<td>Colorectal cancer</td>
<td>Enhanced antitumor effect of oxaliplatin by decreasing M2 macrophages polarization [104]</td>
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<tr>
<td>Apoptosis transporter</td>
<td>Hepatocellular carcinoma</td>
<td>Increased sensitivity to adriamycin-resistant cells to doxorubicin, mitomycin C, vincristine, and cyclophosphamide in vitro and increased apoptosis induction [110,126]</td>
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<td>Increased sensitivity of HBV-associated HCC refractory to sorafenib [127]</td>
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<td>Apoptosis</td>
<td>Ovarian cancer</td>
<td>Bufalin improves cisplatin responsiveness by decreasing HIP-1a [93]</td>
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<td>Gallbladder carcinoma</td>
<td>Bufalin enhances chemotherapeutic sensitivity [44]</td>
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<td>Cervical cancer</td>
<td>Increased the chemotherapeutic efficacy of paclitaxel [132]</td>
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<td>Osteosarcoma</td>
<td>Apoptosis induction in methotrexate-resistant cells [42,63]</td>
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<td>Apoptosis</td>
<td>Glioma</td>
<td>Improved the inhibitory effect of TMZ by activating the mitochondrial apoptotic pathway [133]</td>
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<td>Combination of bufalin with the MEKi PD08059 has a synergistic effect on decreasing proliferation and inducing apoptosis [134]</td>
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<td>Enhanced the chemosensitivity to gemcitabine [105]</td>
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<tr>
<td>Leukemia</td>
<td>Pancreatic cancer</td>
<td>Increased apoptosis induction by the bufalin-HIFU (High Intensity Focused Ultrasound) combination [135]</td>
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Recent study in TNBC identified bufalin as a novel inhibitor of eIF4A-mediated translation by acting on c-MYC translational activity to downregulate eIF4A and eIF4G [113] (Fig. 2).

9. Bufalin interacts with the sodium pump

In relation with its steroid structure, bufalin can act on the Na+/K+ ATPase pump to mediate its anticancer effects [49,82]. More specifically, the main targets of bufalin are the isoforms ATP1A1 or ATP1A3, depending on the cancer type (Table 4). However, the effect seems restricted to caveolae to mediate its impact on the Na+/K+ ATPase signalosome [114] (Fig. 2).

10. Bufalin acts on cancer cell metabolisms

Bufalin may affect the metabolism of cancer cells by 3 main mechanisms impacting mitochondria, glycolysis and lipid metabolism.

1) **Mitochondrial metabolism:** Bufalin is associated with disruption of mitochondrial membrane potential and decreased oxygen consumption and ATP production [49,50,88]. The latter leads to AMPK phosphorylation and activation [50]. Moreover, in glioma, using MitoTracker green, bufalin exposure was linked to scattered mitochondria associated with a relatively loose network and a decreased mitochondria fluorescence. In addition, transmission electron microscopy revealed that bufalin can increase mitochondrial number, density and swelling, and reduce its surface area and volume. Thus bufalin causes mitochondria to split into a number of smaller mitochondria, thereby affecting mitochondrial function [49].

2) **Glycolysis:** Bufalin moderates glucose uptake and lactate production in ovarian carcinoma in vitro and in vivo and is associated with low levels of glycolysis-related proteins, including GLUT4, LDHB and HK2 [119].

3) **Lipid metabolism:** In hepatocellular carcinoma, bufalin modulates sphingolipid and glycerophospholipid metabolism leading to the disruption of tumour cell membranes but mainly located within the non-necrotic areas [120]. Also, bufalin affects the metabolism of a number of amino acids including phenylalanine, histidine, glutamate, aspartate, methionine, glutamine, isoleucine, proline, serine or carnitine (synthesized in the liver from methionine and lysine). As carnitine plays a crucial role in fatty acid transport within the mitochondrial matrix for β-oxidation, bufalin can compromise energy production [121].

11. Bufalin sensitizes resistant cancer cells to conventional therapies

Combination of bufalin with chemo or targeted therapies have proven beneficial compared to each drug alone (Table 5). Moreover, in the context of cancer with resistance to treatment, bufalin was able to reverse the resistance to certain therapies or to kill resistant cells. These effects were often linked to the inhibition of MDR1 pathway. For example, in colorectal cancer, doxorubicin (DOX) uptake experiment indicated that bufalin significantly increased the intracellular drug concentration [122,123]. In multidrug-resistant hepatocellular carcinoma, bufalin enhanced chemosensitivity by inhibiting drug efflux pump activity via the downregulation of MRP1 [61]. A similar MRP1 downregulation by bufalin was observed in leukemia cells with vincristine-acquired multidrug resistance which then led to an increased intracellular levels of adriamycin [124].

Table 6

<table>
<thead>
<tr>
<th>MicroRNAs involved in bufalin antitumor effect.</th>
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<tbody>
<tr>
<td>mIR-522–3P † Involved in NSCLC development [137]</td>
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<tr>
<td>mIR-181a † Involved in apoptosis induction by repressing its target gene Bcl-2 in prostate cancer [138]</td>
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<tr>
<td>mIR-298 † Block apoptosis by increasing its target BAX in gastric cancer [139]</td>
</tr>
<tr>
<td>mIR-497 † Associated with decreased angiogenesis and metastasis in colorectal cancer [140]</td>
</tr>
<tr>
<td>mIR-203 † Associated with decreased stemness in glioma [141]</td>
</tr>
<tr>
<td>mIR-148a † Associated with decreased stemness in osteosarcoma [142]</td>
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†: Increase; †: decrease.
12. Bufalin modulates microRNAs in cancer cells

Bufalin modulates the expression of specific microRNAs responsible for cancer development and has an antitumor effect (Table 6). In prostate cancer, it affected long non-coding RNAs (lncRNA) known to interact with miRNAs. The study reports that bufalin decreased HOTAIR, a crucial lncRNA upregulated in bone metastases, and promotes metastases by binding to miR-520b and thus increasing the oncogene FGFR1 and consequently moderating the metastatic potential of prostate cancer [136].

13. Bufalin induces phenotype switching/EMT towards differentiation and reverses cell stemness

In melanoma, bufalin activates tyrosinase and thus stimulates melanogenesis and pigmentation that is associated with a more differentiated/melanocytic phenotype [143]. Many studies also indicate that bufalin is a potent inducer of differentiation in leukemia cell lines and primary cultures accompanied by a loss of cell proliferation and adherence affecting viability and an increased expression of IL-1β. Similarly, the induction of cell differentiation was enhanced by All-trans retinoic acid known to affect cell growth and apoptosis [144–149].

Cancer stem cells (CSC) possess enhanced tumour-forming capabilities and are resistant to current anticancer therapies. In line with its ability to promote cancer cell differentiation, bufalin attenuates stem cell characteristics in many cancer types (Table 7). This was mainly observed through the inhibition of spheroid formation and the decreased expression of stemness-associated proteins SOX2 and OCT4 and stem cell-surface marker proteins CD133 and CD44 (Table 7). In gallbladder cancer, bufalin also attenuates the self-renewal of cancer stem cells [44]. Furthermore, bufalin reduced the expression of other stemness markers, such as ALDH1, TERT, NANOG, Notch and Bim1 in isolated CSC of osteosarcoma primary cultures. Bufalin-treated CSC conferred a lower ability of xenograft formation in mice [142]. In an in vivo pancreatic cancer model, bufalin inhibited tumour growth and prolonged the duration for tumour formation (subcutaneous xenograft), and pre-treatment with bufalin decreased intestinal and lung metastases after IV tail vein administration [150]. In colorectal cancer, both in in vitro and in vivo models, bufalin abrogated cisplatin induced stemness (associated with increased expression of stemness markers such as CD133, CD44, NANOG, OCT4, SOX2, and ABCG2) [126].

