Anticancer effects of disulfiram: a systematic review of in vitro, animal, and human studies

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Abstract

Background and objectives: Cancer morbidity and mortality rates remain high, and thus, at present, considerable efforts are focused on finding drugs with higher sensitivity against tumor cells and fewer side effects. Disulfiram (DSF), as an anti-alcoholic drug, kills the cancer cells by inducing apoptosis. Several preclinical and clinical studies have examined the potential of repurposing DSF as an anticancer treatment. This systematic review aimed to assess evidence regarding the antineoplastic activity of DSF in in vitro and in vivo models, as well as in humans.

Methods: Two authors independently conducted this systematic review of English and Chinese articles from the PubMed, Embase, and the Cochrane Library databases up to July 2019. Eligible in vitro studies needed to include assessments of the apoptosis rate by flow cytometry using annexin V/propidium iodide, and studies in animal models and clinical trials needed to examine tumor inhibition rates, and progression-free survival (PFS) and overall survival (OS), respectively. Data were analyzed using descriptive statistics.

Results: Overall, 35 studies, i.e., 21 performed in vitro, 11 based on animal models, and three clinical trials, were finally included. In vitro and animal studies indicated that DSF was associated with enhanced apoptosis and tumor inhibition rates, separately. Human studies showed that DSF prolongs PFS and OS. The greatest anti-tumor activity was observed when DSF was used as combination therapy or as a nanoparticle-encapsulated molecule. There was no noticeable body weight loss after DSF treatment, which indicated that there was no major toxicity of DSF.

Conclusions: This systematic review provides evidence regarding the anti-tumor activity of DSF in vitro, in animals, and in humans and indicates the optimal forms of treatment to be evaluated in future research.

Keywords: Disulfiram, Apoptosis rate, Tumor inhibition rate, Progression-free survival, Overall survival

Introduction

Cancer is expected to be the leading cause of death and the foremost contributor to decreased life expectancy in every country worldwide during the twenty-first century and beyond [1]. Although comprehensive therapies prolong survival and improve the quality of life of cancer patients, approximately 96,000,000 cancer deaths occurred in 2018 worldwide [1]. The global community is well aware that new drug development, discovery, and synthesis are a time-consuming process, which involves intensive work and appraisal of the cost-effectiveness of the drug under development [2]. As a result, researchers are allocating considerable efforts for repurposing existing drugs such as disulfiram (DSF).

In the 1800s, DSF was used as an industrial catalyst in the production of rubber [3]. In 1948, DSF was approved by the Food and Drug Administration for treating alcoholism [4]. In 1988, DSF was associated with a decrease in the occurrence of occasional infections in symptomatic patients with human immunodeficiency virus infection [5], prompting the conduct of a wealth of clinical trials, some of which are still ongoing (www.clinicaltrials.gov).

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The antineoplastic activity of DSF was first recorded in 1977 by Dr. Lewison in a 35-year-old female breast cancer patient with systemic metastases who received DSF for her severe alcoholic syndrome and remained clinically free of cancer for 10 years without receiving any form of anticancer therapy [6]. This observation was noted in an era in which the anticancer effect of DSF was being researched. In recent years, a large number of preclinical studies and clinical trials (www.clinicaltrials.gov) of DSF have been conducted to explore the anticancer activities of this drug. Nonetheless, the antitumor effectiveness of DSF remains uncertain owing to existing heterogeneity across different studies with cell lines, animals, and humans. Currently, a systematic review of these studies to assess and clarify the anticancer potential of DSF is lacking.

It is worthy to explore whether there are substantial differences and are appropriate for clinical proposals. Therefore, this study aimed to perform a systematic review of published data on the antitumor activity of DSF. Specifically, this review aimed to assess the apoptosis and tumor inhibition rates of DSF based on data from studies in cell lines and animal models, respectively, and examine the benefit of DSF on progression-free survival (PFS) and overall survival (OS) based on results from clinical studies, regardless of the study design or type of cancer investigated. Meanwhile, it is important for evaluating the anti-tumor effect of disulfiram to include in the side effects. The side effects of disulfiram will be covered in this article.

Materials and methods
The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed to conduct this systematic review [7].

Search strategy
PubMed, Embase, and Cochrane Library databases were searched for relevant studies from their inception to the end of July 2019. The search was performed with a combination of Medical Subject Headings and free words as follows: (neoplasia OR neoplasm OR tumor OR cancer OR malignancy OR malignant neoplasm), and [disulfiram OR bis (diethylthiocarbamoyl)] disulfide OR tetraethylthioperoxydicarbonic diamide OR tetraethylthiuram disulfide OR tetraethylthiuram or antabus OR antabuse tetruram OR dicupral OR esperal OR alcohobin OR anticolin]. The details of the search strategy are presented in the supplement.

