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Review Article

Bromelain and acetylcysteine (BromAc®): a novel approach to the treatment of mucinous tumours

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Abstract: Mucins are a significant extracellular component of neoplastic entities such as pseudomyxoma peritonei and several gastrointestinal adenocarcinomas. Mucinous tumours present a challenge for systemic treatments due to poor drug penetrance and increased resistance. Therefore, the development of an effective mucolytic therapy has significant therapeutic implications for these tumour types. BromAc® is a novel mucolytic agent consisting of bromelain and acetylcysteine. It has demonstrated significant mucolysis and antitumour effects *in vitro* and *in vivo* for several mucinous tumours. It has also exhibited a synergistic potentiation of the effect of several cytotoxic agents on mucinous tumours in preclinical studies. Furthermore, it demonstrates locoregional safety and efficacy in animal and clinical studies. This literature review will summarise the history of BromAc® for mucinous tumours, including its conception, preclinical development *in vitro* and *in vivo*, and clinical evidence. The implications of current data and directions for future research are then discussed.

Keywords: Mucins, BromAc, cancer therapy, chemo-sensitisation, adenocarcinoma, pseudomyxoma peritonei

Introduction

Mucins are a family of glycoproteins heavily expressed in pseudomyxoma peritonei (PMP) and several mucinous adenocarcinomas [1]. Mucinous neoplasms are generally associated with a poorer response to systemic chemotherapies compared to their non-mucinous counterparts [2-4]. Chemoresistance is thought to be derived from the mucin barrier, which confers protection to tumour deposits by hindering drug penetrance and enhancing immune evasion [5]. Therefore, surgical management provides vastly improved outcomes for intra-abdominal mucinous tumours [6, 7]. PMP is a rare syndrome consisting of widely disseminated low-grade tumour throughout the peritoneal cavity, accompanied by a large volume of mucinous ascites. There is limited evidence demonstrating that systemic chemotherapies are efficacious in PMP, given the chemoresistant properties of the mucinous mass, and the poor permeability of the blood-peritoneal barrier limiting intra-abdominal drug concentrations [6]. Hence, the standard of care is cytoreductive surgery (CRS) to remove macroscopic tumours and debulk mucin, accompanied by hyperthermic intraperitoneal chemotherapy (HIPEC) to treat residual microscopic disease [8]. For patients with inoperable disease, the mucinous deposits produce a significant burden, leading to bowel obstruction, malnutrition, sepsis, and respiratory compromise [9]. The development of mucolytic therapies may enhance microscopic cytoreduction in the context of HIPEC, and enable mucin debulking to reduce disease burden and potentiate chemotherapy in the non-operative setting [5].

Mucinous gastrointestinal adenocarcinomas encompass a subtype of adenocarcinomas characterised by abundant mucin secretion [10]. Compared to non-mucinous subtypes, mucinous gastric and colorectal cancers (CRC) are associated with more advanced disease at presentation and higher rates of treatment failure [10-13]. Both secreted (e.g., MUC2, MUC5AC) and transmembrane (e.g., MUC1,

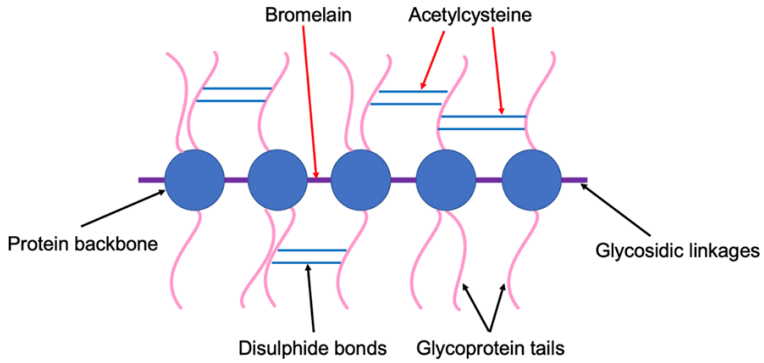

Figure 1. Mechanism of mucolytic action with bromelain and acetylcysteine. Mucin polymers consist of a peptide backbone held together by glycosidic linkages, with glycoprotein tails which interact through disulphide bonds. Bromelain acts on the glycoside linkages within the peptide chain, whilst acetylcysteine cleaves the disulphide bridges between oligosaccharide side chains. The combined effect of bromelain and acetylcysteine synergistically interacts to provide a greater mucolytic effect.

