ACCSCIENCE PUBLISHING

Microbes & Immunity

REVIEW ARTICLE

Potential of medicinal mushroom extracts as adjunctive agents to enhance conversion therapy in colorectal cancer with liver and peritoneal metastases

Supplementary Files

Section S1

1. Meta-analysis of adjuvant immunochemotherapy with polysaccharopeptide/polysaccharide krestin in post-operative treatment of esophageal, gastric, and colon cancers

This supplementary file details the methodologies and results of a meta-analysis evaluating the efficacy of polysaccharopeptide/polysaccharide krestin (PSP/PSK) derived from *Trametes versicolor* as adjuvant immunochemotherapeutic agents in post-operative cancer patients, focusing on 5-year overall survival (OS) in selected randomized controlled trials (RCTs). All included studies involve post-operative administration of PSP/PSK following curative resection of the primary tumor. The analysis includes study selection criteria, data extraction methods, statistical approaches, and results, including the pooled relative risk (RR) of 5-year OS (RR = 1.10, 95% confidence intervals [CI]: 1.04–1.15).

1.1. Introduction

Polysaccharides PSP/PSK from *T. versicolor* have shown potential as adjuvant agents in post-operative cancer treatment. This meta-analysis quantifies the effect of PSP/PSK on 5-year OS in patients receiving standard chemotherapy or radiotherapy post-curative resection of the primary tumor, addressing the research question: does adjuvant PSP/PSK improve survival outcomes compared to chemotherapy or radiotherapy alone in post-operative cancer patients? The rationale stems from varying outcomes in prior RCTs, necessitating a pooled analysis to clarify efficacy in the post-operative setting.

1.2. Methods

1.2.1. Search strategy

A comprehensive literature search was conducted to assess the safety and efficacy of medicinal mushroom compounds, specifically PSP/PSK from *T. versicolor*, in post-operative cancer patients, focusing on gastric, colorectal, and esophageal cancers. Databases included PubMed, Embase, and Cochrane Library, supplemented by evidence-based review summaries from the Memorial Sloan Kettering Cancer Center,[1] the Whole Health Library from the United States Department of Veterans Affairs,[2] and the Physician Data Query summary from the National Cancer Institute.[3] Keywords included: "*Trametes versicolor*," "PSP," "PSK," "immunochemotherapy," "cancer," "gastric cancer," "colorectal cancer," "esophageal cancer," "post-operative," and "randomized controlled trial."

1.2.2. Inclusion and exclusion criteria

Studies were included if they met the following criteria:

- RCTs evaluating PSP/PSK as an adjuvant to standard chemotherapy or radiotherapy in post-operative patients (post-curative resection of the primary tumor)
- With OS as an endpoint
- Included a control group receiving chemotherapy or radiotherapy alone.

Studies were excluded if they:

- Did not report OS
- Were not RCTs
- Did not include a control group.

The final inclusion criteria are reflected in Table S1.

1.2.3. Data extraction

Data were extracted on study design, cancer type, sample size, treatment arms (PSP/PSK + chemotherapy/radiotherapy vs. chemotherapy/radiotherapy alone), and 5-year OS rates. For studies with incomplete group size data, an equal (half-and-half) partition was assumed to estimate survival RR and 95% CI. Extracted studies included Niimoto *et al.*,[4] Torisu *et al.*,[5] Mitomi *et al.*,[6] Nakazato *et al.*,[7] Ogoshi *et al.*,[8] Toge *et al.*,[9] Ito *et al.*,[10] Ohwada *et al.*,[11] Akagi *et al.*,[12] Shichinohe *et al.*,[13] and Ogawa et al.[14]

1.2.4. Quality assessment

A quality assessment was intended using the Cochrane Risk of Bias Tool, which would have evaluated random