Study selection
Studies that implemented the below criteria were included: (1) solid cancer cell lines, animals, or patients treated with DSF; (2) in vitro studies focusing on parameters of the apoptosis index (early apoptosis or early apoptosis plus late apoptosis) using annexin V-fluorescein isothiocyanate/prodium iodide double-staining analysis by flow cytometry, in vivo studies evaluating the tumor inhibition rate in cell-line-derived xenograft animal models, or studies in humans, which included OS and PFS as endpoints, to assess the effect of DSF in cancer patients; and (3) studies published in the English and Chinese language. There were no restrictions on the type of cancer studied. To avoid duplication of data, only the most recent and most comprehensive articles were included. Studies with incomplete data or conference abstracts were excluded. Two investigators (Ling Wang, Run Wan) independently screened the databases for studies based on the eligibility criteria. Any discrepancies were resolved by consulting a third researcher (Cong Zhou).

Data derivation
Two investigators (Ling Wang, Cong Zhou) independently extracted data from the inclusive studies. Inconsistencies between the two investigators were resolved by consulting a third reviewer (Run Wan). When required, we contacted the authors of the research for further information. A pre-designed structured outline was used to abstract data. The outline included the following fields: study type (in vitro, in vivo, clinical study, or case series); general information (first author, publication year, country, and study design); supplement used; anticancer treatment used; and outcomes (i.e., apoptosis rate, tumor inhibition rate, OS and PFS, as applicable). The results of each study included were summarized. Descriptive statistics were used for data analysis. Meta-analysis was not performed owing to substantial heterogeneity across studies.

Results

Study characteristics
The initial search yielded a total of 1278 studies. After excluding 274 irrelevant and duplicate studies, the full texts of 1004 studies were screened. Of these, 148 were considered eligible based on the availability of full texts as well as the description of target outcomes. Ultimately, 113 articles were removed (no full texts, n=43; no target outcomes, n=70), and 35 studies were selected. A detailed description of the steps followed during the retrieval process is provided in Fig. 1.

Of 35 selected studies, 21 were in vitro studies (Table 1), 11 were in vivo studies with animal models (Table 2), and three were clinical trials (Table 3). In vitro studies, the most studied cancer was breast cancer (five studies) [8–12], while the A549 non-small cell lung cancer (NSCLC)
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A cell line was the one most commonly used cell line (four studies) [13–16]. Three studies examined DSF as a single agent [17–19], and 17 studies examined DSF in combination with metal ions (Cu, Ag), chemotherapy, or radiation therapy [8–16, 20–27]. In addition, DSF was encapsulated in nanoparticles (DSF-NPs) in three studies [12, 16, 25].

Of 11 animal studies, Balb/C nude mice were utilized in nine [28–36], whereas the remaining studies used KunMing or female SCID mice [37, 38]. Ten studies used subcutaneous tumor models by injecting cancer cell lines [26, 29, 31, 32, 34–38], and one study used an in situ tumor model [33]. Eleven studies had assessed the dimensions of tumor volume \(V\) using the same formula \(V = 0.5 \times \text{length} \times \text{width}^2\) [28–38], nine studies assessed changes in body weight in mice [26–34, 37, 38], and six studies contained data regarding the toxicity of DSF [28, 29, 32–35]. In addition, eight of the animal studies used DSF by re-synthesizing the molecule with nanoparticles [28–34, 37].

The three human studies included participants with differing characteristics and cancer types. All three clinical trials investigated DSF as a combination therapy with chemotherapy or and radiation therapy [39–41], while two studies reported on adverse events [39, 40].

Outcomes
Three cell lines and one animal study showed that treatment with DSF as a single agent induced apoptosis and increased the rate of tumor inhibition [17–19, 35]. Although the sensitivity between the various cell lines varied, dose-dependency was consistently observed.

The concentration-dependent increase in apoptosis and tumor inhibition rates was augmented by a combination therapy of DSF adding metal ions [copper (Cu), silver (Ag)] in 10 in vitro [8–11, 13–15, 20, 26, 42] and three in vivo studies [36–38]. The synergistic effect of Cis, DOX, TMZ, PTX, Gy, and DSF in induced apoptosis was significantly higher than that of DSF or Cis or DOX or TMZ or Gy alone [8–10, 21, 24, 42]. Tumor cell growth was significantly inhibited when DSF, chemotherapy, and radiation therapy were used simultaneously, as shown in the examined in vivo studies [30, 31, 35, 37].