14. Bufalin modulates migration and invasion of cancer cells

Bufalin regulates the expression of some common markers involved in epithelial-mesenchymal transition (EMT) in different cancer types (Table 8). In hepatocellular and bladder carcinomas, this was associated with the decrease of the transcription factor ZEB1 [78], the increase of mRNA and protein levels of the tissue inhibitor of metalloproteinase (TIMP) 1 and 2 [95] and the decrease in Gli1 and Gli3 proteins within the Hedgehog (Hh) signaling pathway [99]. While in gastric cancer, bufalin-induced inhibition of migration and invasion was associated...
with the inhibition of ASCL2 expression. Indeed, ASCL2 RNAi mimicked bufalin effects on MMPs, E-cadherin and vimentin [152]. In hepatocellular carcinoma, bufalin moderates the phosphorylation of GSK3-β, which plays a key role in tumour invasion, while the total amount of GSK3-β protein was increased [90, 91].

Other more specific mechanisms are also described. In hepatocellular carcinoma, bufalin binds syndecan-4 (SDC4) which is a major endogenous membrane receptor regulating cell cytoskeleton, adhesion, and migration (Fig. 2). Consequently, SDC-4 interaction with its protein substrate DDX23 (Dead-box helicase 12) is increased contributing to genomic instability. A DARTS (Drug Affinity Responsive Target Stabilization) experiment also indicated that bufalin could stabilize SDC4 leading to an enhanced susceptibility to proteolysis. SDC4-DDX23 KO opposes bufalin inhibition of cell migration [78]. In gastric cancer, bufalin suppressed peritoneal dissemination by reducing the phosphorylation of NOS3 [111]. In bladder carcinoma, bufalin inhibits motility and invasiveness by acting on tight junctions (TJs) [95]. In hepatocellular carcinoma and lung cancer, bufalin opposes TGF-β-induced EMT [153, 154] and TGF-β-induced Twist2, zinc finger E-box binding homeobox 2 (ZEB2) as well as the phosphorylation of Smad2 and Smad3 whereas both TGF-β receptor I (TβRI) and TGF-β receptor II (TβRII) were downregulated [154]. In lung cancer, bufalin causes fibroblastic over-expression that, in its turn, reinforces bufalin antimigratory activity [155]. In hepatocellular carcinoma, bufalin reduces APOBEC3F which is overexpressed in tumour tissue having roles in cell growth and cell cycle control [156]. Finally, in lung cancer, bufalin downregulates metastasis-related genes, including the small GTPases RhoA, the Rho-associated kinases ROCK1, and the focal adhesion kinase (FAK) [108].

15. Bufalin impairs neo-angiogenesis

Many studies report that bufalin downregulates HIF-1α notably in colorectal cancer [81], renal cell carcinoma [86] and ovarian carcinoma [93]. In hepatocellular carcinoma hypoxia-induced HIF-1α by CoCl2 could be abrogated by bufalin [153] and moderates VEGF expression both in vitro and in vivo in tumour xenografts in mice [99, 153]. In colorectal cancer, bufalin suppressed tumour microenvironment-mediated angiogenesis by inhibiting the STAT3 signalling pathway in vascular endothelial cells (HUVEC) [157]. In hepatocellular carcinoma, assays on chick chorioallantoic membrane and rat aortic rings demonstrated that bufalin enhanced anti-angiogenic effect of sorafenib via modulating the AKT/VEGF signalling pathway [78, 158]. Moreover, bufalin decreased HUVEC proliferation and migration. Accordingly, as vascular endothelial cells liberate various cytokines to affect angiogenesis, conditioned medium (CM) from bufalin-treated HUVEC significantly inhibited both HUVEC migration and blood vessel formation [78, 158].