Table S1. Detailed meta-analysis data

Study ID	Risk ratio (RR)	95% CI	ln (RR)	SE	Weight (w_i)	Patients
Torisu *et al.*[5]	1.47	0.94–2.31	0.38526240	0.22936809	19.00789345	111
Mitomi *et al.*[6]	1.12	1.01–1.26	0.11332869	0.05641872	314.16189795	448
Nakazato *et al.*[7]	1.22	1.02–1.45	0.19885086	0.08973493	124.18722837	262
Ogoshi *et al.* (RT+PSK)[8]	1.06	0.64–1.75	0.05826891	0.25660788	15.18658138	87
Ogoshi *et al.* (RT+CT+PSK)[8]	1.28	0.70–2.34	0.24686008	0.30786374	10.55073971	87
Toge and Yamaguchi[9]	1.10	0.99–1.22	0.09531018	0.05329112	352.11967071	751
Ito *et al.*[10]	1.03	0.95–1.13	0.02955880	0.04426299	510.40917595	446
Ohwada *et al.*[11]	1.13	0.96–1.34	0.12221763	0.08507439	138.16635904	205
Subtotal (pooled)	1.10	1.04–1.15	0.09209178	0.02596055	1,483.78954656	2,397

sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. However, this assessment could not be performed due to a lack of access to the complete published articles or reports for all 11 RCTs listed in the meta-analysis. Access to supplementary materials or trial protocols, which may contain critical details, was also unavailable. As a result, the inclusion of studies was based solely on the reported 5-year OS data and study design criteria, without formal risk of bias evaluation.

1.2.5. Statistical analysis

The meta-analysis estimated the pooled RR of 5-year OS, comparing PSP/PSK + chemotherapy/radiotherapy (p_1) to chemotherapy/radiotherapy alone (p_2). The RR was calculated as:

$$RR = \frac{p_1}{p_2} \quad \text{(I)}$$

Where $RR > 1$ favors PSP/PSK. The 95% CI was derived using the delta method approximation for the log-transformed RR:

$$Var\left(ln\left(p\right)\right) \approx \frac{1-p}{np}, Var\left(ln\left(RR\right)\right) = \frac{1-p_1}{n_1 p_1} + \frac{1-p_2}{n_2 p_2} \quad \text{(II)}$$

$$SE\left(ln\left(RR\right)\right) = \sqrt{Var\left(ln\left(RR\right)\right)} \quad \text{(III)}$$

$$95\%\,\text{CI} = \left(e^{ln(RR)-1.96\times SE\left(ln(RR)\right)}, e^{ln(RR)+1.96\times SE\left(ln(RR)\right)}\right) \quad \text{(IV)}$$

The pooled RR was calculated using a fixed-effect model, with weights (w_i) as the inverse of the variance:

$$w_i = \frac{1}{Var\left(ln\left(RR\right)\right)} = \frac{1}{SE^2} \quad \text{(V)}$$

$$ln\left(RR_{pooled}\right) = \frac{\sum w_i ln\left(RR_i\right)}{\sum w_i} \quad \text{(VI)}$$

Heterogeneity was assessed using Cochran's Q statistic:

$$Q = \sum w_i \left(ln\left(RR_i\right) - ln\left(RR_{pooled}\right)\right)^2 \quad \text{(VII)}$$

Between-study variance (τ^2) was estimated as:

$$\tau^2 = \frac{Q-\left(k-1\right)}{\sum w_i - \frac{\sum w_i^2}{\sum w_i}} \quad \text{(VIII)}$$

(set to 0 if $Q < k - 1$). The I^2 statistic quantified heterogeneity:

$$I^2 = \frac{Q-\left(k-1\right)}{Q} \times 100\% \quad \text{(IX)}$$

The standard error of the pooled estimate was:

$$\text{SE}_{ln\left(RR_{pooled}\right)} = \sqrt{\frac{1}{\sum w_i}} \quad \text{(X)}$$

The 95% CI for the pooled RR was:

$$\ln\left(RR_{pooled}\right) \pm 1.96 \times \text{SE}_{ln\left(RR_{pooled}\right)} \quad \text{(XI)}$$

All calculations were performed directly using the above equations.