MUC4) mucins contribute to the carcinogenesis and chemoresistant phenotype of mucinous adenocarcinomas [14]. Whilst secreted mucins provide a physical barrier to protect tumours, transmembrane mucins can transduce growth signals, activating several intracellular pro-survival pathways which impede the cytotoxic effects of chemotherapy [14-16]. There is a significant amount of laboratory and clinical data to support the significance of the mucin barrier in producing a chemoresistant phenotype. BromAc® is a unique mucolytic therapy consisting of bromelain and acetylcysteine, developed in light of the mucin-related treatment difficulties in PMP and other adenocarcinomas. The mechanism of action of BromAc® surrounds the breakage of the structural framework of mucin, being peptide and disulphide bonds. Additionally, as a thiol donor, acetylcysteine contributes to regeneration of the active component within bromelain, thereby potentiating its effect on the glycoprotein [17]. This review aims to provide an overview of the preclinical and clinical evidence for BromAc® in the treatment of mucinous tumours.

Preclinical development of BromAc®

To address the pivotal role of mucins in treatment resistance and carcinogenesis, a variety of agents were screened for their mucolytic efficacy. Mucin polymerises and solidifies via disulphide and glycosidic linkages, forming a viscoelastic mass [18]. Over 150 compounds were screened as single agents for their mucolytic activity, including sodium bicarbonate, dextran and dextran sulphate, streptokinase, ascorbic acid, hydrogen peroxide, bromelain, papain, and acetylcysteine. However, all monotherapies demonstrated limited efficacy in liquefying mucin from PMP samples, likely owing to the composite nature of PMP mucin [19]. Therefore, combination therapies were explored in hopes of achieving adequate efficacy in cancer-associated mucin. The combination of bromelain and acetylcysteine demonstrated marked synergism in mucolytic activity and cytotoxicity.

Acetylcysteine is a reducing agent which readily dissolves mucin in respiratory diseases (e.g., cystic fibrosis) via the cleavage of disulphide bonds [20]. Bromelain is a proteinase extract from the stem of the pineapple plant consisting of thiol endopeptidases, phosphatases, peroxidases, and several other enzymatic components. Mechanistically, bromelain cleaves the glycosidic linkages between amino acids within the MUC peptide chain, which complements the action of acetylcysteine on the disulphide bridges between oligosaccharide groups (**Figure 1**). Therefore, the combination of bromelain and acetylcysteine (BromAc®) underwent preclinical evaluation to characterise its mucolytic potential, antitumour effects, and chemo-sensitising effects in different mucinous tumours [17].

Mucolytic efficacy of BromAc®

Mucolytic activity in PMP mucin: The *in vitro* mucolytic activity of BromAc® was examined through incubation with patient samples of PMP mucin. Due to heterogeneity in mucin consistency, the samples were divided into three grades of hardness: soft, semi-hard, and hard mucin. A formulation of BromAc® consisting of 300 μg/mL bromelain and 250 mM acetylcysteine was incubated with 1 gram of mucin at 37°C for 4 hours. BromAc® was able to dissolve 100% of soft mucin, whilst semi-hard and hard mucin was solubilised to 57% and 50% respectively (**Figure 2A**). However, residue from the semi-hard and hard mucin samples was com-

