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Short communication

Synergistic immunosuppressive effects of the mTOR inhibitor sirolimus and the phytochemical curcumin

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ABSTRACT

The immunosuppressant sirolimus and curcumin, the main principle of the turmeric spice, have shown antiproliferative effects on many human and not-human cell lines. Whereas the antiproliferative effect of sirolimus is mainly mediated by inhibition of mTOR, curcumin is described to affect many molecular targets which makes it unpredictable to appraise if the effects of these both substances on cell proliferation and especially on immunosuppression are additive or synergistic. To answer this question we investigated the interaction of both these substances on OKT3-induced human peripheral blood mononuclear cell (PBMC) proliferation. OKT3-induced human PBMC proliferation was determined by measuring ^{3}H-thymidine incorporation. Influence of curcumin on interleukin-2 (IL-2) release and IκB-phosphorylation in PBMC was determined by ELISA and western blot, respectively. Curcumin-induced apoptosis and necrosis was analyzed by FACS analysis. Whereas curcumin completely inhibited OKT3-induced PBMC proliferation in a dose-dependent manner with an IC_{50} of 2.8 μM, sirolimus could reduce PBMC proliferation dose-dependently only to a minimum of 28% at a concentration of 5 ng/ml (IC_{50} 1.1 ng/ml). When curcumin was combined at concentrations of 1.25–2.5 μM with sirolimus at concentrations from 0.63 to 1.25 ng/ml the effects were synergistic. Combination of curcumin (1.25–2.5 μM) with sirolimus (5 ng/ml) showed additive effects. The effects after combination of curcumin at 5 μM with each sirolimus concentration and sirolimus at 10 ng/ml with each curcumin concentration were presumably antagonistic. We conclude that the immunosuppressive effects of curcumin and sirolimus in low concentrations are synergistic in OKT3-activated PBMC. Whether curcumin and sirolimus have also synergistic antiproliferative effects in tumor cells has to be shown in further experiments including animal models.

Introduction

The immunosuppressant sirolimus was the first mTOR inhibitor discovered (Zhou et al., 2010). Additionally, its anti-proliferative effect has been investigated in numerous murine and human cancer cell lines derived from rhabdomyosarcoma (Hosoi et al., 1999), neuroblastoma, glioblastoma (Geoerger et al., 2001), small cell lung cancer (Seufferlein and Rozengurt, 1996), osteosarcoma (Ogawa et al., 1998), pancreatic cancer (Grewe et al., 1999), breast cancer, prostate cancer (van der Poel et al., 2003; Pang and Faber, 2001), murine melanoma and B-cell lymphoma (Busca et al., 1996; Muthukkumar et al., 1995).

Curcumin (diferuloylmethane) as principal ingredient in turmeric spice is consumed daily by approximately a quarter of the world's population (Dickinson et al., 2004). Over the last 60 years, more than 3000 studies have demonstrated that curcumin has antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, proapoptotic and anti-atherosclerotic effects, exerting medicinal benefits against neurodegenerative diseases, arthritis, allergy, inflammatory bowel disease, nephrotoxicity, AIDS, psoriasis, diabetes, multiple sclerosis, cardiovascular disease, and lung fibrosis (Zhou et al., 2011; Aggarwal and Harikumar, 2009; Pari et al., 2008; Agrawal and Mishra, 2010; Epstein et al., 2010).

Modern scientific research demonstrated that curcumin is a highly pleiotropic molecule that interacts with numerous molecular targets. This pleiotropy makes it unpredictable to say whether

Abbreviations: C, controls; Con A, concanavalin A; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell scanner; IC_{50}, halfmaximal inhibitory concentration; IL-2, interleukin-2; IκB, inhibitor of κB; mTOR, mammalian target of rapamycin; NFκB, nuclear factor κB; OKT3, mouse anti-human CD3 antibody; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PI, propidium iodide; PMA, phorbol myristate acetate.