Compared with free molecule, DSF encapsulated with nanoparticles significantly induced selective death-dependent apoptosis, especially in acidic conditions (pH = 6.5) in cancer cell lines. Eleven animal studies demonstrated that DSF modified by particular nanoparticles increased the tumor inhibition rate and that the anticancer activity was more obvious when chemotherapy (Cis) was combined with nanoencapsulated DSF [32].

Changes in body weight during the whole study period were analyzed in nine animal studies. With the exception of three reports of weight changes in DSF-treated or DSF-modified groups [30, 33, 36], other studies recorded that there was no noticeable body weight loss after DSF treatment or no significant difference in body weight changes across different groups [28–32, 34, 36, 38], which
Table 1  Effects of disulfiram on cell apoptosis rates from in vitro studies

| Reference | Country | Tumor                        | Intervention time | Negative control | Positivitive control | Cell lines | Negative control | Positive control | Treatment group         |
|-----------|---------|------------------------------|-------------------|------------------|----------------------|------------|------------------|------------------|-----------------------|
| You et al.| China   | Colorectal cancer            | 48 h              | Saline           | DOX (8.5 μM)         | HCT116     | 0.27 ± 0.24      | 29.2 ± 4.1       | DSF/Cu0.05 μM: 8.55 ± 2.3, 0.1 μM: 24.02 ± 3.6, 0.2 μM: 38.4 ± 7.9, 0.4 μM: 58.3 ± 7.7 |
|           |         |                              |                   |                  | HCT8                 |            | 2.1 ± 1.6        | 32.3 ± 4.1       | DSF/Cu0.05 μM: 29.5 ± 4.4, 0.1 μM: 28.1 ± 9.5, 0.2 μM: 38.6 ± 10.3, 0.4 μM: 56.4 ± 10.2 |
|           |         |                              |                   |                  | SW620                |            | 2.21 ± 0.5       | 48.4 ± 9.5       | DSF/Cu0.05 μM: 20.1 ± 7.7, 0.1 μM: 30 ± 4.2, 0.2 μM: 42 ± 6.3, 0.4 μM: 43.4 ± 8.3 |
| Yang et al.| Germany| Breast cancer                | 48 h              | Control          | CIS (5 μM)           | MCF-7      | 25.31            | 31.67            | DSF 1 μM: 36.6, DSF 1 μM + CIS 5 μM: 57.4 |
|           |         |                              |                   |                  | MDA-MB-435S         |            | 5.843            | 5.447            | DSF 1 μM: 13.56, DSF 1 μM + CIS 5 μM: 29.4 |
|           |         |                              |                   |                  | SKB-R3               |            | 3.023            | 11.46            | DSF 1 μM: 5.6, DSF 1 μM + CIS 5 μM: 7.71 |
| Wu et al. | China   | Triple-negative breast cancer | 24 h              | DMSO             | PAX (5 nM)           | SUM102 ALDH- | 2.22             | 5.83             | DSF/Cu0.75 μM: 23.53 |
| Guo et al.| Germany | Ovarian cancer               | 72 h              | Control          | _                    | KROVI      | 10.32            | _                | DSF/Cu0.75 μM: 20.9 |
|           |         |                              |                   |                  |                      |            |                  |                  | Cu 1 μM: 15.3, DSF 1 μM: 25.46, DSF/Cu: 47.65 |
|           |         |                              |                   |                  | SKOV3IP1            |            | 8.69             | _                | Cu 1 μM: 7.1, DSF 0.1 μM: 15.99, DSF/Cu: 55 |
|           |         |                              |                   |                  | SKOV3               |            | 3.65             | _                | Cu 1 μM: 1.91, DSF 1 μM: 43.2, DSF/Cu: 50.4 |
| Wu et al. | China   | Non-small cell lung cancer   | 24 h              | Control          | _                    | A549       | 2.5              | _                | Cu 1 μM: 3.8, DSF 1.4 μM: 4.8, DSF/Cu: 35.4 |
|           |         |                              |                   |                  |                      | H460       | 4.7              | _                | Cu 1 μM: 3.7, DSF 8 μM: 4.9, DSF/Cu: 21.4 |
|           |         |                              |                   |                  |                      | H1299      | 8.7              | _                | Cu 1 μM: 10.3, DSF 4 μM: 7.1, DSF/Cu: 37.9 |
| Chen et al.| China  | Non-small cell lung cancer   | 24 h              | Control          | _                    | A549       | 3.35             | _                | Ag 1.25 μM: 4.34, DSF 1.25 μM: 5.14, DSF/Ag: 42.81 |
| Butcher et al.| UK     | Non-small cell lung cancer   | 16 h              | Vehicle          | _                    | A549       | 6.3              | _                | CuCl2 10 μM: 6.5, DSF 1 μM: 15.2, DSF/CuCl2: 47.2 |
| Reference        | Country | Tumor                      | Intervention time | Negative control | Positive control | Cell lines | Negative control | Positive control | Treatment group |
|------------------|---------|----------------------------|-------------------|------------------|------------------|------------|------------------|------------------|-----------------|
| Albers et al.    | Germany | Head and neck squamous cell carcinoma | 48 h              | Control          | CIS (1μM)+10Gy | HNSCC cell lines | 11.35           | CIS 1 μM: 24.12, 10Gy: 23.47 | DSF 3 μM/Cu 0.1 μM: 20.87, DSF 3 μM + CIS 1 μM: 38.35, DSF 3 μM/Cu 0.1 μM + CIS 1 μM: 51 |
| Yang et al.      | China   | Nasopharyngeal cancer      | 6 h               | Control          | _                | CNE-2Z     | 4.41             | _                | DSF 0.2 μM/Cu 10 μM: 24.08, DSF 0.4 μM/Cu 10 μM: 58.9 |
| Marwa et al.     | Egypt   | Colon cancer               | 72 h              | Control          | _                | DCECs      | 1.58             | _                | DSF 9.5 ± 0.9 μg/mL: 60.3 ± 1.2, UC-NPs 1548 ± 25 μg/mL: 12.1 ± 0.47, C-NPs 3122 ± 39 μg/mL: 2.6 ± 0.07 |
|                  |         |                            |                   |                  |                  | CDCECs     | 0.28             | _                | DSF 23.9 ± 0.1 μg/mL: 57.8 ± 0.34, UC-NPs 77.7 ± 1.4 μg/mL: 54.7 ± 1.24, C-NPs 93.8 ± 0.4 μg/mL: 47.5 ± 0.31 |
|                  |         |                            |                   |                  |                  | Caco-2     | 0.05             | _                | DSF 39.6 ± 0.3 μg/mL: 53.6 ± 0.53, UC-NPs 97.9 ± 0.5 μg/mL: 53.49 ± 0.59, C-NPs 148 ± 3 μL: 0.1 μg/mL: 40.28 ± 0.24 |
| Wang et al.      | China   | Non-small cell lung cancer | 24 h              | Control          | _                | A549       | 0.45             | _                | DSF-LP-PLGA-MP 1, 3, 5, 7 days: 93.2, 27.1, 28.2, 49.18 |
| Yang et al.      | China   | Breast cancer              | 24 h              | Control          | _                | MCF-7      | 0.29             | _                | DSF 0.2 μM/CuCl₂ 10 μM: 27.15, DSF 0.25 μM/CuCl₂ 10 μM: 86.8 |
| Reference       | Country | Tumor                                | Percentage of apoptosis (%) | Treatment group                                                                 |
|-----------------|---------|--------------------------------------|-----------------------------|--------------------------------------------------------------------------------|
|                 |         |                                      | Intervention time           | Negative control | Positive control | Cell lines | Negative control | Positive control | |
| Kim et al.      | Korea   | HER2-positive breast cancer           | 24 h                        | DMSO              | SKBR3           | 3.16       | _               | _               | Cu 1 μM: 2.91, DSF 1 µM: 26, DSF/Cu: 30.21 |
|                 |         |                                      |                             |                   | BT474           | 2.49       | _               | _               | Cu 1 μM: 2.88, DSF 1 µM: 8, DSF/Cu: 40.76 |
| Sharma et al.   | India   | Prostatic cancer                     | 48 h                        | Control           | STA (3mM)       | PC3        | 8.34±2.2       | 26.31±5.5       | DSF 1 μM: 15.04±3.14, DSF 2 μM: 19.71±4.2, DSF 3 μM: 32.06±6.16 |
|                 |         |                                      |                             |                   | DUI45           | 13.67±2.66 | 41.31±4.47     |                 | DSF 1 μM: 10.89±1.56, DSF 2 μM: 42.81±4.56, DSF 3 μM: 47.23±4.85 |
| Zhao et al.     | China   | Pituitary adenomas                   | 24 h                        | Control           | TMZ (100μM)     | Pituitary adenoma cells | 0.29±0.09 | 0.81±0.23       |                 | DSF 25 μM: 0.31±0.10, DSF 25 μM + TMZ 100 µM: 1.64±0.16 |
| Zhang et al.    | China   | Hepatocellular carcinoma             | 24 h                        | Control           | Hep G2 cells    | 1.3        | _               |                 | DSF-S-LNCs (PH = 7.4): 9.4, DSF-S-LNCs (PH = 6.5): 16.5 |
| Duan et al.     | China   | Breast cancer                        | 24 h                        | Control           | 4T1             | 1.07       | _               |                 | DSF 1 μg: 34.77, DnMs (DSF 1 μg): 34.37, DCM (DSF 1 μg): 41.11 |
| Rezk et al.     | USA     | Ovarian cancer                       | 72 h                        | Control           | A2780DK         | 4.15       | _               |                 | DSF 5 μM: 36.4 |
| Dastjerdi et al.| Iran    | Pancreatic cancer                    | 24 h                        | Control           | PANC-1          | 27         | _               |                 | DSF 5 μM: 51, DSF 10 μM: 84, DSF 13 μM: 92 |
| Han et al.      | China   | Pancreatic cancer                    | 72 h                        | Control           | SW1990          | 1.5        | _               |                 | DDTC–Cu(I) 1 μM: 6.4, DDTC–Cu(I) 3 μM: 17.7, DDTC–Cu(I) 5 μM: 24.8 |
| Cen et al.      | USA     | Melanoma                             | 48 h                        | Control           | BSO (100M)      | C81-46A    | 12.057±0.72    | 13.194±1.11     | DSF 50 ng/ml + BSO 100 M: 54.78 ± 2.83 |

