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RESEARCH LETTER

Effects of combination treatment with sirolimus and mitotane on growth of human adrenocortical carcinoma cells

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Introduction

Adrenocortical carcinoma (ACC) is a rare but aggressive solid cancer with a 5-year survival rate of <15 % at the metastatic stage [1]. Surgery remains the only curative treatment in patients diagnosed at an early stage [1, 2].

Mitotane, is currently the only drug approved in Europe and in the United States for the treatment of advanced ACC [2]. As monochemotherapy, a response rate of mitotane between 13–35 % has been reported [1, 2]. However, patients achieving a plasma mitotane level above 14 mg/L show a higher response rate and an improved survival [1, 2]. In combination with chemotherapy, response rates of mitotane range between 14–55 % [1–3]. No novel treatment option has emerged in the last four decades [1, 2], underlining the urgent need of new therapeutic options for patients affected by this malignancy.

The mammalian target of rapamycin (mTOR) pathway is considered a target for antineoplastic therapy, and in preclinical models of ACC, mTOR inhibitors such as sirolimus, temsirolimus, and everolimus inhibit cell proliferation in a dose- and time-dependent manner [4–6]. However, different human ACC cell lines and primary cultures show a variable response to mTOR inhibitors [7, 8]. In the absence of a clear predictor of the effectiveness of mTOR inhibitors in this malignancy, it is difficult to define selection criteria for patients, who are candidates for these drugs [1, 7–9]. Therefore, combination of mTOR inhibitors with mitotane could be a more prudent clinical approach than the use of these inhibitors as monotherapy in unselected ACC patients.

This study aimed at evaluating the effects of mitotane in combination with sirolimus in the two human ACC cell lines H295 and the SW13.

Materials and methods

Two human ACC cell lines the NCI-H295R (H295) and the SW13, were obtained and cultured as previously described [7]. Mitotane was purchased from Sigma-Aldrich and dissolved in ethanol as a concentrated (10^{-2} M) stock solution (stored at −20 °C) and diluted in ethanol prior to use. The mTOR inhibitor sirolimus was obtained from LC Laboratories and used as previously described [7]. The doses of sirolimus used were selected on the bases of the previously reported dose–response curves of sirolimus in the used cell lines [7]. The measurement of total DNA content was used as a measure of cell proliferation and performed as previously described [7]. For the statistical analysis, statistical software of GraphPad Prism version 5 (GraphPhad Software, San Diego, CA) was used.

Results

In both H295 and SW13, mitotane significantly inhibits cell proliferation in a dose-dependent manner (Fig. 1). Mitotane was slightly, but significantly more potent in H295 (IC_{50} 4.5×10^{-6} M) than in SW13 ($IC_{50}1.6 \times 10^{-5}$ M) ($p < 0.01$).

