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Conflict of interest
The authors declare that they have no conflict of interest.

Abstract

Tumour progression allows for aberrant angiogenesis. Consequently, cancer-associated thrombosis is a prevalent complication that is coupled with poor prognosis. Anticoagulants have therefore been prescribed with chemotherapeutic agents to target potential thrombo-embolic risk. A systematic review was carried out to summarise existing evidence on the interactions between anticoagulants and oral cancer. This treatment paradigm has demonstrated beneficial results in some oncology patients, thus associating anticoagulants with anticancer effects. Increasing prevalence of oral cancer presents a need to source alternative therapeutic means to prevent disease progression, and thus the use of anticoagulants in these patients may provide an avenue

for this to occur. The paucity of evidence regarding the interactions between oral squamous cell carcinoma and anticoagulants emphasises the urgency with which further research should be conducted.

Keywords: anticoagulants, warfarin, heparin, anticancer agents, oral cancer

Introduction

Oral squamous cell carcinoma (OSCC) is the most common epithelial cancer of the head and neck. Current treatment strategies for OSCC include surgery, radiation therapy and adjuvant therapy, the latter involving chemotherapeutic agents among which 5-FU and cisplatin are the most commonly used.

There is mounting evidence to suggest that several components of the coagulation system directly affect carcinogenesis. In particular, angiogenic processes are favoured in carcinogenesis, owing to certain cell-to-cell interactions, localised hypoxia, and the expression of certain cytokines and growth factors [1]. Because of the links between coagulation, cancer biology and prognosis, interest has grown in the potential benefit of anticoagulants, for the prevention or treatment of cancer.

Anticoagulant use has been steadily increasing globally [2]. Their use is of particular concern to cancer patients, and it is important to elucidate their potential effects on tumour biology and

interactions with chemotherapeutics. The most commonly prescribed anticoagulant is warfarin, a vitamin K antagonist (VKA) [3], followed by Heparin. New oral anticoagulants (NOACs) such as rivaroxaban, dabigatran, apixaban are now available [3]. Studies have shown that low-molecular-weight heparin (LMWH) and warfarin interfere with cancer cell growth and development, inhibit angiogenesis, and alter host-immune responses [4,5]. In contrast, drug interactions between anticancer drugs and NOACs are still not fully understood.

This systematic review sought to examine the hypothesis that anticoagulants may affect the invasive phenotype of OSCC and the antineoplastic properties of common chemotherapeutic agents used in its treatment.

Specific questions addressed included:

1. Do anticoagulants have anti-neoplastic effects on OSCC cells?
2. Is there a pharmacodynamic interaction between anticoagulants and anticancer chemotherapeutic agents with an effect on prognosis/outcome of OSCC?

Methods

This systematic review was collated according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting standards .

A comprehensive search of electronic databases (MEDLINE/EMBASE/EBM REVIEWS/ Web of Science) was conducted on 24 April 2020 (**Table S1)**.

Only studies written in English discussing OSCC were included, while no restrictions were placed on the date of publication. Retracted studies, review articles, commentaries, conference abstracts, opinion articles, letters to the editor were excluded.

Results were imported and compiled into Microsoft Excel. Duplicates were removed manually by two blind reviewers (L.M. & J.L.), and screened by title. Included papers were screened by abstract. Three reviewers (Z.L., K.M. & L.R.L.) individually screened the remaining

full-text articles. Inter-rater reliability was calculated using Fleiss' kappa. All discrepancies were discussed and resolved. Data extracted from each paper is displayed in **Table 1**.

Results

The initial search strategy yielded 2882 citations (**Figure 1**). 407 duplicates were removed, and 2475 articles were selected for title screening (Cohen's kappa: 0.8734, C.I. 95% = 0.8532-0.8947). 2224 articles were removed during title screening and 251 articles were selected for abstract screening (Cohen's kappa: 0.8600, C.I. 95% = 0.7913-0.9287). Of the 79 articles selected for full-text screening, 1 article was eligible for further data extraction (Fleiss' kappa: 0.6535, C.I. 95% = 0.1794-1.1276).

Only the single study by Ueda et al. [6] was deemed suitable for review. This study investigated the effects of unfractionated heparin (UFH) on cell growth and apoptosis in four OSCC cell lines, and the mechanisms underlying its actions.

Discussion

Investigating the effects of anticoagulants on OSCC appears to be novel, with Ueda et al. [6] the sole study exploring their consequences on OSCC cell lines. The main findings of this study are highlighted in **Table 1**. Importantly, a significant reduction in cell viability was noted alongside an increase in apoptosis; suppression of the AKt signalling pathway led to heparin-induced apoptosis, caused an increase in cisplatin sensitivity, and reversed drug resistance, thus demonstrating heparin's potential as a chemotherapy sensitiser. Ueda et al. [6] emphasises the need for more clinical intervention studies using dual heparin, in order to extend and apply their results in a clinical setting as a potential adjunct in human cancer treatment.