Abbreviations: DOX Doxorubicin, CIS Cisplatin, PTX Paclitaxel, STA Staurosporine, TMZ Temozolomide, BSO Buthionine-sulfoximine, DnMs DSF-loaded noncrosslinked micelles, DCM DSF-loaded redoxsensitive shell crosslinked micelle, DSF-LP-PLGA-MP Disulfiram-loaded porous PLGA microparticle, UC-NPs Uncoated NPs, C-NP Coated NPs, DDTC–Cu(I) Diethylthiocarbamate-Cu(I)
### Table 2 Effects of disulfiram on tumor inhibition rates from animal studies

| Reference | Country | Tumor      | Information of reference | Information of animals | Intervention and tumor inhibition rate | Toxicity evaluation |
|-----------|---------|------------|---------------------------|------------------------|----------------------------------------|---------------------|
| Peng et al. | China | Lung cancer | Female Balb/C nude mice   | 4–5                    | 1.0 × 10^6 A549 cells, SC, right flank | Every 4 days with 4 times, iv | TSR% = 16.6% TSR% = 51.6% | No significant weight loss |
| Parikshit et al. | China | Breast cancer | Female Balb/C nude mice   | 4–5                    | 1.0 × 10^5 4T1 cells, SC, left armpit | Every 3 days with 6 times, iv | TSR% = 8.49% TSR% = 29.2% TSR% = 48.24% | No noticeable body weight loss |
| Ji et al. | China | Breast cancer | Female Balb/C nude mice   | _20±2                  | 8.0 × 10^5 4T1 cells, SC, right flank | Everyday with 2 weeks, iv or every day with 2 weeks, iv | TSR% = 55.01% | Weight increased slightly |
| Zhou et al. | China | Liver cancer | KunMing mice              | 5–6                    | 1.5 × 10^7 H-22 cells, SC, left axilla | Every 3 days with 4 times, iv | TSR% = 26.8% TSR% = 35.5% TSR% = 50.3% | – |
| Reference | Country | Tumor | Information of reference | Information of animals | Intervention and tumor inhibition rate | Toxicity evaluation |
|-----------|---------|-------|--------------------------|------------------------|---------------------------------------|-------------------|
| Tao et al. | China | Breast cancer | | Female Balb/C nude mice | | |
| | | | | Old (weeks) | 20 ± 2 | |
| | | | | Weight (g) Animal tumor model | 3.0 × 10^6 4T1 cells; SC, right flank | |
| | | | | Intervention methods | Every 2 days with 4 times, iv | |
| | | | | Negative control | Saline | |
| | | | | Positive control | DOX (5 mg/kg) | |
| | | | | Treatment group | DSF 5 mg/kg + DSF 5 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 348.1% | |
| | | | | Outcome | TIR% = 80.92% TIR% = 89.27% | |
| | | | | Safety | No significant difference in body weight change | |
| Song et al. | China | Lung cancer | | Female Balb/C nude mice | | |
| | | | | Old (weeks) | 6 | 200 | 2.0 × 10^6 A549 DDP cells, SC, right flank | |
| | | | | Intervention methods | Every 2 days with 4 times, iv | |
| | | | | Negative control | Saline | |
| | | | | Positive control | PGA-CisPt 5.0 mg/kg | |
| | | | | Treatment group | PGA-CisPt 5.0 mg/kg + NPs-DSF 10 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 45.6% | |
| | | | | Outcome | TSR% = 75.4% | |
| | | | | Safety | No body weight changes | |
| Hamidreza et al. | Iran | Breast cancer | | Female Balb/C nude mice | | |
| | | | | Old (weeks) | 5 | | 1.0 × 10^6 4T1 cells; mammary fat pad | |
| | | | | Intervention methods | 2 weeks; iv | |
| | | | | Negative control | Blank NPs | |
| | | | | Positive control | DSF 10 mg/kg | |
| | | | | Treatment group | DSF 10 mg/kg + DS-P-NPs 10 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 17.07% | |
| | | | | Outcome | TSR% = 66.67% TSR% = 75% | |
| | | | | Safety | No sign | |
| Song et al. | China | Breast cancer | | Balb/C mice | | |
| | | | | Old (weeks) | 5-6 | | 2.0 × 10^6 4T1 cells; SC, right flank | |