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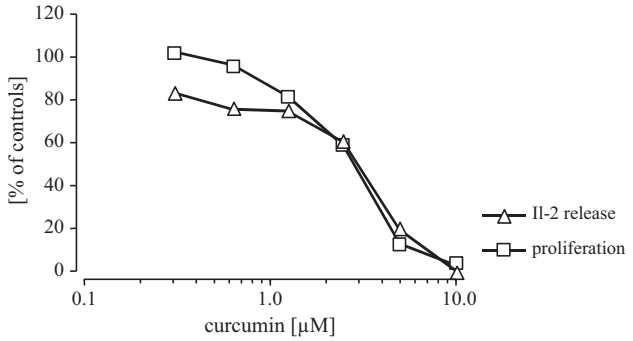

Fig. 1. Influence of curcumin on proliferation and IL-2 release of OKT3-induced PBMC. Means ± S.E.M. of 3 experiments each. S.E.M. was maximal ± 16% of the given values and not shown in the present figure.

the combined effects of sirolimus and curcumin on cell proliferation and especially on immunosuppression may be synergistic or not. To answer this question we investigated the interaction of these both substances on OKT3-induced human peripheral blood mononuclear cell (PBMC) proliferation. OKT3-induced human PBMC proliferation was determined by measuring ^{3}H-thymidine incorporation (Vieregge et al., 1999; Deters et al. 2008). Influence of curcumin on interleukin-2 (IL-2) release and IκB-phosphorylation in PBMC was determined by ELISA and western blot, respectively. The interactions were evaluated by the algorithm of Berenbaum (Berenbaum, 1977). Curcumin inhibited OKT3-induced PBMC proliferation in a dose-dependent manner with an IC_{50} of 2.8 μM (Fig. 1). This value is comparable to values observed in experiments with PMA-/CD28-, and PHA-induced proliferation of human T-lymphocytes with IC_{50} of 3.5 μM and 7.7 μM, respectively (Ranjan et al., 1998). The inhibitory effect of the commercially available curcumin powder of Roth (77% curcumin, 19% desmethoxy-, 4% bisdesmethoxycurcumin) was the same as of purified curcumin. Therefore, the commercial powder was used for all further experiments.

Table 1
Values of interactions of curcumin with sirolimus. Calculation was performed according to the algorithm of Berenbaum. A value of lower than 1 indicates synergistic effects (bold-faced data). If the value is 1 the effects of two combined substances are additive and a value higher than 1 can be found if the effects of the substances are antagonistic.

Sirolimus (ng/ml)	Curcumin (μM)		
	1.25	2.50	5.00
0.63	**<0.66**	**<0.78**	<1.06
1.25	**<0.65**	**<0.63**	<1.13
2.50	**<0.74**	**<0.75**	<1.25
5.00	**<0.89**	<1.00	<1.50
10.00	<1.38	<1.50	<2.00

The inhibition of OKT3-induced cell proliferation in PBMC by curcumin was paralleled by a decrease of IL-2 release (Fig. 1). The correlation was statistically significant ($p < 0.05$) with a correlation coefficient of 0.975. Curcumin decreased phosphorylation of IκB in a dose-dependent manner (data not shown). A statistically significant ($p < 0.05$) correlation between diminished phosphorylation of IκB and inhibition of OKT3-induced PBMC by curcumin with a correlation coefficient of 0.83 was observed. Stainining by propium iodide and FACS analysis revealed that the antiproliferative effects of curcumin were caused neither by curcumin-induced cytotoxicity nor by apoptosis. The OKT3-induced PBMC cell proliferation was decreased by the half at a sirolimus concentration of 1.1 ng/ml (Fig. 2A). At higher sirolimus concentrations the cell proliferation could only be reduced to 28%. When sirolimus was combined with curcumin the IC_{50} of sirolimus was lower than 0.63 ng/ml and the cell proliferation could be inhibited almost totally by the combination of curcumin (5 μM) with sirolimus (Fig. 2A). When curcumin was combined at concentrations of 1.25–2.5 μM with sirolimus at concentrations from 0.63 to 1.25 ng/ml the antiproliferative effects were synergistic (Table 1 and Fig. 2B and C). