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Research paper

Development of disulfiram-loaded vaginal rings for the localised treatment of cervical cancer

Peter Boyd [a], Ian Major [b], Weiguang Wang [c], Christopher McConville [d,*]

[a] School of Pharmacy, Medical Biology Centre, Queen's University of Belfast, Belfast, UK
[b] Materials Research Institute, Athlone Institute of Technology, Westmeath, Ireland
[c] Research Institute in Healthcare Science, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK
[d] Department of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, UK

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ABSTRACT

Cervical cancer is the third most prevalent cancer in women and disproportionately affects those in low resource settings due to limited programs for screening and prevention. In the developed world treatment for the disease in the non-metastasised state usually takes the form of surgical intervention and/or radiotherapy. In the developing world such techniques are less widely available. This paper describes the development of an intravaginal ring for the localised delivery of a chemotherapeutic drug to the cervix that has the potential to reduce the need for surgical intervention and will also provide a novel anti-cancer therapy for women in low resource settings. Disulfiram has demonstrated antineoplastic action against prostate, breast and lung cancer. Both PEVA and silicone elastomer were investigated for suitability as materials in the manufacture of DSF eluting intravaginal rings. DSF inhibited the curing process of the silicone elastomer, therefore PEVA was chosen as the material to manufacture the DSF-loaded vaginal rings. The vaginal rings had an excellent content uniformity while the DSF remained stable throughout the manufacturing process. Furthermore, the rings provided diffusion controlled release of DSF at levels well in excess of the IC50 value for the HeLa cervical cancer cell line.

1. Introduction

Cervical cancer is the third most prevalent cancer in women, with 529,000 new cases diagnosed each year of which 275,000 result in death. 85% of new cases occur in developing countries due to a lack of cervical cancer prevention and screening programs. Where as in developed countries, where women have access to resources capable of detecting and treating precancerous lesions, the number of cases is reduced by approximately 80% [1]. Sexual transmission of the human papillomavirus (HPV) is the main cause of cervical cancer, with 15 types of HPV being classed as carcinogenic or high risk. Types 16 and 18 are the most carcinogenic and are the main contributors to cervical cancer [2] and persistent HPV infections have the potential of causing the development of precancerous lesions and cervical intraepithelial neoplasia (CIN) that may lead to cervical cancer [3].

How cervical cancer is treated will depend on the women's general health as well as the type, stage and grade of the cancer and usually involves a combination of surgery, chemotherapy and/or radiotherapy [4,5]. All of these treatment options are either very invasive and involve extended stays in or repeated visits to the hospital and in the case of chemo and radiotherapy can result in significant side-effects, reducing the patients overall quality of life during the treatment. The location of the cervix makes it easily accessible through the vagina and allows for non-invasive localised delivery of chemotherapeutic drugs offering a number of advantages over systemic administration such as direct delivery to the site of action resulting in a lower dose being required as well as a reduction in systemic side effects and increased drug stability as it remains in the delivery device until released [6].

The vagina has been used to deliver drugs for a range of clinical and research applications, including contraception, vaginal infections and HIV prevention, with many different vaginal formulations such as gels, creams, pessaries, suppositories rings, films

Abbreviations: CIN, cervical intraepithelial neoplasia; DDC, diethyldithiocarbamate; DSC, differential scanning calorimeter; DSF, disulfiram; HPV, human papillomavirus; IVRs, intravaginal rings; PEVA, poly ethylene vinyl acetate; ROS, reactive oxygen species; SDS, sodium dodecyl sulphate.

* Corresponding author. Department of Pharmacy, Faculty of Science and Engineering, University of Wolverhampton, Wulfrana Street, Wolverhampton WV1 1LY, UK. Tel.: +44 01902 322615.
E-mail address: C.Mcconville@wlv.ac.uk (C. McConville).

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and tablets available [7–15]. However, only a small number of these delivery systems have been investigated for the localised delivery of chemotherapeutic drugs to the cervix [16–20].

Intravaginal rings (IVRs) are torus-shaped drug delivery devices that are capable of providing controlled delivery of substances to the vagina for up to a period of 1–12 months where it slowly releases one or more drugs to provide either a local or systemic effect [21–24]. IVRs have already seen clinical and commercial success in contraception (Nuvaring®) [23,25,26] and oestrogen replacement therapy (Estring® and Femring®) [21,27]. Femring® and Estring® are both manufactured from silicone elastomer, whereas Nuvaring® is manufactured from ethylene–vinyl-acetate copolymer (EVA). The clinical and commercial success of these rings makes them an ideal candidate for the delivery of chemotherapeutic drugs to the cervix. The IVR overcomes many of the disadvantages associated with more traditional vaginal drug dosage forms, such as gels, tablets and pessaries, which are often messy, interfere with intercourse and are poorly retained within the vagina. However, the major advantage of the IVR is its ability and versatility in providing long-term, continuous release of drug(s) at constant pre-determined rates, thereby increasing cost-effectiveness, patient compliance and therapeutic efficacy. Furthermore the vaginal ring is user controlled and thus does not require minor surgery or a physician for it to be placed in the vagina.