| | | | | Intervention methods | Every 2 days with 6 times, iv | |
| | | | | Negative control | Saline | |
| | | | | Positive control | DSF 15 mg/kg + NP4/5/1 15 mg/kg | |
| | | | | Treatment group | DSF 15 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 0 | |
| | | | | Outcome | TSR% = 43.2% | |
| | | | | Safety | No obvious body weight loss | |
| Jennifer et al. | USA | Breast tumor | | Female SCID mice | | |
| | | | | Old (weeks) | _ | | 1.0 × 10^6 SUM149 cells, SC, flank | |
| | | | | Intervention methods | Daily, iv | |
| | | | | Negative control | Vehicle | |
| | | | | Positive control | DSF 50 mg/kg | |
| | | | | Treatment group | DSF 50 mg/kg + Cu 0.5 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 75% | |
| | | | | Outcome | TSR% = 84% | |
| | | | | Safety | No noticeable body weight change | |
| Choi et al. | Korea | Atypical teratoid/rhabdoid tumors | | Female Balb/C nude mice | | |
| | | | | Old (weeks) | 7 | | 1.0 × 10^6 AT/RT cells, SC, _ | |
| | | | | Intervention methods | Every 5 consecutive days with 3 weeks, ip | |
| | | | | Negative control | DMSO | |
| | | | | Positive control | DSF 100 mg/kg | |
| | | | | Treatment group | DSF 100 mg/kg | |
| | | | | Inhibit Rate Parameter | TSR% = 72.23% | |
| | | | | Outcome | _ | No major | |
| Reference | Country | Tumor | Strain and gender | Old (weeks) | Weight (g) | Animal tumor model | Intervention methods | Negative control | Positive control | Treatment group | Inhibit Rate Parameter | Outcome |
|-----------|---------|-------|-------------------|------------|-----------|-------------------|-------------------|------------------|-----------------|----------------|---------------------|---------|
| Vino et al. | China | Malignant Pleural Mesothelioma | Female Balb/C nude mice | 5 | _ | 0.5 × 10^6 AB12 cells, SC, right flanks | Daily with 17 days, ip | Vehicle | _ | DSF/Cu 50 mg/kg | TSR% = 71.5% | Weight of DSF-Cu group was 75% lower than that of vehicle group |

**Abbreviations:** DOX Doxorubicin, Cis Cisplatin, 5-Fu 5-fluorouracil, TV Volume, L Length = longest diameter of the tumor, W Width = shortest diameter of the tumor, SC Subcutaneous, iv Intravenous injection, TGI Tumor growth inhibition rate — TGI% = (\( Vc_1 - Vt_1 \)/\( Vc_0 - Vt_0 \)) × 100%, TIR Tumor inhibition rate — TIR% = (\( Vc - Vx \)/\( Vc \)) × 100%, TSR Tumor suppression rate — TSR% = (\( Vc - Vx \)/\( Vc \)) × 100%, Vc Mean tumor volume of the negative control group, Vt Mean tumor volume of certain administration group, Vc1 Mean tumor volume in the negative control group at the time of tumor extraction, Vt1 Mean tumor volume in the treatment groups at the time of tumor extraction, Vc0 Mean tumor volumes in the negative control group, Vt 0 Mean tumor volumes in the treatment group, NPs Nanoparticles, NSps Nanosuspensions, NLC Nanostructured lipid carriers, TPGS D-alpha-Tocopheryl polyethylene glycol succinate, PNPL-D5F/Cu Polymeric nanoparticles loading copper(II) diethylthiocarbamate (DSF/Cu 1:1), Cu(OI)2-S Administration of copper oleate solution, Cu(OI)2-L Administration of copper oleate liposome, NP4/5/1 The feed ratio of mPEG-PLGA/PCL/DSF was 4/5/1 in mass, PLGA Poly(lactide-co-glycolide), PEG Poly(ethylene glycol), mPEG-PLGA Methoxy poly(ethylene glycol)-b-poly(lactide-co-glycolide), PCL Polycaprolactone, DCC N,N'-Dicyclohexylcarbodiimide, DCM Dichloromethane, NHS Sulfo-N-hydroxysuccinimide, DS-PPF-NPs Disulfiram encapsulated PLGA PEG-folate NPs, DS-P-NPs Disulfiram encapsulated PLGA NPs.
### Table 3  Effects of disulfiram on progression-free survival and overall survival from human studies