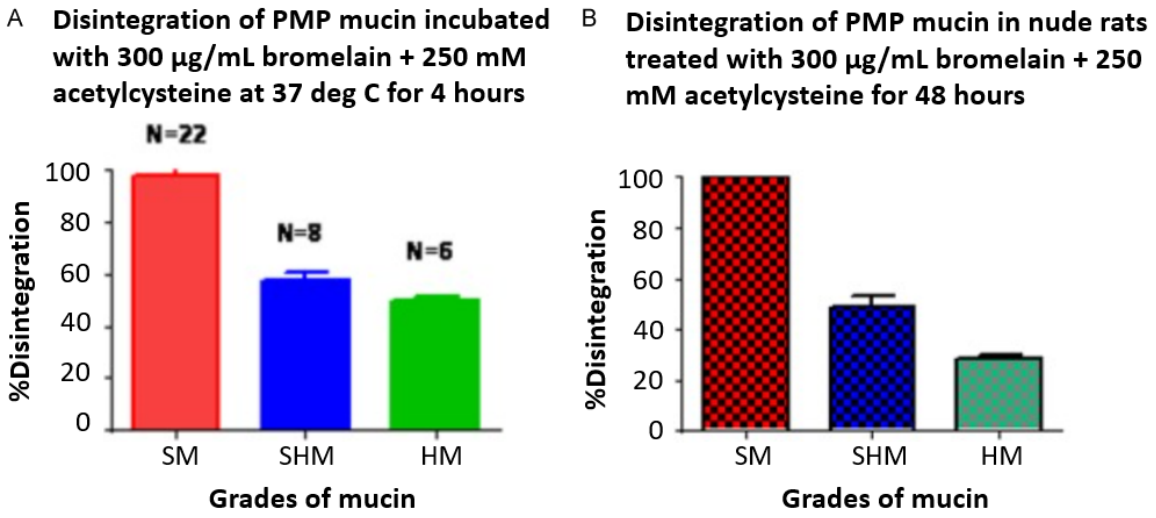

Figure 2. Mucolytic activity of BromAc® in vitro and in vivo. A. Demonstrates the absolute dissolution of 3 grades of mucin when incubated with BromAc® for 4 hours in vitro. B. Demonstrates the absolute dissolution of 3 grades of mucin implanted intraperitoneally in nude mice after treatment with BromAc® for 48 hours; SM = soft mucin; SHM = semi-hard mucin; HD = hard mucin. Figure taken from [21].

prised of cellular material with a complete absence of mucin, indicating that effective mucolysis had been achieved for all 3 mucin grades [21]. Given the potential role of BromAc® in HIPEC, analysis of mucolytic activity was repeated at an incubation temperature of 41°C. This revealed no significant difference in the time taken for mucin dissolution between the two incubation temperatures, suggesting that BromAc® efficacy would not be affected by the temperatures used in HIPEC [17]. Mucolysis was examined *in vivo* via intraperitoneal PMP mucin implants in nude mice. Intraperitoneal BromAc® dissolved 100% of soft, 48.67% of semi-hard, and 28.67% of hard mucinous tumour deposits (**Figure 2B**). This data suggests that the efficacy of BromAc® on semi-hard and hard mucin is reduced *in vivo*. This is potentially due to the greater dispersal of BromAc® throughout the peritoneal cavity and absorption into surrounding tissues, compared to the consistent exposure achieved *in vitro* [21].

Mucolytic activity in other adenocarcinomas: BromAc® has also demonstrated mucolytic activity in a range of mucinous adenocarcinomas. Mucinous gastric and colorectal adenocarcinomas express variable amounts of mucin subtypes, including MUC1, MUC2, and MUC-5AC. As a transmembrane mucin, MUC1 is a critical driver of carcinogenesis, metastasis, and chemoresistance via the upregulation of survival pathways, apoptosis inhibition, and chemoresistance mechanisms [22, 23]. MUC2 and MUC5AC are secreted mucins which facilitate the mobilisation of tumour cells during peritoneal dissemination, and serve as a physical barrier to immunologic and pharmacologic attack. In the human gastric carcinoma cell lines KATO III and MKN45, treatment with BromAc® significantly reduced the expression of MUC1 and MUC5AC. In the LS174T CRC cell line, MUC2 and MUC5AC were attenuated following treatment with BromAc® (**Figure 3**). *In vivo*, the treatment of intraperitoneal deposits of MKN45 and LS174T with BromAc® in nude mice reduced the mass of the tumour in a dose-dependent manner. Significantly, tumour mass was reduced by approximately 30% for either bromelain or acetylcysteine monotherapy but reached near-total dissolution with BromAc®, reflecting the synergistic interaction between these two agents. Periodic Acid-Schiff's staining of tumour sections demonstrated a reduction in mucin, particularly with combination therapy [24].

Antitumour effects of BromAc®

In vitro evidence: Given the role of mucins in carcinogenesis and tumour survival, the potential antitumour properties of BromAc® were investigated. A summary of the fifty-per-

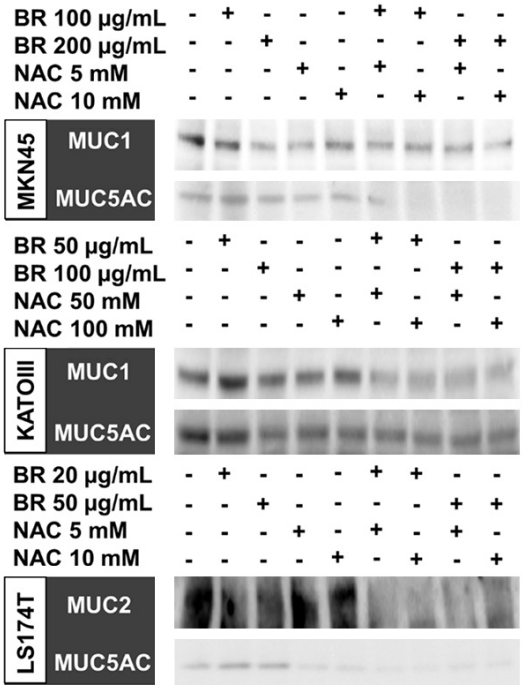

Figure 3. Western blot analysis of mucin expression in gastrointestinal carcinoma cells. Attenuation of MUC1, MUC2, and MUC5AC was observed in gastric and colorectal adenocarcinoma cell lines with treatment of bromelain, acetylcysteine, or their combinations. A significantly greater effect was observed with combination therapy. BR = bromelain; NAC = Acetylcysteine. Figure taken from [24].