1.2.6. Relevant studies not evaluated in the meta-analysis

Among the relevant studies excluded from the meta-analysis, Tsang *et al.*[15] investigated the effects of PSP on patients with advanced non-small cell lung cancer (NSCLC). Conducted as a double-blind, placebo-controlled trial, it involved 34 patients who had completed conventional treatment. After 28 days of PSP administration, results showed significant improvements in blood leukocyte and neutrophil counts, serum immunoglobulin (Ig) G and IgM levels, and body fat percentage in the PSP group compared to the control group. Notably, fewer PSP patients withdrew from the study due to disease progression (5.9% vs. 23.5% in controls). No adverse reactions were reported. The findings suggest that PSP may slow the progression of advanced NSCLC, although it did not improve NSCLC-related symptoms. Miyake *et al.*[16] in a phase III trial compared oral adjuvant uracil and tegafur plus protein-bound polysaccharide K (UFT/PSK) to uracil and tegafur plus leucovorin (UFT/LV) in patients with stage IIB and III colorectal cancer. The study aimed to demonstrate the non-inferiority of UFT/PSK in terms of 3-year disease-free survival (DFS). A total of 357 patients were randomized, revealing a 3-year DFS of 82.3% for UFT/LV and 72.1% for UFT/PSK, with a difference of −9.06% (90% CI: −17.06% to −1.06%), failing to meet the non-inferiority margin. OS rates were 95.4% for UFT/LV and 90.7% for UFT/PSK. While UFT/PSK was not non-inferior to UFT/LV, the study was not designed to definitively establish the superiority of UFT/LV, leaving its statistical superiority unconfirmed in this context.

1.3. Results

1.3.1. Study characteristics

Eleven RCTs were evaluated, with seven studies included in the meta-analysis based on their focus on 5-year OS in post-operative patients (post-curative resection of the primary tumor) with gastric, colorectal, or esophageal cancer. Study details are summarized in Table S1. Excluded studies (e.g., Akagi *et al.*,[12] Shichinohe *et al.*,[13] Ogawa *et al.*,[14]) did not meet the 5-year OS endpoint criterion, or it could not be determined from the publicly available data.

1.3.2. Statistical results

The pooled RR for 5-year OS was 1.10 (95% CI: 1.04–1.15), indicating a survival benefit for post-operative PSP/PSK + chemotherapy/radiotherapy compared to chemotherapy/radiotherapy alone. Individual study data, including RR, 95% CI, natural log of RR (ln[RR]), standard error ($SE=\frac{\ln(\text{Upper CI})-\ln(\text{Lower CI})}{2\times1.96}$), and weights ($w_i = 1/SE^2$), are presented in Table S1. The fixed-effect model yielded a pooled ln(RR) of 0.09209178, with a standard error of 0.02596055. A forest plot visualizing these results is presented in Figure 1.

The weights are allocated based on the inverse of the variance of the log-transformed RR ($1/SE^2$), reflecting the precision of each study's estimate. Studies with smaller standard errors (e.g., study by Ito *et al.*[10] with an SE of 0.04426299 and a weight of 510.40917595) contribute more to the pooled estimate due to their higher precision, while those with larger standard errors (e.g., study by Ogoshi *et al.*[8] (RT + CT + PSK) with an SE of 0.30786374 and a weight of 10.55073971) contribute less. This weighting ensures that the meta-analysis prioritizes more reliable data.

The heterogeneity test Q statistic (Q = 5.585853; p=0.592) assesses the variation between study results beyond chance. A non-significant p-value (>0.05) indicates no evidence of heterogeneity, suggesting that the effect sizes are consistent across studies. The I^2 of 0% and τ^2 of 0 further reinforce homogeneity, indicating that all variability is due to sampling error rather than true differences between RCTs. Clinically, this homogeneity supports the generalizability of the pooled RR (1.10) across the included post-operative cancer cohorts, although the lack of heterogeneity does not preclude potential subgroup differences that could be explored in future analyses.

Section S2

2. Analysis of silymarin's potential as a hepatoprotector and antimetastatic agent

Silymarin, a flavonoid derived from milk thistle (*Silybum marianum*), has been extensively investigated for its therapeutic potential in supporting liver health. Both silymarin and certain mushroom-derived bioactive compounds possess anticancer, immunomodulatory, and tissue-protective properties through distinct yet potentially complementary mechanisms. Exploring their combined use in metastatic colorectal cancer may offer opportunities to enhance therapeutic efficacy while limiting treatment-associated toxicity. This note provides a comprehensive examination of whether silymarin can prevent hepatic metastasis while simultaneously protecting the liver from chemotherapy-induced toxicity in healthy cells, without compromising the chemotherapeutic efficacy against malignant cells. The analysis is grounded in a review of recent literature, encompassing *in vitro, in vivo,* and clinical studies.

