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Data Article

Data describing the effects of the Macrolide Antibiotic Clarithromycin on preclinical mouse models of Colorectal Cancer

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ABSTRACT

Macrolide antibiotics, such as Clarithromycin (Cla), have been proven to exert anti-tumour activity in several preclinical models of different types of cancer. Cla can exert its anti-tumour effects through different mechanisms, e.g. by blocking the autophagic flux, inducing apoptosis or inhibiting tumour-induced angiogenesis. The clinical benefit of Cla in treating various tumours in combination with conventional treatment was confirmed in extensive clinical studies in patients suffering from non-small cell lung cancer, breast cancer, multiple myeloma and other haematological malignancies. Data regarding the anti-cancer effect of Cla on Colorectal Cancer (CRC) are still lacking. This article shares data on the *in vivo* efficacy of Cla in two xenograft models of CRC. Our results show that Cla treatment reduces tumour growth and increases the overall survival in CRC mouse xenograft models. Moreover, the Western blot analysis of autophagic and apoptotic markers suggests that the anti-tumour effects of Cla are related to a modulation of both cellular processes. The data suggest that it will worth consider Cla as treatment option for CRC patients.

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Specifications Table

Subject	Medicine
Specific subject area	Cancer research, Pharmacology and Toxicology
Type of data	Graph Figure Time course of tumour growth Overall survival Western blot
How data were acquired	Tumour growth was monitored by external measurement using calipers. WB images were acquired with an Epson 3200 scanner. Densitometric analysis was performed using ImageJ.
Data format	Raw and analysed data
Parameters for data collection	Subcutaneous experiments: mice were treated with 100 μ l of saline (Control) or Cla (15 mg kg ⁻¹) by oral gavage for 2 weeks daily, starting 1 week after cell inoculum. Experimental groups comprised: control group, n = 4 mice (n = 8 tumour masses); Cla-administered group, n = 5 mice (n = 10 tumour masses). Survival experiments: mice were treated as above. Each experimental group comprised n = 10 mice.
Description of data collection	Tumour growth was monitored by external measurement using calipers. The volume of tumour masses measured at sacrifice (3 weeks after inoculum) was calculated by applying the ellipsoid equation, while during the experiment it was estimated with the following equation: 0.5 x Length x Width ² . Whole protein lysates were obtained from a portion of excised tumour masses at sacrifice. Samples were homogenized in cold protein extraction buffer and sonicated for 30 min. Survival was monitored until death.
Data source location	Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy
Data accessibility	With the article

Value of the data

- The *in vivo* effect of Clarithromycin has been previously described for different cancer model, except for Colorectal Cancer.
- This research analysed the *in vivo* effect of Clarithromycin in two different xenograft models of Colorectal Cancer: with the subcutaneous model we provide new insights about the effect of the drug on both tumour growth and expression of autophagic and apoptotic proteins; with the intraperitoneal model we evaluated the overall survival and the toxicity of the drug.
- Valuable for researchers interested in the impact of Macrolide Antibiotics on Cancer treatment.

1. Data

The *in vivo* efficacy of Cla was evaluated in two preclinical CRC xenograft models, both employing human CRC cells: HCT116 cells xenografted subcutaneously, and LS174T cells injected intraperitoneally. In both cases, immunodeficient mice were used. Compared to the control group, the volumes of the subcutaneous tumours were significantly ($P = 0.0001$) reduced after 2 weeks of Cla treatment (15 mg Kg⁻¹, daily, by oral gavage) (Fig. 1A). The same treatment schedule produced a significant increase of the overall survival of LS174T-injected mice (Fig. 1B) ($P = 0.0355$), without inducing toxicity. No death or significant body weight change was observed in Cla-treatment group (Fig. 1C).