cent inhibitory concentrations (IC_{50}) of BromAc® in different cell lines is provided in **Table 1**. *In vitro*, BromAc® demonstrates cytotoxicity against human gastric and colorectal carcinoma cell lines. Both bromelain and acetylcysteine as monotherapies significantly inhibit the proliferation of MKN45, KATO-III, HT29-F12, HT29-5M21, and LS174T cells in a dose-dependent relationship. However, combination therapy produces a significantly greater cytotoxic effect, with median effect analysis demonstrating a predominantly synergistic drug-drug interaction. Mechanistically, treatment with BromAc® resulted in greater apoptotic activity, demonstrated by an increase in caspase-3/7/8 on Western blot analysis, and an increase in apoptotic bodies on terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) assay. Additionally, increased autophagy-related proteins including Atg3/5/7/12 and Beclin-1 suggest that autophagy is another important component of BromAc®-induced cell death. Finally, prosurvival kinase pathways were attenuated, reflected by a reduction in phosphorylated Akt, limiting the proliferative and malignant potential of treated tumour cells [20]. These antitumour mechanisms are believed to be mediated by the disintegration of mucin subtypes involved in regulating apoptosis, autophagy, and proliferation, which normally contribute to the carcinogenic phenotype of mucinous tumours [25].

In vivo evidence: In vivo, the treatment of nude mice models of peritoneal carcinomatosis demonstrated a significant reduction in tumour burden. Intraperitoneal administration of BromAc® to nude mice with implanted LS174T and MKN-45 peritoneal deposits resulted in a significant reduction in tumour mass and number of peritoneal nodules (**Figure 4**). Immunohistochemical analysis of tumour sections revealed that Ki-67 expression was dramatically reduced in treatment groups, indicating reduced proliferative activity. This suggests that BromAc® debulks mucinous tumour masses by both dissolving mucin and inhibiting tumour growth [24]. Similarly, results from a patient-derived xenograft mouse model of mucinous appendiceal adenocarcinoma revealed a reduction in tumour mass and number of intra-abdominal tumour deposits following treatment with intraperitoneal BromAc® [26]. In subcutaneous deposits of the pancreatic AsPC-1 cell line, the intraperitoneal administration of BromAc® significantly reduced tumour weight and volume. Again, immunohistochemistry demonstrated a reduction in Ki67, highlighting the antitumour effect of BromAc®. This was consistent with Western blot analysis which revealed a reduction in cyclin proteins and PARP, mediators of cell cycle progression. Additionally, there was a downregulation of the antiapoptotic proteins Bcl-2 and NF-κB, and the pro-metastatic proteins MMP-9 and TGF-B. Importantly, the intraperitoneal administration of BromAc® was able to exert a significant antitumour effect on subcutaneous tumour deposits at distant sites, suggesting that BromAc® may have efficacy with systemic administration [27].

Chemo-sensitising effects of BromAc®

The interaction of BromAc® with other chemotherapeutics was investigated as a potential form of chemo-sensitisation, given the role of mucins in chemoresistance. Peritoneal carcinomatosis is a notoriously difficult target for systemic therapies due to the poor penetrability of

Table 1. Fifty-percent inhibitory concentration (IC_{50}) values of Bromelain and Acetylcysteine in different cell lines