16. Bufalin modulates immune response

To date, many studies focused on the effect of bufalin on tumour cells and many things are known regarding its cytotoxic activities. However, there are not many studies focusing on the impact of bufalin on the immune system in a context of cancer while a recent study published in May 2022 in the Journal for Immunotherapy of Cancer reported a stronger effect of bufalin on decreasing tumour volume and growth rate in immunocompeotent C57BL/6 mice compared to immunodeficient nude mice. Moreover, in this context, the depletion of T cells with anti-CD4 and anti-CD8 neutralizing antibody or the macrophage deprivation decreased the anti-tumour activity of bufalin in hepatocellular carcinoma (HCC) xenograft models indicating a predominant role of the immune system in bufalin anti-cancer properties [159]. We report in this section the data on the implication of bufalin on macrophages, lymphocytes, and inflammation.

16.1. Macrophages

Bufalin promotes macrophage phagocytosis. Indeed, blood samples from mice treated with bufalin revealed that bufalin treatment led to increased phagocytosis by macrophages isolated from peripheral blood mononuclear cells or from the peritoneal cavity [160]. In colorectal cancer, the anti-inflammatory/pro-tumoral macrophages M2 play a key role in oxaliplatin chemoresistance. Bufalin reverses this chemo-resistance by moderating M2 macrophages polarization through inhibition of SCR-3 and its target MIF that is crucial for M2 polarization [104]. In HCC xenograft immunocompetent mice, bufalin increased the recruitment of macrophages to the tumour site and induced their polarization toward an anti-tumour M1 phenotype. This M2 to M1 polarization is linked to the activation of the NF-κB pathway by bufalin. Ex vivo, HCC cells co-cultured with Bone Marrow-Derived Macrophages (BMDMs) differentiated such macrophages into M2 phenotype, while bufalin abrogated this mechanism and increased the M1/M2 ratio. The IL-12/IL-10 ratio was also increased by bufalin (from 0.3 to 7) indicating an increased M1 polarization. Finally, the delivery of these bufalin-primed BMDMs to mice suppressed HCC development [159].

16.2. Lymphocytes

In hepatocellular carcinoma, bufalin exerts its immunomodulatory effects by modifying the balance between stimulatory and inhibitory receptors on the surface of natural killer (NK) cells. Moreover, bufalin promotes MICA expression on the cell membrane and decreases its secretion, which contributes to the induction of cytolytic activity of the NK cells. In hepatocellular carcinoma, low MICA expression in tumours is associated with a poor survival [161]. In leukaemia mouse models, bufalin stimulates NK cell cytotoxic activity as well as B-cell and T-cell proliferation [160]. A study in HCC indicated that bufalin did not directly induce T cell immune response. Indeed, splenic CD4+ and CD8+ T cells did not proliferate faster in the presence of bufalin. However, in bufalin treated mice, M1 polarization by bufalin promoted the migration and accumulation of T cells in the tumour. Ex vivo, the treatment of BMDMs by bufalin increases IL-12 and TNF-α and decreased IL-10 and TGF-β and these changes were responsible for T cell proliferation and activation. Moreover, the co-culture of bufalin treated-BMDMs and CD8+ T cells enhanced their killing activity. The delivery of BMDM-primed with bufalin to HCC xenografts mice upregulated the presence T cells in the tumour and provoke their cytotoxic activity [159].

Regarding its impact on immunotherapy, this study on HCC, which is usually not a good candidate for immunotherapy due to its strong immunosuppressive tumour microenvironment, reported that bufalin could increase the efficacy of anti-PD1 antibody in vivo by modifying the microenvironment [159].

16.3. Inflammation

In in vivo colorectal cancer models, bufalin inhibited NF-κB and GSK-3β pathways resulting in suppression of the expression of pro-inflammatory mediators COX-2, TNFa, IL-1β, IL-6, CXCL1–2–5 [28]. Similarly, in a murine model of bone cancer, an anti-inflammatoryatory effect of bufalin was associated with a decrease in cancer-induced bone pain and destruction [162]. Another study confirmed that bufalin exerted its anti-inflammatory and analgesic effects by downregulating iNOS, COX-2, IL-1β, IL-6, and TNFa [163].