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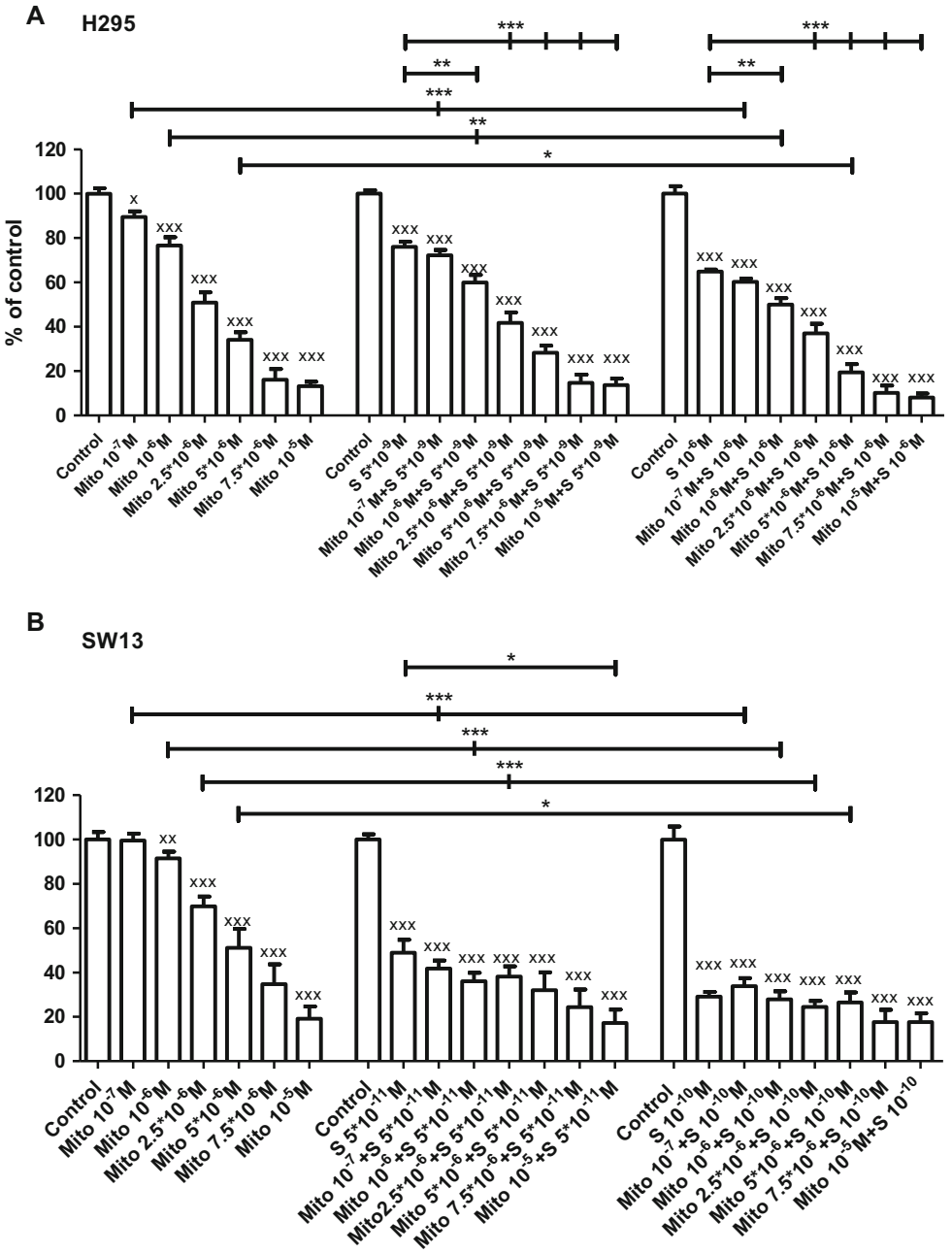

Fig. 1 Combined effects of a 6-day treatment with increasing concentrations of mitotane (Mito) and selected concentrations of sirolimus (S) in two human ACC cell lines: H295 (*panel* **a**) and SW13 (*panel* **b**). At some of the combinations tested significant additive inhibitory effects on cell growth are observed in both cell lines. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$; $^{x}p < 0.05$, $^{xx}p < 0.01$ and $^{xxx}p < 0.001$ versus control

In both H295 and SW13, the selected concentrations of sirolimus significantly inhibited cell proliferation (24–45 % and 57–70 %, respectively; $p < 0.001$).

When mitotane was used at low concentrations (between 10^{-7} and 5×10^{-6}M), sirolimus had a statistically significant additive effect, when compared with mitotane alone (Fig. 1).

In H295, as compared with sirolimus alone, mitotane at a concentration higher than 10^{-7} M had significant additive effects when combined with both the concentrations of sirolimus tested ($p < 0.01$ or $p < 0.001$; Fig. 1a). In SW13, as compared with sirolimus alone, mitotane had significant additive effects only when the highest concentration of mitotane (10^{-5} M) was combined with the lowest concentration of sirolimus (5×10^{-11} M) ($p < 0.05$; Fig. 1b).

Discussion

The current study demonstrates that in human ACC cell lines, sirolimus has additive antiproliferative effects when combined with low mitotane doses.

Mitotane is a referral drug in the treatment of ACC patients, but unfortunately there are patients who do not

respond and/or tolerate the drug, raising the requirement of new treatment options [1, 2].

