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Original Article

A Randomised, Double Blind, Placebo-Controlled Pilot Study of Oral Artesunate Therapy for Colorectal Cancer☆,☆☆

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ABSTRACT

Background: Artesunate is an antimalarial agent with broad anti-cancer activity in *in vitro* and animal experiments and case reports. Artesunate has not been studied in rigorous clinical trials for anticancer effects.
Aim: To determine the anticancer effect and tolerability of oral artesunate in colorectal cancer (CRC).
Methods: This was a single centre, randomised, double-blind, placebo-controlled trial. Patients planned for curative resection of biopsy confirmed single primary site CRC were randomised (n = 23) by computer-generated code supplied in opaque envelopes to receive preoperatively either 14 daily doses of oral artesunate (200 mg; n = 12) or placebo (n = 11). The primary outcome measure was the proportion of tumour cells undergoing apoptosis (significant if >7% showed Tunel staining). Secondary immunohistochemical outcomes assessed these tumour markers: VEGF, EGFR, c-MYC, CD31, Ki67 and p53, and clinical responses.
Findings: 20 patients (artesunate = 9, placebo = 11) completed the trial per protocol. Randomization groups were comparable clinically and for tumour characteristics. Apoptosis in >7% of cells was seen in 67% and 55% of patients in artesunate and placebo groups, respectively. Using Bayesian analysis, the probabilities of an artesunate treatment effect reducing Ki67 and increasing CD31 expression were 0.89 and 0.79, respectively. During a median follow up of 42 months 1 patient in the artesunate and 6 patients in the placebo group developed recurrent CRC.
Interpretation: Artesunate has anti-proliferative properties in CRC and is generally well tolerated.

1. Introduction

Colorectal cancer (CRC) contributes 9–10% of the annual global cancer burden in men (746,000 cases) and women (614,000 cases) (Ferlay et al., 2012). In the UK, 110 new cases are diagnosed daily, with older patients particularly at risk of death (UK CR, 2014) and with >50% of newly diagnosed cases having locally advanced disease (T3/T4). Resection is the only curative treatment for non-metastatic CRC but this has to be combined with neo-adjuvant chemo- and/or radio-therapy, to downstage more advanced presentations.

Prognosis with best available treatments does not increase disease free or overall survival beyond ~60% at 5 years after diagnosis. For most patients, access to advanced treatment modalities is lacking, too expensive to be widely available, or associated with significant morbidity thereby further compromising their survival. There is therefore a continuing and urgent need to develop new, cheap, orally effective and safe CRC therapies. One approach is to study existing drugs that already have some anticancer properties in experimental settings, and to assess their safety and efficacy in *in vivo* studies.

Artesunate is derived from artemisinin, which is extracted from *Artemisia annua* L. and is a widely used antimalarial that can be administered by oral, rectal and parenteral routes (Gomes et al., 2009; Kremsner and Krishna, 2004; Kremsner et al., 2012; Nealon et al., 2002; Hien et al., 1994, 1992; Jiang et al., 1982). Soon after the isolation of artemisinin by a Chinese government's programme, the anticancer properties of artemisinins were first reported (Efferth et al., 2007;

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Krishna et al., 2008). Subsequently, many studies of artemisinins using *in vitro* and animal models have confirmed their remarkable capacity to exert broad anti-cancer effects (Efferth et al., 2007). They reduce cell proliferation and angiogenesis and trigger apoptosis (Anfosso et al., 2006; Efferth et al., 2001, 1996).

There have only been isolated case reports in humans of anti-cancer effects of artemisinins (reviewed Krishna et al., 2008). These include cases of metastatic uveal melanoma (Berger et al., 2005) laryngeal squamous cell carcinoma (Singh and Verma, 2002) and pituitary macroadenoma (Singh and Panwar, 2006). An open-label Chinese study treated non-small cell lung cancer patients and showed prolonged time to cancer progression compared with controls when artesunate was added to conventional treatment (Zhang et al., 2008), but no benefit on mortality. An open-label pilot study of patients receiving artesunate for advanced cervical cancer suggested that it was well tolerated and improved symptoms (Jansen et al., 2011). There has been a phase II trial on the activity of artesunate in non-resectable tumours of dogs (Rutteman et al., 2013) and efficacy of extracts of *A. annua* in 5 veterinary sarcomas (Breuer and Efferth, 2014). This study examines anti-CRC effects and tolerability of artesunate used as monotherapy in a rigorous study design.

2. Methods

2.1. Ethics

The trial was approved by Wandsworth Ethics Committee (Wandsworth UK, Ref: 08/H0803/3) and was registered (ISCRTN05203252).