Cell line	Bromelain IC_{50} (µg/mL)	Acetylcysteine IC_{50} (mg/mL)	BromAc® IC_{50} (Br µg/mL; Ac mg/mL)	Combination index range	Primary drug-drug interaction
Gastric Cancer					
MKN45	15.69	26.69	Br (5) + Ac (2.5)	0.50-1.1	Synergism/additivity
KATO-III	142.9	57.74	-	0.25-1.1	Synergism/additivity
Colorectal cancer					
LS174T	27.76	22.49	Br (20) + Ac (10)	0.80-1.1	Synergism/additivity
HT29-5F12	30.02	15.4	-	0.50-0.90	Synergism/additivity
HT29-5M21	34.6	15.99	Br (10.6) + Ac (1)	0.60-0.80	Synergism/additivity
Pancreatic cancer					
AsPC-1	22.85	2.5	Br (7) + Ac (0.577)	0.37-0.84	Synergism/additivity
CFPAC	15.52	17.88	Br (6) + Ac (13.8)	0.43-0.70	Synergism/additivity
PANC-1	14.21	25	Br (11.66) + Ac (5)	-	-
Hepatocellular carcinoma					
Hep-3B	11.8	13.8	Br (15) + Ac (0.60)	0.36-0.39	Synergism/additivity
Hep G2	24.44	2.16	Br (1.09) + Ac (1)	0.15-0.79	Synergism/additivity
Ovarian cancer*					
A2780	10.37	8.97	Br (7.45) + Ac (5)	-	-
OVCAR-3	13.23	2.6	Br (9.09) + Ac (1)	-	-
SKOV-3	>100	>50	Br (25) + Ac (19.89)	<0.9	Synergism/additivity
Breast cancer*					
MCF-7	23.98	45.91	Br (7) + Ac (20)	<0.9	Synergism/additivity
MDA-MB-231	100	10	-	-	-
T47D	4.89	19.96	Br (3.06) + Ac (10)	<0.9	Synergism/additivity
Lymphoma*					
U937	37.02	8.43	Br (10) + Ac (10)	>1.1	Antagonism
Jurkat	167.9	20.73	-	>1.1	Antagonism
Mesothelioma					
REN	30.3	33.8	Br (19.56) + Ac (10)	<0.9	Synergism/additivity
Glioblastoma*					
U87	23.3	27.3	Br (10) + Ac (3.05)	<0.9	Synergism/additivity
LN18	15.86	17.89	Br (10) + Ac (6.75)	<0.9	Synergism/additivity
Sarcomas					
HT1080 (fibrosarcoma)	6	7.8	Br (3) + Ac (4)	0.22-0.90	Synergism/additivity
SW872 (liposarcoma)	>100	30	Br (10) + Ac (20)	0.34-0.99	Synergism/additivity
SW982 (biphasic synovial)	7.57	16.5	Br (5) + Ac (5)	0.40-0.76	Synergism/additivity
VA-ES-BJ (epithelioid sarcoma)	8	17.53	Br (5) + Ac (5)	0.43-0.66	Synergism/additivity

Combination index data derived from published studies [20, 31] represent a range of bromelain and acetylcysteine concentrations. *refers to unpublished data, collected from methodology as published in [20]. Br, Bromelain. Ac, Acetylcystei.

the blood-peritoneal barrier [28]. Therefore, microscopic cytoreduction of these tumours requires the use of locoregional chemotherapies. In mucinous tumours however, the efficacy of locoregional therapies is hindered by the mucin barrier. Furthermore, the upregulation of survival pathways by transmembrane mucins often allows for tumour cells to escape the effects of cytotoxic drugs [29]. BromAc® may therefore enhance the efficacy of locoregional chemotherapies, such as in the context of HIPEC or early postoperative intraperitoneal chemotherapy (EPIC).

In vitro evidence: In the colorectal LS174T and gastric KATO-III cell lines, BromAc® pre-treatment sensitises tumour cells to cisplatin, 5-fluorouracil (5-FU), paclitaxel, and vincristine. This occurred in a dose- and time-dependent manner, as higher concentrations of BromAc® and longer incubation times resulted in a greater potentiation of the chemotherapeutic agents. Furthermore, the concomitant treatment of chemotherapies and BromAc® results in a greater chemo-potentiating effect compared to pre-treatment with BromAc®. The combination index (CI) revealed that BromAc® and the

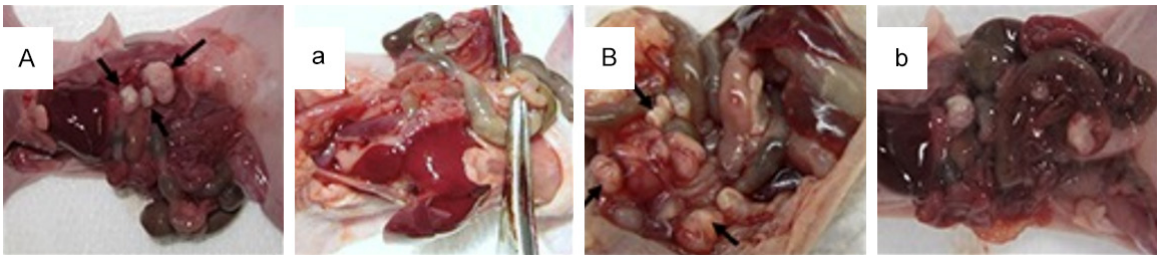

Figure 4. Effects of intraperitoneal BromAc® on mucinous tumour burden. A and B. Represent untreated controls of LS174T and MKN45 xenografts in nude mice respectively, whilst a and b represent the high dose BromAc® treatment group. There is a significant reduction in mucinous tumour mass with intraperitoneal BromAc® treatment to both LS174T and MKN45 xenografts. Figure taken from [24].