| Reference          | Country | Study design                              | Study participants                                                                 | Study protocol                                                                 | OS       | PFS       | Adverse events                                                   |
|--------------------|---------|-------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------|----------|---------------------------------------------------------------|
| Huang, et al.      | USA     | Phase II, open-label, single-arm study    | Recurrent GBM who had developed unequivocal progression after RT and concurrent TMZ as per the RANO criteria while receiving adjuvant TMZ or within 3 months from the last dose of TMZ’ | DSF 80 mg and Cu Glucosinate 1.5 mg TID by mouth approximately 4–8h apart.     | 7.1 months (95% CI 5.8–8.5) | 1.7 months (95% CI 1.4–1.9) | Nausea/vomiting (17%) followed by dizziness (9%) grade. Only one patient (4%) had a possible DLT with grade 3 elevated alanine transaminase on day 31, which required study therapy to be held. The liver function test subsequently recovered after 4 weeks. |
| Huang, et al.      | USA     | Phase I, open-label, single-arm, single-institution study | Adjuvant TMZ in newly diagnosed adult GBM patients after standard chemoradiation therapy | 7 patients at DSF 500 mg per day, 5 patients at DSF 1000 mg per day, 6 patients at DSF 500 mg per day with Cu 2 mg | 14.0 months (95% CI 8.3–196) | 4.5 months (95% CI 0.8–8.2) | One with delirium after 1.6 months (without Cu), one with motor neuropathy after 2.6 months (without Cu) and one with diarrhea and nausea after 0.5 months (with Cu). All symptoms resolved shortly after dose reduction. |
| Nechushtan, et al. | Israel  | Phase II, multicenter randomized double-blinded study | Newly diagnosed NSCLC patients were recruited. Patients with either stage IV or what was considered at the time “wet IIIb” (since 2009, these patients have been considered stage IV) were recruited. The patients were treated with only chemotherapy, and none were treated with either surgery or chemoradiation. | controls: six cycles of cisplatin and vinorelbine (plus placebo tablets), experimental groups: the same plus disulfiram (40mg three times daily). | 10.0 versus 7.1 months | 5.9 versus 4.9 months | — |

**Abbreviations**: GBM Glioblastoma, NSCLC Non-small cell lung cancer, TMZ Temozolomide, TID Three times per day, DLT Dose-limiting toxicity, RANO Radiologic Assessment in Neuro-Oncology
indicated that there was no major toxicity of DSF [28, 29, 32–35].

Many clinical trials have mentioned the use of DSF for solid tumors (www.clinicaltrials.gov). One study clearly analyzed the difference in PFS (5.9 versus 4.9 months) and OS (10.0 versus 7.1 months) between control and experimental groups [42]. PFS and OS both improved in the experimental groups. Two studies described PFS and OS of the entire research cohort, and the treatment efficacy seemed to be in contrast to historical data [39, 40]. Our systemic review included two single-arm trials in glioblastoma (GBM) patients and a randomized controlled trial in NSCLC patients. Although the two single-arm clinical trials did not compare treatment with a control group, positive effects were observed; e.g., a 40-year-old woman with unmethylated isocitrate dehydrogenase wild-type GBM had good health without any signs of tumor recurrence 33 months after study initiation.

Among the reported adverse effects, none were serious, and they were of grades 2–3. Adverse effects were reported in two studies and included diarrhea, nausea, dizziness, vomiting, motor neuropathy, and elevated alanine transaminase levels. Symptoms resolved quickly when the dose was reduced [39, 40].

All three studies show that DSF is safe and seems to prolong survival of cancer patients. Because of individual differences in patients, the response to DSF was also varied [39, 40, 42]. The optimal concentration and sensitivity type should be further explored by in vitro and animal studies.

Discussion

DSF is decomposed into diethyldithiocarbamate in the body and exhibits anticancer activities [43]. Considering that the loss of cellular proliferation control leads to the development of cancer, effective clinical therapies of cancer have been developed based on the principle of inducing apoptosis [44]. In the included animal studies, the tumor inhibition rate was utilized to evaluate antitumor efficiency by calculating tumor volume. Most studies included in this review revealed enhanced apoptosis and tumor inhibition rates with DSF treatment (Table 4).

