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Review

Time to use a dose of Chloroquine as an adjuvant to anti-cancer chemotherapies

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ABSTRACT

Chloroquine, a drug used for over 80 years to treat and prevent malaria and, more recently, to treat autoimmune diseases, is very safe but has a plethora of dose-dependent effects. By increasing pH in acidic compartments it inhibits for example lysosomal enzymes. In the context of cancer, Chloroquine was found to have direct effects on different types of malignancies that could potentiate chemotherapies. For example, the anti-malaria drug may inhibit both the multidrug-resistance pump and autophagy (mechanisms that tumor cells may use to resist chemotherapies), intercalate in DNA and enhance the penetration of chemotherapeutic drugs in cells or solid cancer tissues. However, these activities were mostly demonstrated at high doses of Chloroquine (higher than 10 mg/kg or 10 mg/l *i.e. ca.* 31 μM). Nevertheless, it was reported that daily uptake of clinically acceptable doses (less than 10 mg/kg) of Chloroquine in addition to chemo-radio-therapy increases the survival of glioblastoma patients (Sotelo et al., 2006; Briceno et al., 2007). However, the optimal dose and schedule of this multi-active drug with respect to chemotherapy has never been experimentally determined. The present article reviews the several known direct and indirect effects of different doses of Chloroquine on cancer and how those effects may indicate that a fine tuning of the dose/schedule of Chloroquine administration versus chemotherapy may be critical to obtain an adjuvant effect of Chloroquine in anti-cancer treatments. We anticipate that the appropriate (time and dose) addition of Chloroquine to the standard of care may greatly and safely potentiate current anti-cancer treatments.

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1. Introduction

Chloroquine (N′-(7-chloroquinolin-4-yl)-N,N-diethyl-pentane-1,4-diamine), a chemically synthesized compound that is structurally related to the natural product Quinine from Cinchona bark, can cure malaria. Although Quinine has been used since the 17th century to treat fever and malaria, Chloroquine, which has a higher activity and is entirely synthetic, arrived on the market during World War II. Chloroquine remains a widely used drug for the prophylaxis and treatment of malaria. Interestingly, the mechanisms of action of this "old" drug on *Plasmodium* are still being revealed (Schlitzer, 2007). However, it is agreed that the main toxic activity of Chloroquine for the parasite is due to the binding of the drug to ferriprotoporphyrin IX, which comes from the digestion of hemoglobin imported in the pathogen's digestive vacuole where Chloroquine accumulates (Chloroquine becomes protonated at acidic pH levels and thus accumulates in all acidic cellular compartments). Ferriprotoporphyrin IX is toxic to the parasite and is usually disposed by the formation of an insoluble polymer called hemozoin. By preventing this disposal, Chloroquine induces death in *Plasmodium*.

The usual dose of Chloroquine to control malaria ranges from 100 mg in one uptake a week (prophylaxis) to 300 mg daily (therapy), which could be considered as a maximal dose of approximately 5 mg/kg/day. Although safe below 10 mg/kg/day, a cumulative total dose of 50–100 g in long term usage (*i.e.,* over 160 days of daily uptake of 300 mg) has been associated to retinopathy. Above 20 mg/kg, Chloroquine can cause serious toxic effects. The lowest oral lethal dose of Chloroquine is 86 mg/kg (Taylor and White, 2004). In the remainder of the review, I suggest that 10 mg/kg (10 mg/l *i.e.* ca. 31 μM) is the maximum realistic clinical dosage of Chloroquine. Thus, effects of Chloroquine (Table 1) recorded *in vitro* only at concentrations higher than 10 mg/l (higher than 31 μM) or in animals only at dosages higher than 10 mg/kg/day may generally not be relevant for translational studies. In the following, for clarity reasons and to allow direct comparisons between *in vitro* and *in vivo* results, all Chloroquine concentrations will be given in mg/l and mg/kg respectively, knowing that 1 mg/l of Chloroquine is approximately 31 μM.

2. Chloroquine and cancer

2.1. Chloroquine dose and cancer

In vitro tumoricidal activities of Chloroquine were reported in the seventies in the range of approximately 30 mg/l for lymphoma cells and approximately 20 mg/l for melanoma cells (Bedoya, 1970). Marked inhibition of cancer cell growth (reaching IC_{50} values) in 24 h *in vitro* assays by Chloroquine is found at doses above 10 mg/l (A549 human lung cancer cells (Fan et al., 2006), CT26 mouse colon carcinoma cells (Zheng et al., 2009), 4T1 mouse mammary carcinoma cell line (Jiang et al., 2010), MCF-7 and T-47D human breast cancer cells, HeLa human cervical cancer cells, Caco-2 and HCT116 human colon cancer cells, HEp-2 human larynx cancer cells, HepG2 human larynx cancer cells, HepG2 human liver cancer cells and PC3 human prostate cancer cells (Abdel-Aziz et al., 2014), and several breast cancer cell lines (Jiang et al., 2008)). Alternatively, some concentrations of Chloroquine stimulate the growth of tumor cells *in vitro* (Rossi et al., 2007).

In vivo, high doses of Chloroquine are required to detect (moderate) the inhibition of tumor growth: 50 mg/kg daily can enhance the survival of BALB/c mice inoculated with the CT26 colon carcinoma cells (Zheng et al., 2009), of BALB/c mice inoculated with the 4T1 mouse mammary carcinoma cells (Jiang et al., 2010) and of C57BL/6 mice inoculated with the B16 melanoma cells (Inoue et al., 1993). Thus, at doses used for humans (up to 10 mg/kg), Chloroquine alone is not expected to be efficient to treat cancer. However, the physiological impact of Chloroquine on cancer even at a clinically acceptable dose that may not lead on its own to the clinically relevant arrest of tumor development is one of the rationales to use the anti-malaria drug in combination with standard of care radio-chemo-therapies to obtain synergistic anti-cancer effects (see below). In addition, several indirect (on the tumor microenvironment or immune cells) activities of Chloroquine justify the combination approach and are detailed below. In this context, a critical feature that is rarely highlighted or studied is the schedule of the combination: should Chloroquine be given before, during or after radio-chemo-therapies? As the drug has a plethora of opposing effects (immunostimulating versus immunosuppressing; promoting or limiting tumor cell proliferation) that are dose dependent, what is the optimal dose/schedule of Chloroquine to be used in combination treatments? The following article aims to review the potential adjuvant effect of Chloroquine on the standard of care with regard to those dose/schedule aspects.

2.2. Direct effects of Chloroquine on cancer cells

2.2.1. Induction of apoptosis

Similar to hydroxychloroquine (Boya et al., 2003), Chloroquine at high doses (above 10 mg/l) induces apoptosis in a number of cancer cell lines *in vitro* (Fan et al., 2006; Jiang et al., 2008; Zheng

Table 1
Required dose of Chloroquine to detect the reported effect.