Two weeks after treatment with Cla, mice subcutaneously injected with HCT116 were sacrificed, and tumour masses were collected, in order to analyse the effects of the drug on the autophagic process studying the expression of two autophagic markers: (1) the conversion of the soluble form of the microtubule-associated protein 1-light chain 3 (LC3-I), to the lipidated autophagosome-associated form, LC3-II, and (2) the ubiquitin-binding protein p62/sequestome 1 (p62/SQSTM1) [1]. Compared to control, the LC3-II/LC3-I ratio was significantly increased and the p62/SQSTM1 levels were significantly decreased in Cla-treated tumours (Fig. 2A). Then we studied the activation of caspase 3 in tumour masses, as a marker of apoptosis induction [2]. Significant reduction in protein levels of procaspase 3, correlated to increase in cleaved (activated form) caspase 3, was detected in the tumour masses of Cla-treated group (Fig. 2A). Finally, phosphorylation of Erk 1/2 and Akt, pathways involved

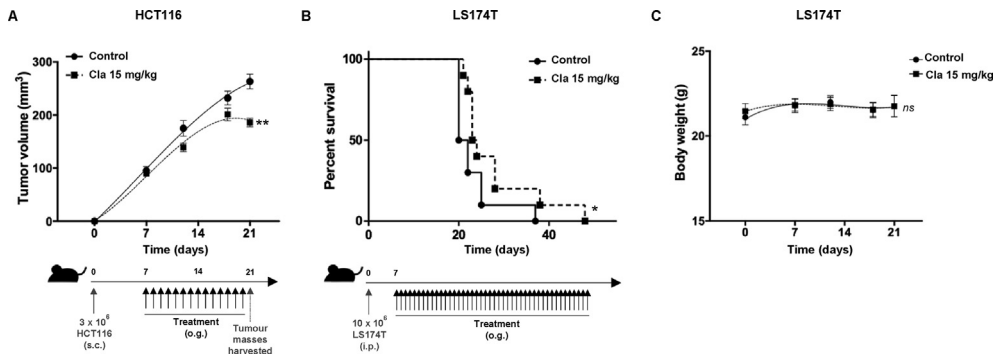


Fig. 1. Efficacy of Clarithromycin in two xenograft models of Colorectal Cancer. (A) Time course of tumour growth in control and Cla (15 mg Kg⁻¹) treated nude mice, subcutaneously (s.c.) injected with HCT116 cells (3×10^6 /flank). Seven days after the inoculum, mice were treated with saline (control) and Cla. Cla was daily administered by oral gavage (o.g.) for 14 days, as reported in the schematic representation of treatment regime. Data presented as mean \pm SEM of 8 masses for control group ($n = 4$ mice) and 10 masses for Cla group ($n = 5$ mice). **, $P = 0.0001$ (Student's t-test). (B) Nude athimic mice were injected with LS174T cells (10×10^6 cells i.p.) and starting from day 7, animals were treated daily with saline (control, $n = 10$) and Cla (15 mg kg⁻¹, by o.g., $n = 10$), as reported in the schematic representation of treatment regime. Survival curves of each experimental group, estimated by Kaplan and Meier analysis are reported. *, $P = 0.0355$ (Gehan-Breslow-Wilcoxon Test). (C) Body weight of LS174T xenograft-bearing nude mice, treated as in (B). Data presented as mean \pm SEM ($n = 10$ mice for each treatment group). *ns* = not significant (Student's t-test).

both in the modulation of autophagic [3–6] and apoptotic processes [6,7], was significantly inhibited by Cla treatment (Fig. 2B).

2. Experimental design, materials, and methods

2.1. In vivo tumour xenograft models

Mice experiments were performed at the Laboratory of Genetic Engineering for the Production of Animal Models (LIGeMA) at the Animal House of the University of Florence (Ce.S.A.L.). Mice were housed in filter-top cages with a 12-h dark-light cycle, and had unlimited access to food and water. Procedures were conducted according to the laws for experiments on live animals (Directive 2010/63/EU), and approved by the Italian Ministry of Health (authorization n° 114/2016-PR and n° 1279/2015-PR).