Conversely, the role of heparin on other epithelial cancers is more established. Heparin induces over-expression of Thioredoxin Interacting Protein, which represses proliferation of human hepatocellular carcinoma (HCC) [7]. Heparin can inhibit c-Myc signalling in vascular smooth muscle cells [8], suggesting that heparin may act as an anticancer molecule.

Heparin has been shown to decrease the invasive phenotype and metastasis of multiple cancers, including pancreatic cancer [9] and HCC, the latter via inhibition of c-Met pathway [10]. Activation of c-Met is critical for enhanced OSCC proliferation, invasion and metastasis; these factors correlate with decreased survival, increased recurrence rates and poor patient prognosis. Hence, the anti-metastatic properties of heparin should be evaluated in relation to OSCC treatment.

When used as an adjunct to cisplatin, heparin significantly increases OSCC cell apoptosis, possibly attributable to the ability of heparin to inhibit AKt signalling [6]. This poses the possibility of using heparin as a chemotherapeutic adjunct in managing OSCC patients that are deemed to have a heparin-sensitive phenotype.

While there is no study investigating the effect of warfarin on OSCC, McCulloch and George [11] highlight a potential influence of warfarin on the Gas-6-dependent Axl signalling pathway. Regulation of tumorigenesis and cancer invasiveness via this pathway has been demonstrated in OSCC [12]. This highlights the potential for further research into the antineoplastic effects of warfarin in OSCC, focussing on the Gas-6-dependent Axl pathway.

There are opposing results about the effectiveness of NOAC use in cancer treatment. Rivaroxaban and dabigatran failed to show statistically significant inhibition of tumourigenesis and tumour metastasis in breast cancer patients [13]. Conversely, pancreatic tumours showed a reduction in growth when treated *in vivo* with apixaban [14]. This contrast in antineoplastic activity for different cancer types suggests that further research is necessary for NOACs, particularly in the field of oral oncology.

Conclusion

This systematic review found that there is a significant dearth of information surrounding interactions between anticoagulant therapy and OSCC. However, there is sufficient evidence to suggest there may be a positive effect of anticoagulant therapy on OSCC treatment. The general

paucity of information combined with the anticancer potential of anticoagulants suggests the importance of further research in this field.

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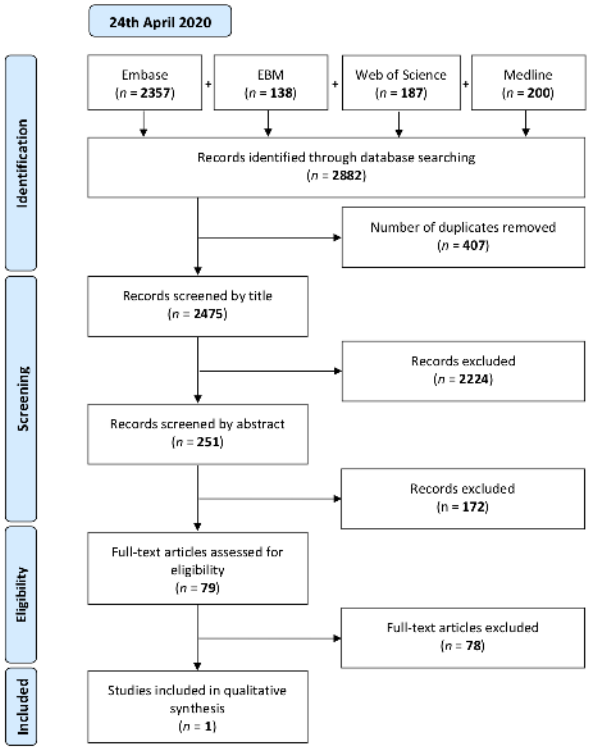
Figure Legends:

Fig. 1: Selection of studies for the systematic review of the effects of anticoagulants on OSCC

Table 1. Findings from Ueda et al. (19).

Aim/s of study	Investigate the effects and mechanisms of unfractionated heparin (UFH) on cell growth and apoptosis in four OSCC cell lines.
Experimental Model	In vitro
Methods	Cell culture, MTT assay, apoptosis assay, cell fractionation, western blotting
Anticoagulant	UFH
Chemotherapeutic agents	Cisplatin, Ly294002 of AKt/PI3-I inhibitor
Cell Lines	Human OSCC cell lines of HSC-4, KOSC2, KON and HO-N1 with varying heparin sensitivities
Pathway/s	AKt (AKt 1 [Ser473] and AKt 2 [Thr308]), BAD, Bcl-xL, Cytochrome C, pro- and cleaved caspase 9 and β-actin
Major findings	1. UFH induces cell apoptosis by targeting the AKt-BAD pathway. 2. Inactivation of Akt inactivates PI3K, causing dephosphorylation of BCL-2-associated agonist of cell death (BAD) protein and binding of Bcl-xL, thereby activating the mitochondria caspase cascade and resulting in cell apoptosis. 3. Clinically available concentrations (20 U/ml) of UFH significantly reduced cell viability in heparin-sensitive cancer cell lines. 4. UFH enhanced cytotoxicity of cisplatin, with greater apoptosis occurring in cell lines treated with combined cisplatin and UFH than with cisplatin alone.



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