tested chemotherapies primarily exhibited synergism and additivity. However, antagonism was observed with cisplatin in the MKN45 gastric carcinoma cell line [30]. This may be due to the antioxidant effect of acetylcysteine interfering with the generation of reactive radicals by cisplatin. In the pancreatic cell lines AsPC-1 and CFPAC, the cytotoxic efficacies of gemcitabine, 5-FU, doxorubicin, and oxaliplatin were all potentiated by BromAc®. Additionally, BromAc® is able to potentiate both gemcitabine and 5-FU in the HEP3B and HEPG2 hepatocellular carcinoma cell lines. Significantly, the high doses of acetylcysteine used in this study indicate that these results may only be clinically applicable to locoregional treatment, as systemic delivery may lead to toxicity [31].

The use of BromAc® in sarcomas has also demonstrated significant chemo-potentiating properties. Synergism was observed with the addition of BromAc® to doxorubicin in a variety of sarcoma cell lines, including HT1080, SW-872, VAESBJ, and SW982. This effect corresponded with a reduction in MUC1 and MUC4 expression on immunofluorescence. Additionally, Western blot analysis demonstrated that combination therapy increased the concentration of apoptotic caspase proteins in the SW8-72 cell line, which was associated with an increase in PARP cleavage [32]. A previous study of the PARP inhibitor olaparib with doxorubicin in osteosarcoma demonstrated that PARP inhibition significantly potentiated the cytotoxicity of doxorubicin [33]. Therefore, BromAc® may sensitise sarcoma cells to doxorubicin through PARP cleavage. Another potential mechanism of synergy includes p-glycoprotein cleavage, an efflux transporter associated with doxorubicin resistance [34]. However, p-glycoprotein expression was not analysed in this study [32].

The chemo-potentiating properties of BromAc® have been corroborated by Dilly and colleagues in vitro. The sensitivity of LS174T cells to oxaliplatin was significantly increased through 2 hours of BromAc® pre-treatment, with a corresponding increase in intracellular oxaliplatin concentrations. The combination of BromAc® and mitomycin C exhibited greater apoptosis in mucinous appendiceal tumour explants compared to mitomycin C monotherapy. Significantly, the potentiation of mitomycin C by BromAc® was far greater in mucinous appendiceal tumours compared to non-mucinous appendiceal tumours, highlighting the significance of mucolysis in this method of sensitisation [26].

In vivo evidence: The chemo-sensitising effect of BromAc® in mice inoculated with the LS174T cell line was evaluated with several cytotoxic agents. The combination of BromAc® with either oxaliplatin or 5-FU was ineffective in suppressing tumour growth [35]. The lack of synergy between BromAc® and oxaliplatin at the concentrations and volumes examined is consistent with the aforementioned hypothesis that acetylcysteine antagonises the oxidative stress generated by platinum agents [31]. Additionally, bromelain may degrade the copper transporter 1 and organic cation transporters 1-3, which are implicated in the uptake of oxaliplatin [36]. However, in patient derived PMP mouse xenografts, intraperitoneal BromAc® was able to enhance the efficacy of oxaliplatin [26]. The discrepancy in chemosensitisation potential may relate to several factors, including the administration technique, volume of drug delivery, inconsistent peritoneal exposure times, and differences in the degree and type of mucin expression. Regarding 5-FU, the proteolytic action of bromelain may be responsible for the lack of sensitisation observed in

LS174T tumours, by interfering with its enzymatic activation or cellular uptake through protein transporters. This contradicts *in vitro* evidence in hepatic, gastric, and colorectal cancer cell lines, which report synergy between BromAc® and 5-FU However, one limitation of this study is the administration of single-agent 5-FU, as clinically it is co-administered with oxaliplatin for chemopotentiation. Therefore, examining combination therapies *in vivo* would provide more clinically relevant conclusions [20, 31].