In recent years, metal-based complexes have been reported to exhibit anticancer activity [45]. Silver complexes demonstrate anti-tumor activity and display low toxicity in humans. The mechanism of action is related to their interaction with nucleic acids and proteins [46]. Metabolites of DSF chelate with metal ions, leading to alterations in the intracellular levels of metal ions, enhancement of oxidative stress, inhibition of the activities of superoxide dismutase or matrix metalloproteinases, inactivation of essential sulfhydryl groups by protein carbamoylation, and alteration of cancer cell invasion, tumor angiogenesis, and metastasis [47, 48]. The observation that the combination of DSF with metal ions (Cu, Ag) leads to enhanced anticancer effectiveness is in accordance with the observations of in vitro and animal experiments [11, 14].

In different cancer cell lines, the lethal concentration of DSF was different. The lethal concentration was reduced when DSF combined with metal ions or nano-reconstructed DSF.

The additive/synergistic action of DSF with other chemotherapy agents in inhibiting tumor cell growth and cytotoxicity is mediated through the enhancement of cellular oxidative stress, inhibition of P-glycoprotein (P-gp) activity, and dysregulation of the NF-κB signaling pathway [8, 49, 50].

In the examined studies, anti-tumor activity, as evidenced by higher apoptosis and tumor inhibition rates, was enhanced with DSF-NPs in various ways. At the pH of 7.4, the half-life of DSF is 1–1.5 min [47]. The half-life was improved by nanomaterial packaging of DSF, with the anti-tumor effects increasing under acidic conditions (pH = 6.5) [51]. DSF-NPs enhanced cellular uptake, induced high levels of reactive oxygen species, activated the MAP-kinase pathway, sustained drug supply, and blocked copolymer micelles, such as the P-gp inhibitor [14, 20, 52]. Evidence supports that DSF-NPs ameliorate the instability and low treatment efficacy of free DSF.

Event-free survival (EFS) means that there are no adverse events since the start of treatment, including change of regimen, adverse side effects, intolerance, disease progression, and patient death. EFS represents a direct measure of the ability of the treatment to achieve a response, the durability of the response achieved, and its capacity to prolong life [53]. It was found that the doses of disulfiram significantly increased EFS [39].

| Table 4  | The summary of the findings |
|---------|----------------------------|
|          | Studies | Evaluation indicator | Results                | Side effects |
| Cells studies | 21      | Apoptosis rate        | From 4.8 to 92%        | N/A          |
| Animals studies | 11      | Tumor inhibition rate | From 8.49 to 89.27%    | Safety       |
| Human studies  | 3       | PFS and OS            | Be prolonged           | Low          |
Although our results may be more reliable than those of single studies, the present study has certain limitations. First, only articles published in English and Chinese were included; the non-inclusion of articles published in other languages may have had an effect on the results. Second, only some solid tumors were included, not referred to non-solid tumor (hematological malignancy). Third, the scarcity of the studies in general (35 in total) and the fact that they are performed on different cancers may make any specific conclusions difficult. Finally, no quality evaluation was conducted, and the majority of studies were animal and cell experiments; thus, the translation of these results to benefits in the clinic needs to be determined.

In conclusion, many studies have investigated the antineoplastic activity of DSF. This systematic review provides evidence of the antineoplastic activity of DSF in vitro, in vivo, and in humans. DSF could induce cancer cell apoptosis in cell experiments and inhibit cancer cell growth in animal experiments. Administration of DSF as a combination therapy or as a nanoparticle-encapsulated molecule seems to enhance its effectiveness. Meanwhile, DSF hardly affect the animal weight. Above all, DSF is effective and safe. These findings may serve as the basis for designing clinical studies of DSF in the future.

**Abbreviations**

DSF: Disulfiram; PFS: Progression-free survival; OS: Overall survival; EFS: Event-free survival; TIR: Tumor inhibition rate.

**Supplementary Information**

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**Authors’ contributions**

All authors read and approved the final version of this article. Ling Wang conceptualized the review, conducted the literature search, and was responsible for data extraction, data analysis, and for writing the original draft of this manuscript. Cong Zhou was responsible for data extraction. Run Wan conducted the literature search and served as an independent reviewer of the data extracted from the studies, responsible for solving any inconsistencies between Ling Wang and Cong Zhou. Yang Yu contributed to the analysis and interpretation of study data. Yumin Li conceptualized the review, supervised the process, and was responsible for project administration and manuscript review.

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**Availability of data and materials**

Not applicable.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Competing interests**

Ling Wang, Yang Yu, Cong Zhou, Run Wan, and Yumin Li declare that they have no competing interests.

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