Disulfiram (DSF), which is currently used for treating alcohol abuse, has shown potential anti-tumour activity by inducing apoptosis in some cell lines and reducing cell growth in certain tumours [28]. This anticancer effect has been demonstrated in prostate cancer, breast cancer, lung cancer, leukaemia and cervical adenocarcinoma [29–37] and has been shown to be copper (Cu) dependent [21,38,39] as Cu plays a crucial role in redox reactions and triggers the generation of reactive oxygen species (ROS) which induce apoptosis in human cells [40]. The transport of Cu into the cell is strictly mediated by the trans-membrane Cu transporter (Ctr115) and due to its strong divalent metal ion chelating properties, DSF can chelate Cu(II) forming a DSF/Cu complex which improves the transport of Cu into cancer cells. Furthermore, the DSF/Cu complex is a much stronger ROS inducer than Cu alone [41,42] and is also an inhibitor of ALDH [29], which is involved in detoxifying a wide range of aldehydes. Aldehyde accumulation induces lipid peroxidation and generation of highly reactive free radicals leading to protein and DNA cross-linking and cell death. ALDH also plays a critical role in scavenging ROS and reducing UV-induced oxidative stress [43]. Therefore, inhibition of ALDH by the DSF/Cu complex will result in ROS accumulation leading to apoptosis. Drug induced ROS accumulation is usually counterbalanced by the activation of NFκB, an anti-apoptotic factor inhibiting ROS and ROS-induced cytotoxicity [44]. However, DSF is also capable of inhibiting activity of NFκB [29]. The DSF/Cu complex has been shown to induce ROS and inhibit NFκB activity in brain, colon and breast cancer cell lines [29,45,46]. Furthermore, it has been demonstrated that DSF can potentiate the cytotoxic effect of other anticancer drugs and ionising radiation *in vitro* while protecting normal cells in the kidneys, gut and bone marrow *in vivo* thus having the potential to increase the therapeutic index of other anticancer treatments.

In this study we describe, for the first time, the development and characterisation of a DSF-loaded vaginal ring that has the potential to be used for the localised treatment of cervical cancer.

2. Materials and methods

2.1. Materials

The poly ethylene vinyl acetate (PEVA) copolymers Elvax 40 (40% vinyl acetate content) and Elvax 150 (32% vinyl acetate content) were purchased from Dupont (Delaware, USA). The tin catalysed silicone MED8-6382 was purchased from NuSil Technology (Carpinteria, CA). Stannous octoate, sodium dodecyl sulphate (SDS) and DSF were purchased from Sigma–Aldrich, (Dorset, UK). All were used as supplied.

2.2. Determination of the thermal stability of DSF

50 mg samples ($n = 4$) of DSF was weighed into separate vials. The vials were placed into an oven at various temperatures (60, 70, 80, 100, 120, 140 and 160 °C) where they remained for 5, 10, 15, 30, 45 or 60 min. After removal from the oven the contents of each vial was dissolved in 10 mL of ethanol and analysed using a DSF stability indicating HPLC method.

2.3. Shore-A hardness determination of post-cured DSF-loaded MED8-6382 silicone elastomers

10 g of MED8-6382 silicone elastomer containing various loadings (0, 1, 2, 3, 5 and 10% w/w) of DSF were manufactured by weighting the appropriate amounts of silicone and DSF into a sealed plastic container and speed mixing them for 30 s at 3500 rpm (SpeedMixer™ DAC 15FVZ-K, Synergy Devices). Varying amounts (0.5%, 1.0%, 1.5%, 2.5%, 5% and 10%) of stannous octoate catalyst was then added to the silicone elastomers, which were subsequently poured into 81 × 81 × 18 mm square moulds before being placed in an oven at 60 °C for 1 h. Upon removal from the oven the silicone elastomers were allowed to cool overnight when their shore-A hardness was measured at five different places along the surface using a HBA 100-0 Shore-A durometer hardness tester (SAUTER, Balingen, Germany).

2.4. Rheological evaluation of the curing rate of DSF-loaded MED8-6382 silicone elastomers

3 g of MED8-6382 silicone elastomer containing various loadings (1, 2 and 5% w/w) of DSF were manufactured by weighting the appropriate amounts of silicone and DSF into a sealed plastic container and speed mixing them for 30 s at 3500 rpm (SpeedMixerTM DAC 15FVZ-K, Synergy Devices). 2.5% w/w of stannous octoate catalyst was added to the silicone mix which was immediately placed onto the lower plate of a TA Instruments AR2000 Rheometer using a disposable plastic syringe. The upper 40 mm diameter crosshatch parallel plate was lowered to produce a gap between the plates of 1000 lm and the excess silicone mix removed before the oscillation experiment was begun. A stress of 15 Pa and a frequency of 1 Hz were selected based on previously published work [47] and used for the subsequent cure analysis. Samples were analysed at both 60 and 80 °C.

2.5. Manufacture of 5% w/w DSF-loaded PEVA vaginal rings

47.5 g of PEVA (either Elvax 40 or Elvax 150) was weighed into a sealed plastic container along with 2.5 g of DSF. The container was manually shaken for approximately 10 min to mix the PEVA and DSF. The Elvax 150/DSF active mix was compounded at 80 °C and 50 rpm screw speed using a Thermo Electron HAAKE minilab extruder, while the Elvax 40/DSF active mix was compounded at both 65 and 80 °C. The compounded PEVA/DSF active mixes were pelletised using a Thermo Scientific VARICUT pelletiser and compounded a second time. 5% w/w DSF-loaded PEVA vaginal rings were produced by feeding the pellets into a Babyplast 6/10P micro-moulding machine with a barrel temperature of either 65 or 80 °C (depending on the active mix) and a mould temperature of 40 °C. The mould temperature was used as a processing aid to ensure the polymer melt filled the mould cavity before freezing off.