Gemcitabine was potentiated in LS174T-implanted mice when combined with BromAc®. The CI indicated a synergistic interaction between gemcitabine and BromAc® (CI = 0.08) with a significant reduction in tumour mass by 71% [35]. Mechanistically, BromAc® may cleave transmembrane MUC4 which has been implicated in gemcitabine resistance. In pancreatic cancer, MUC4 has been associated with the downregulation of hCNT1 and hCNT3 transporters which facilitate gemcitabine entry into cells [37, 38]. Therefore, aside from dissolving the physical mucin barrier, BromAc® potentially promotes a more chemo-sensitive molecular phenotype for gemcitabine. The treatment of pancreatic cells with combinations of BromAc® and gemcitabine was investigated *in vivo* with subcutaneous AsPC-1 deposits in nude mice. No synergy was observed when combining gemcitabine with BromAc®, contrary to data from *in vitro* studies of pancreatic cell lines. Consequently, there was similar efficacy between the gemcitabine alone, BromAc® alone, and BromAc® plus gemcitabine groups, with significant tumour weight regression [27].

Safety profile in animal studies

In vivo studies in nude mice have repeatedly demonstrated no signs of toxicity with the intraperitoneal administration of BromAc®. Early investigations into the safety profile of intraperitoneal BromAc® were performed with a dose escalation of 30 mg/kg bromelain in nude mice implanted with intraperitoneal PMP. This revealed no toxicity in a follow-up of 55 days, measured via appearance, behavioural, and weight parameters [17]. Necropsy in nude mice with intraperitoneal LS174T or MKN45 tumour deposits after treatment with intraperitoneal BromAc® demonstrated no macroscopic or microscopic signs of hepatic or colonic toxicity [24].

Clinical evidence

BromAc® has undergone phase I evaluation for mucinous tumours and has also been published in case studies. Currently, BromAc® is classed as an orphan drug for PMP and has also been available for compassionate use in multiple countries. A phase I trial of percutaneous BromAc® for inoperable mucinous tumours was performed. Patients unfit for cytoreductive surgery have limited therapeutic options, given the inadequacy of systemic therapies [39]. Percutaneous administration of BromAc® to mucinous tumour masses is intended to dissolve the mass, followed by aspiration to reduce the intra-abdominal disease burden in non-operative patients. This treatment was anticipated to improve symptoms associated with inoperable high-volume disease, including pain, bowel obstruction and malnutrition, and general, progressive decline. The primary outcome of the trial was the safety profile of percutaneous BromAc®. In total, twenty patients underwent treatment with percutaneous BromAc®; six had low grade PMP, ten had appendix adenocarcinoma, three had mucinous ovarian tumours, and one had colon cancer. BromAc® formulations varied between patients and were determined based on estimated tumour volume and site of administration (intraperitoneal vs. intratumoural). BromAc® was left in situ and aspirated at 24 hours, and the volume of tumour removed was measured to consider repeat treatment [40].

Overall, this trial demonstrated the relative safety of percutaneous BromAc®, with all adverse events being manageable. Seventeen out of twenty patients developed adverse events with fever, pain, and nausea being the most common [40]. The low-grade fever was consistent with a rise in inflammatory markers, with many patients exhibiting an elevated C-reactive protein and white cell count. This acute phase reaction is expected when solubilising a large tumour volume within the abdominal cavity. Importantly, there were no derangements in coagulation profile, despite the known anticoagulant properties of bromelain [41]. Serious adverse events included intra-abdominal sepsis (n = 1), fistulae (n = 2), and hypovolaemia (n = 1). The development of fistulae after treatment may be due to pre-existing fistulous connections secondary to tumour invasion which became patent after the tumour was solu-