2.2. Trial Design

This was a single-centre, double-blind, placebo-controlled trial with balanced randomisation of patients (1:1) conducted at the St George's University of London, UK and St. George's Healthcare NHS Trust.

2.3. Participants for Inclusion

Eligible participants were with biopsy confirmed single primary site colorectal adenocarcinoma; aged 21–90 years; with all stages amenable to surgical treatment and not requiring neoadjuvant treatment; with planned curative resection; and with written, informed consent.

2.4. Exclusion Criteria

These were: contraindication to use of artesunate due to hypersensitivity; pregnancy; history of hearing or balance problems; immunosuppression or concomitant medication known to interact with artesunate (see below); weight <50 kg or >100 kg; severe anaemia (haemoglobin <8 g/dL); other planned intervention, apart from standard of care; inability to give informed consent; inability or unwillingness to take effective contraception in women of child-bearing age; chronic kidney disease of NKF D/QOFI stage 3 or above (eGFR < 60 mL/min); bilirubin >2 of the upper limit of normal without haemolysis or known chronic liver disease.

2.5. Recruitment

Recruitment was at St George's Healthcare NHS Trust in London from 9 March 2009 to 15 October 2012.

2.6. Interventions

Patients received two weeks of experimental medication (artesunate or placebo) just before surgery and standard care. Artesunate (Arinate® 100 mg) was manufactured by Famar Italia S.p.A and matching placebo tablets were manufactured by MPF in The Netherlands under a manufacturing licence in accordance with EU cGMP certified by Dafra Pharma (Belgium). Study medication was packaged, labelled and certified by B&C CliniPack (Belgium) and was in pack sizes of 30 × 100 mg and was received, stored and dispensed by the Pharmacy at St George's Healthcare NHS Trust.

The dose of artesunate for the study was 200 mg orally, daily for fourteen days, with medication stopped 48–72 h prior to surgery.

Medication was provided in blister packs with one patient box provided 14 doses, sufficient for the duration of the study.

There was no delay in surgery if patients entered into this study, nor any other change in clinical management, and the 62 day rule (requiring treatment within this time period after confirmation of diagnosis) was strictly adhered to.

2.7. Outcomes

The primary endpoint of the trial was the presence or absence of significant apoptosis in the epithelial cells of the tumour specimen defined as >7% of cells with apoptotic features.

Secondary outcomes included seven immunohistochemical stains applied to the paraffin-embedded tumour specimens and quantified in both epithelial cells and fibroblasts: vascular endothelial growth factor (VEGF), c-MYC status and EGF-receptor status; microvessel density determining the quantity of the cluster of differentiation 31 (CD31) protein; proliferative activity assessed with Ki67 staining and p53 tumour suppressor protein expression. Each stain in each patient was generally evaluated in 6 microscopic areas with a semiautomatic system (in a few cases 7 or 8 areas were evaluated, and in some – especially for fibroblasts – measurements less than 6 or no areas could be evaluated).

2.8. Blood Samples

Three blood samples were taken: (1) at baseline, (2) after one week of medication (following protocol amendment for enhanced safety monitoring) and (3) after ending the two week medication (just before surgery). In each sample the safety measures included assay of potassium, sodium, creatinine, urea, albumin, alkaline phosphatase, ALT, bilirubin, haemoglobin, platelet count and white cell count. Carcinoembryonic antigen (CEA), was monitored where available in patients at baseline and after randomization.

2.9. Secondary Outcomes

These were measures of safety and tolerability (both clinical and laboratory) according to conventional criteria assessed by comparing baseline blood test results and those during or after treatment and anti-cancer efficacy (with markers described above).

2.9.1. Changes to Outcomes

There were no changes to predefined endpoints.

2.10. Sample Size

An indicative sample size calculation, given the pioneering nature of this pilot study, was carried out on the primary outcome before starting the trial based on the assumption that colorectal cancer is unlikely to exhibit significant apoptosis if untreated. Most patients in the placebo group (more than 95%) were anticipated to have less than 7% of cells with apoptotic features. The majority of patients (greater than 60%) in the artesunate group were anticipated to have significant apoptosis. This large difference was derived from published baseline estimates of apoptotic indices (Yamamoto et al., 1998; Ikenaga et al., 1996; Bendardaf et al., 2003). With equal group sizes a sample size of 2*11 was estimated to have 80% power and accepting a Type I error of 5% for superiority, bearing in mind that in most pilot studies the aim is to demonstrate proof-of-concept (Arain et al., 2010) rather than exclusively test a hypothesis.