The mitotane therapeutic window is very narrow. Therefore, monitoring mitotane plasma levels during treatment is very important. A response rate of up to 66 % has been reported in patients achieving mitotane plasma level above 14 mg/L, but a higher rate of adverse effects is reported when the plasma mitotane level exceeds 20 mg/L. A rapid achievement and long-term maintenance of this therapeutic range (14–20 mg/L) has been suggested as a predictor of mitotane response in patients with ACC [1, 2, 10, 11]. However, this clinical goal is sometimes difficult to reach because of the complex pharmacokinetic of mitotane which causes a large variation in individual drug availability. Additionally, the onset of adverse events can preclude a fast drug escalation or the use of a high mitotane dose. Treatment strategies combining mitotane with other drugs could increase the response rate of patients, as compared with monotherapy, for several reasons. The combination could have additive antiproliferative effects that potentially increase the in vivo antineoplastic effects. On the other hand, in absence of appropriate predictors of a clinical response that can help to select patients for the most appropriate treatment, the combination of two drugs acting with different mechanisms, could increase the chance of patients to get a clinical benefit from at least one of the two treatments. Additionally, in particular in the case of mitotane, the combined treatment with other active drugs could reduce the risk of tumor progression during the treatment initial mitotane dosage titration.

Recently, mTOR inhibitors have been suggested as a new potential treatment for ACC. Preclinical data suggest sirolimus, temsirolimus, and everolimus can inhibit ACC cell proliferation [4–6]. However, preliminary experience with the use of everolimus as salvage treatment in few ACC patients did not show promising results [12], whereas combined treatment of an insulin like growth factor 1 receptor (IGF1R) antibody with temsirolimus in a phase I study including ACC patients, showed more promising results [13]. Therefore, combination treatment with mTOR inhibitors and other drugs might have higher effects than mTOR inhibitors alone. To our knowledge, there are no in vitro studies evaluating the effects of mitotane in combination with mTOR inhibitors.

In the current study, all the concentrations of mitotane used (from 10^{-7} to 10^{-5} M) were lower than the concentrations (4.3–6.3 $\times$ 10^{-5} M) corresponding to the mitotane plasma level at the therapeutic range [14]. The addition of sirolimus to these low concentrations of mitotane showed higher antiproliferative effects than mitotane alone suggesting that combined treatment might have additive effects to the antineoplastic action of mitotane, permitting to reduce the dose required to obtain desired clinical effects. This additivity was higher when the concentration of mitotane used were lower, suggesting that combined treatment might be particularly useful during the phases of treatment in which mitotane plasma level are below the therapeutic range, such as during the initial dose titration and/or for those patients in which the therapeutic range of mitotane is hardly maintained due to low tolerance or other reasons.

With the exception of 10^{-6} M sirolimus used in H295 cells, all the tested concentrations where within the range of blood drug levels reached in humans during sirolimus treatment (maximal concentration about 10^{-7} M) [15, 16]. In both cell lines, the addition of mitotane to sirolimus showed a significant additive inhibitory effect as compared with sirolimus alone, but only at some of the experimental conditions tested. Particularly, in SW13 cells that are more sensitive to sirolimus, but less sensitive to mitotane than H295, the addition of mitotane showed a significant additive inhibitory effect as compared to sirolimus alone, only when the highest mitotane concentration was combined with a very low sirolimus concentration. This might be related to the potent inhibitory effects of sirolimus alone in SW13 cells, which induced already a near maximal inhibition of cell proliferation. While H295 cells are well accepted as a good model of ACC, a debate is still open about the appropriateness of SW13 cells as a model for this type of cancer [17]. Taking the other potential limitations of ACC cell lines as preclinical model of ACC, the results of the current study might suggest that among ACC patients it could be possible to find a subgroup of patients with a high sensitivity to sirolimus, in which the use of combined treatment could induce an important antineoplastic effect even in the absence of mitotane effect, such as in mitotane-resistant patients.

Among the potential limitations of the current study, there is the lack of possibility to explore pharmacokinetic interactions between drugs. In the in vivo setting, mitotane is a well known inducer of microsomal liver enzyme cytochrome P450 (CYP3A4/5) [18]. The induction of this enzyme can reduce the circulating concentrations of drugs metabolized by it, including mTOR inhibitors [19]. This pharmacokinetic interaction should be kept in mind when translating the in vitro results to the potential in vivo applications, and attention should be used in monitoring the blood concentrations of drugs to reach the desired therapeutic levels.

In conclusion, this study suggests a potential advantage of combining mitotane with sirolimus, but these data are still preliminary. Clearly, additional studies, including animal models, are mandatory before moving from the bench to the bedside.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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