For subcutaneous xenografts, female nude mice (Envigo Laboratories) aged five to six weeks were injected subcutaneously in either flanks with 3×10^6 HCT116 cells, resuspended in 100 μ l complete medium. After cell inoculation, mice were monitored daily to ensure they did not show any signs of suffering or disease (such as weight loss, abdominal distension, impaired movement, edema in the injection area). Mice were treated with 100 μ l of saline or Cla (15 mg kg⁻¹) by oral gavage (o.g.) for two weeks daily, starting one week after inoculation. Experimental groups comprised: control group, $n = 4$ mice ($n = 8$ tumour masses); Cla-administered group, $n = 5$ mice ($n = 10$ tumour masses). Tumour growth was monitored by external measurement using calipers. The volume of tumour masses measured at sacrifice (3 weeks after inoculum) was calculated by applying the ellipsoid equation, while during the experiment it was estimated with the following equation: $0.5 \times \text{Length} \times \text{Width}^2$.

For survival experiments, female nude mice aged five to six weeks were injected intraperitoneally (i.p.) with 10^7 LS174T cells, resuspended in 100 μ l EMEM plus 20% Matrigel (20 mg/ml). Mice were treated with 100 μ l of saline or Cla (15 mg kg⁻¹) by o.g. daily, starting one week after inoculation, and survival was monitored until death. Each experimental group comprised $n = 10$ mice.

2.2. Western blot (WB) analysis

For tumour lysates, whole protein lysates were obtained from a portion of excised tumour masses from mice injected with HCT116 cells. Samples were homogenized in cold protein extraction buffer (1X Cell Lysis Buffer; Cell Signaling Technology, #9803) and sonicated for 30 min.

HCT116 Tumour Masses

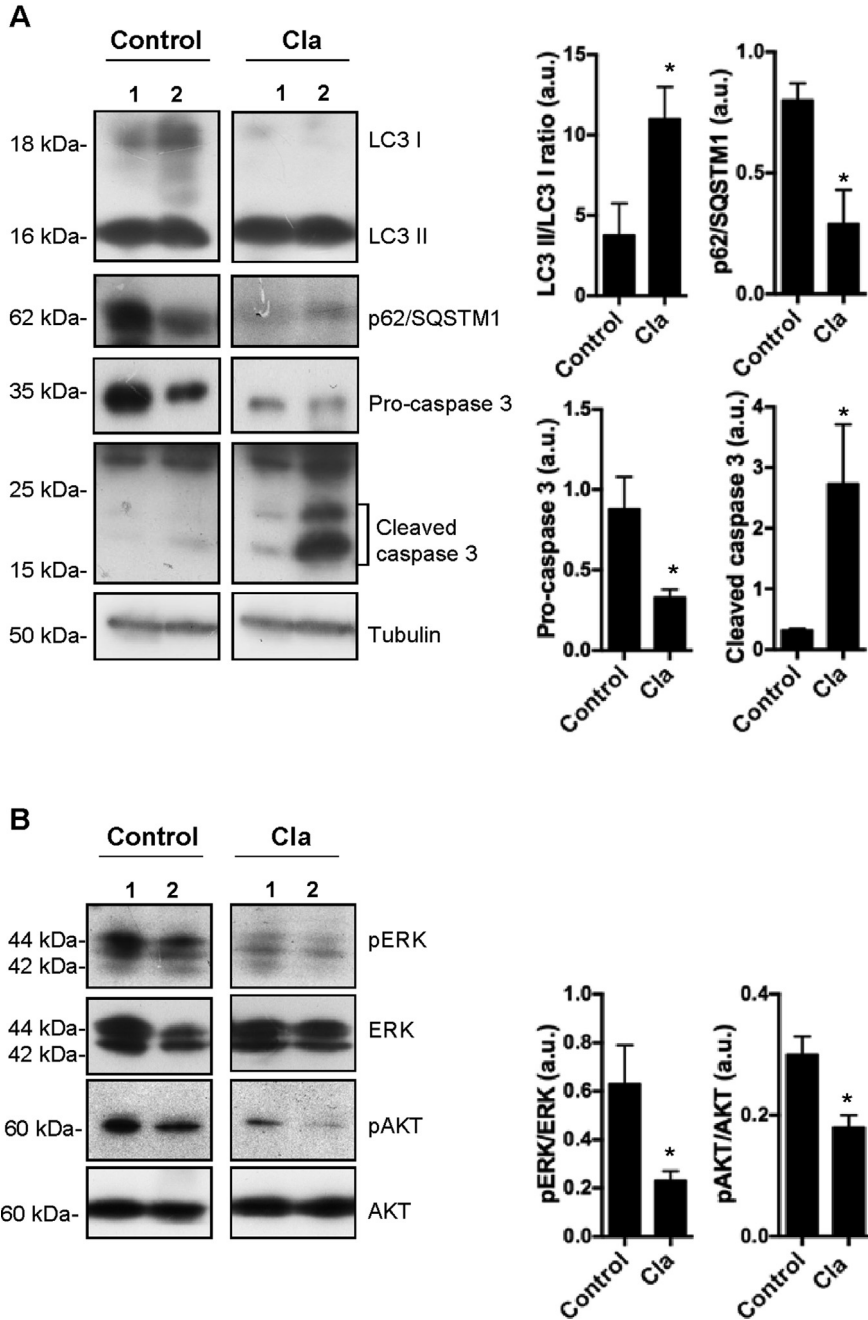


Fig. 2. Clarithromycin-induced modulation of markers of autophagy and apoptosis in tumour masses. (A-B) WB analysis of the protein levels of phospho-ERK1/2^{Thr202/Tyr204}, phospho-Akt^{Thr308}, pro-caspase 3, cleaved caspase 3, LC3 and p62/SQSTM1 in the total lysates of HCT116 tumour xenografts of mice treated as in (Fig. 1A). The membranes were reprobed with anti-ERK1/2, anti-Akt or anti-tubulin antibodies. The corresponding densitometric results are given in the bar graphs; data shown as mean \pm SEM ($n = 4$ different tumour masses for each treatment group). *, $P < 0.05$ (Student's t-test).

The following primary antibodies were used for WBs, at the indicated concentrations. The rabbit pAb against LC3A/B (#4108; dilution 1:1000), the rabbit mAb against SQSTM1/p62 (clone D5E2, #8025; dilution 1:1000), the rabbit mAb against Caspase 3 (clone 8G10, #9665; dilution 1:1000), and the rabbit pAb against phospho-44/42 MAPK (Erk1/2) (Thr202/Tyr204, #9101; dilution 1:1000) were purchased from Cell Signaling Technology. The mouse mAb against phospho-AKT1/2/3 (clone B-5, sc-271966; dilution 1:500), the rabbit pAb against AKT1/2/3 (clone H-136, sc8312; dilution 1:500), and the rabbit pAb against ERK1/2 (clone H-72, sc-292838; dilution 1:200) were purchased from Santa Cruz Biotechnology. The anti- α -tubulin mouse mAb (T9026; dilution 1:500) was used as loading control. Anti-rabbit IgG peroxidase-conjugated (A0545; dilution 1:10000) and anti-mouse IgG peroxidase antibodies (A4416; dilution 1:5000) were used as secondary antibodies.

WB images were acquired with an Epson 3200 scanner. Densitometric analysis was performed using ImageJ on two different scans, after background subtraction, from at least three different experiments, as previously described [8].

2.3. Statistical analysis

Data are given as mean values \pm standard error of the mean (SEM). The number of independent experiments is indicated in the Figure legends as *n*. Statistical comparisons were performed with GraphPad Prism 6.0e (GraphPad Software, San Diego, USA). Unless otherwise indicated, for comparisons between Cla-treated and Control group, we used Student's t-test and *P* values are reported as *, *P* < 0.05.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2019.104406>.

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