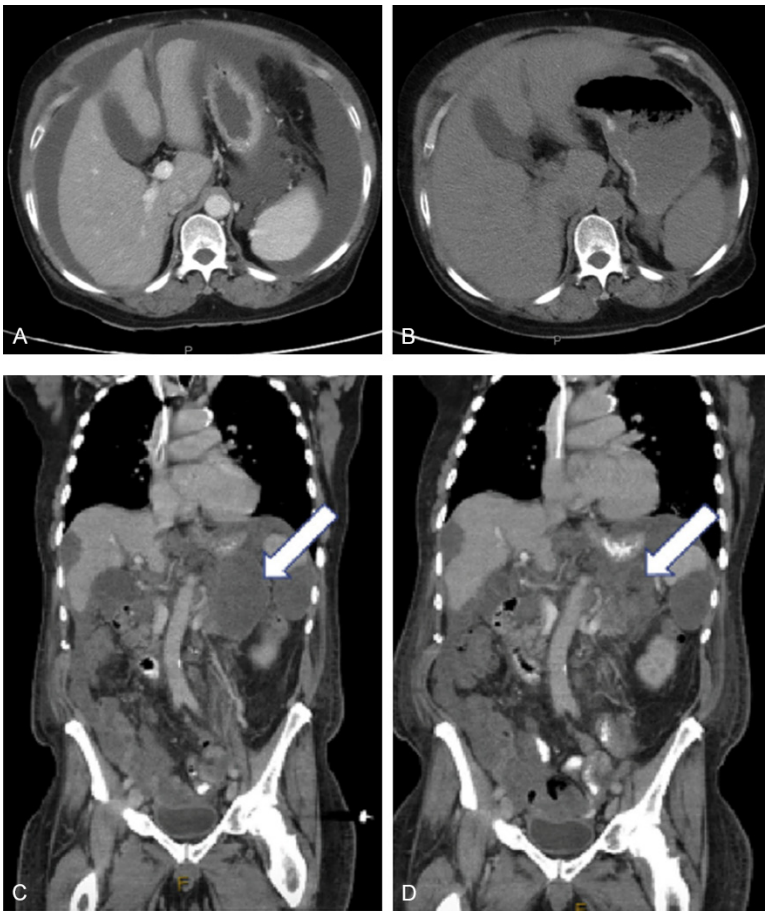

Figure 5. Computed tomography of patients pre- and post-treatment (1 month) with percutaneous BromAc®. A, B. Represent the pre- and post-treatment scans respectively of a patient with ovarian cancer who underwent intraperitoneal BromAc® treatment (×2). C, D. Represent the pre- and post-treatment scans respectively of a patient with appendix adenocarcinoma complicated by gastric compression from the mucinous mass (arrow). Figures taken from [40].

bilised. Similarly, after eroding the bowel wall, tumour deposits may act as barriers preventing bacterial translocation and intra-abdominal sepsis. Therefore, patients with suspected fistulae or extensive bowel erosion should be approached with caution. Whilst this study focused on safety, it should be noted that several patients remained clinically well at one-year post-trial, and that percutaneous BromAc® was able to solubilise a large, mucinous tumour burden (**Figure 5**) [40].

Preliminary evidence suggests a differential response to BromAc® depending on tumour hardness. A case report from Lam et al. of BromAc® for inoperable PMP describes the response to BromAc® in two patients; one 41-year-old male and one 68-year-old male, who both presented with pleural PMP recurrence. In the first patient, percutaneous BromAc® allowed for the dissolution and drainage of the mucinous tumour mass, accompanied by relief of respiratory symptoms, pain and mobility. Progress CT imaging revealed a significant reduction in tumour size. The patient did not experience severe adverse effects. However, in the latter patient, drain insertion was complicated by the hardness of the tumour, and minimal mucin was aspirated with BromAc® treatment [42]. This suggests that there is a difference in efficacy with different tumour hardness, likely due to drug dispersion, which confers with observations from *in vitro* studies of PMP mucin [21]. Further data is required to delineate the relationship between tumour hardness and clinical outcomes with BromAc®. This may improve patient selection for percutaneous BromAc® treatment.

Future direction

Given the chemo-sensitising properties of BromAc®, a promising avenue to pursue is the addition of BromAc® to HIPEC or EPIC formulations, improving microscopic cytoreduction. However, intraoperative or early post-operative BromAc® may hinder wound healing as a consequence of the proteolytic activity of bromelain. Consequently, before clinical trials of BromAc® in HIPEC, a safety study investigating the effect of BromAc® on colonic anastomoses in rats was performed. In this rat model, colon anastomosis was performed before intraperitoneal treatment with either BromAc® or saline (control), hyperthermia, and combinations with chemotherapy. Macroscopic inspection of the abdominal cavity for signs of anastomotic dehiscence, including abscesses or collections, was performed. The conditions of the anastomoses were compared through the anastomotic bursting pressure and tissue histology. The

results of these studies will inform the safety of BromAc® administration during or after cytoreductive surgery. Additionally, the systemic safety and efficacy of BromAc® requires further characterisation. Whilst a percutaneous drain would be required to aspirate mucinous masses, the use of BromAc® solely as a chemopotentiating agent for chemotherapies would benefit from systemic delivery.

Summary

BromAc® has demonstrated *in vitro* and *in vivo* efficacy of mucolysis, cytotoxicity, and chemopotentiation in a range of mucinous tumours. Early clinical studies have revealed safety with locoregional use, and preliminary data suggests that BromAc® is efficacious in dissolving mucinous tumour deposits in humans. Evidence from animal and clinical studies is limited to locoregional administration, with planned studies on intravenous BromAc®.

Disclosure of conflict of interest

Sarah J Valle and David L Morris are shareholder founders of Mucpharm Pty Ltd.

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