17. Toxicity of bufalin on healthy tissues and in vivo models

Bufalin showed no significant toxicity in leucocytes and lymphocytes obtained from healthy donors [58, 164] and no apoptosis induction even at 1 μM of bufalin in polymorphonuclear cells [165]. Other studies also showed no damages to normal ovarian and endometrial tissues and cells
whereas 0.2 μM bufalin eliminated almost all tumour cells sparing healthy cells [89,93,132]. It was also the case with immortalized hepatocytes where up to 1 μM concentration almost eliminated all cancer cells while merely reducing healthy cells by only 20% [83]. Bufalin also exerts marginal effect on normal gastric mucous epithelium cell line up to 200 mmol/l [60] as well as on human mesangial and breast epithelial cell lines [34]. Finally, bufalin exhibits lower toxicity towards human oesophageal squamous cells compared to cancer cells, supporting its high selectivity [166].

Moreover, the effect of bufalin has been assessed in vivo in many cancer animal models with promising results reporting inhibition of tumour development and metastases (Table 9). None of these studies (Table 9), reported changes in animal weight or specific acute or delayed toxicities. Moreover, a study indicated no morphological changes in the myocardium, brain, liver and kidney tissues [167].

### 18. Bufalin pharmacokinetics and bioavailability

One of the major concerns for the implementation of bufalin in the clinic is linked to its poor pharmacological properties. Indeed, like other steroids, bufalin harbours a poor water solubility linked to its hydrophobic chemical structure. Moreover, as other cardiotonic steroids, it has a narrow therapeutic window linked to the modification in sodium and potassium balances when it affects the Na+/K+-ATPase pump which is ubiquitously expressed in human cells and is crucial for the proper functioning of the heart and the cardiovascular system [169, 170].

Some studies also reported a fast distribution in rat and mice (half-life of 0.0693 min in blood after i.v. injected) [80,171]. Bufalin clearance occurs through the hepatic (by CYP3A4 [172–174]) and renal routes with a half-life of 510 min [171].

As bufalin has a poor water solubility and is rapidly distributed and metabolized, many formulation studies are now focusing on developing tools to improve bufalin bioavailability by increasing its water solubility and tumour uptake while decreasing its cardiac toxicity (Table 10). In this context, tumor-vectorized bufalin-grafted magnetic iron oxide nanoparticles could constitute an interesting therapeutic strategy combining improved tumour uptake of unmetabolized bufalin and possibility of magnetic resonance imaging [175].
19. Discussion and perspectives

Firstly, we will focus on the advantages of bufalin as a potential future cancer treatment.

All the data discussed in the current review indicated that bufalin could act on many hallmarks of cancer which could make it a candidate of choice for the treatment of such diseases. Indeed, actual treatment, such as targeted therapy mainly focus on only one specific oncogenic mechanism which is the main cause of resistance development. As bufalin target many oncogenic pathways, the development of resistance should be delayed or blocked, making combination more effective.

Among the mechanisms of action of bufalin, one even more interesting is its ability to reverse stemness which is often associated with resistance and metastasis development. Its main target is the α-subunit ATP1A1 of the sodium pump which is overexpressed in many cancers. By acting on ATP1A1, bufalin induced the inactivation of the SRC protein kinase and of many downstream signalling pathways, inhibiting cell survival. Another important target of bufalin is the co-activator SRC-3 of transcription factors. Indeed, by inhibiting SRC-3, bufalin is able to affect the transcription of many genes, including genes coding for specific proteins involved in the translation complex toward cancer-associated proteins (Fig. 2). Hence, targeting ATP1A1 and SRC-3, bufalin can impact signalling pathways, gene transcription and translation, all important regulatory processes in cancer.