2.1. Background on silymarin

Silymarin is a complex of flavonolignans, including silybin, also known as silibinin, silidianin, isosilybin, and silychristin, with silybin identified as the most bioactive

constituent. Historically, it has been employed to safeguard the liver against conditions, such as alcoholic cirrhosis and toxin-induced hepatic damage. Recent research has expanded its scope to include applications in malignant diseases, particularly those affecting the gastrointestinal system, due to its antiproliferative, anti-inflammatory, and antimetastatic properties.[17]

2.2. Potential to prevent hepatic metastasis

Studies suggest that silymarin exhibits antimetastatic activity by modulating tumor characteristics, including cell cycle arrest, apoptosis, and angiogenesis. For example, research on malignant colon cell lines (e.g., LoVo and DLD-1) demonstrated that silymarin inhibits cellular proliferation and induces apoptosis, potentially reducing metastatic burden.[18] Furthermore, its antiangiogenic effects—evidenced by the suppression of endothelial cell migration and capillary tube formation—may restrict the vascular support required for metastasis.[19] Although specific studies on the prevention of colorectal-derived hepatic metastasis in animal models remain limited, several investigations highlight silymarin's broader antimetastatic effects.[20]

2.3. Protection of the liver against chemotherapy-induced toxicity in healthy cells

Chemotherapy frequently induces hepatotoxicity, impairing liver function. Silymarin's hepatoprotective properties are well-documented, with pre-clinical and clinical data indicating its capacity to mitigate oxidative stress and shield hepatocytes from cytotoxic damage. It has been shown to modulate Phase I and Phase II xenobiotic-metabolizing enzymes in a manner that both protects healthy cells from chemotherapy-induced toxicity and fosters chemosensitivity. At the same time, the concentrations of silybin following the consumption of milk thistle extract are insufficient to have a significant impact on P450 function.[21] An RCT in breast cancer patients demonstrated silymarin's ability to lower hepatic enzyme levels (e.g., bilirubin and alkaline phosphatase) during treatment, suggesting potential benefits for individuals with colon cancer.[22] This protective effect is attributed to its antioxidant and anti-inflammatory actions that may preserve healthy hepatocytes during therapy. In leukemia patients undergoing chemotherapy, silymarin (420 mg/day) combined with diammonium glycyrrhizinate reduced hepatic enzyme levels and exhibited less drug-induced liver injury compared to the control group receiving only glycyrrhizinate, indicating its safety and utility in this context.[23,24] Similar findings were observed in breast cancer patients in a randomized, triple blind, placebo-controlled clinical trial of 30 patients who received doxorubicin/cyclophosphamide–paclitaxel chemotherapy. In the silymarin group, the grade of hepatic involvement assessed through ultrasonography significantly decreased following the intervention (p=0.012).[25,26]

2.4. Non-inhibition of chemotherapy effects on metastatic cells

A critical consideration is ensuring that silymarin does not shield metastatic tumor cells from chemotherapy, an effect that may diminish treatment efficacy. Research suggests that silymarin may enhance, rather than inhibit, chemotherapeutic effects. Studies combining silymarin with doxorubicin or paclitaxel in colon tumor cell lines (e.g., LoVo and LoVo/DX) revealed synergistic antiproliferative activity, particularly in non-multidrug-resistant cells, indicating that it does not confer protection to tumor cells and may, in fact, enhance their sensitivity to chemotherapy.[27] This is supported by evidence that silymarin inhibits ATP-binding cassette transporters, counteracting chemoresistance, and enhances apoptosis in tumor cells.[21] Consequently, silymarin appears to protect healthy hepatocytes while maintaining or improving chemotherapy's effectiveness against metastatic cells. In addition, at therapeutic doses, it exhibits no significant interactions with CYP450 enzymes, minimizing the risk of drug–herb interference and ensuring compatibility with treatment.[24] It may also enhance chemosensitivity by modulating antiapoptotic pathways.[21,25]

2.5. Conclusion

Based on the present research, silymarin demonstrates potential in preventing hepatic metastasis in colon cancer through its antiproliferative, apoptotic, and antiangiogenic effects. It also effectively protects the liver from chemotherapy-induced toxicity in healthy cells, as evidenced by its hepatoprotective properties in clinical trials. Moreover, rather than inhibiting chemotherapy, silymarin may potentiate its effects on metastatic cells, offering a dual strategy in cancer management.

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