On the other hand, bufalin is a cardiotonic steroid, as digoxine which has also demonstrated interesting anti-tumour properties [192]. Moreover, epidemiological studies have highlighted lower risks of developing leukemia and renal tumours as well as lower risks of breast cancer recurrence in patient treated with cardiotonic steroids for other heart disorders [102]. But the major problem with these molecules is their potential cardiac toxicity as well as their low solubility and their fast distribution and metabolization observed in in vivo studies. There are a few reasons to hope that bufalin used at low doses should not induce cardiotoxicity as in vivo studies did not observe any morphological changes in the myocardium of mice or rats treated with this compound. Moreover, as explained above, bufalin is endogenously expressed in humans; so if used at physiological doses, it should not induce toxicity. To counteract its low bioavailability, it is crucial to better understand how bufalin is synthesized in the human body and how it is stored, and to try to stimulate its endogenous production. On the other hand, the I.V. administration of bufalin-coupled nanoparticles could be an effective alternative strategy to improve bioavailability of the molecule for cancers.

Regarding the possibility of cancer treatment with bufalin and knowing the current necessity of treatment combinations, it is difficult to imagine a treatment relying only on bufalin today. Nevertheless, bufalin could be used at small doses to improve the efficacy of other conventional chemotherapy or targeted treatments as it already showed synergy with many drugs in different cancer types. In addition, in the context of the development of immunotherapy, bufalin could be a very interesting molecule to switch the immunosuppressive tumour microenvironment into a more immunocompetent one and to improve the response to immunotherapeutic strategies.

20. Conclusions

In the past few years, bufalin has been largely studied in the context but this level could be sufficient to fight cancer development as the concentrations used in the literature to decrease tumour development in vitro and in vivo range from 1 to 10 nM. Moreover, bufalin levels are significantly decreased in cancer patients [12]. This major observation shed a completely new perspective suggesting that restoring normal bufalin levels in cancer patients could limit and delay tumour spread.

Then, we will emphasis on the major disadvantage of bufalin and how we could work to improve its use in clinics.

The major drawbacks of bufalin are its potential cardiotoxicity as well as its low solubility and its fast distribution and metabolization observed in in vivo studies. There are a few reasons to hope that bufalin used at low doses should not induce cardiotoxicity as in vivo studies did not observe any morphological changes in the myocardium of mice or rats treated with this compound. Moreover, as explained above, bufalin is endogenously expressed in humans; so if used at physiological doses, it should not induce toxicity. To counteract its low bioavailability, it is crucial to better understand how bufalin is synthesized in the human body and how it is stored, and to try to stimulate its endogenous production. On the other hand, the I.V. administration of bufalin-coupled nanoparticles could be an effective alternative strategy to improve bioavailability of the molecule for cancers.
of oncology in many cancer types for its major regulatory role in various tumorigenic pathways. Taken together, all data discussed within the present review support that bufalin exerts its antitumor effect by acting on all the hallmarks of cancer (Fig. 3). Indeed, bufalin acts on tumour growth by inducing cell death, blocking cell cycle, stimulating senescence, or inhibiting proliferative signaling and protein translation. It also modulates metastatic spread by inhibiting cell migration and invasion as well as by blocking angiogenesis. Bufalin also modifies other aspects of cancer cells such as their metabolism, phenotype, or level of oxidative stress. Finally, bufalin could improve the effect of current anticancer therapies by overcoming resistance and/or stimulating immune response. Despite that bufalin could be seen as a molecule of choice to improve cancer treatment, the major problem is linked to a narrow therapeutic window because of a possible cardio toxicity, that can, however, be easily bypassed by using prodrugs, drug formulations to improve tumour delivery or/and repeated low dose administration.

CRediT authorship contribution statement

Laura Soumoy: Investigation, Writing – original draft, Review & editing, Visualization. Ghaneem Ghaneem: Writing – original draft, Funding acquisition. Sven Saussez: Writing – original draft, Funding acquisition. Fabrice Journe: Writing – original draft, Review & editing, Supervision, Funding acquisition, Visualization.

Competing interests

The authors have no competing interests.

Data Availability

No data was used for the research described in the article.

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Bufalin inhibits cancer growth by blocking cell cycle progression and inducing apoptosis through HIF-1α-mediated pathways.


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15


16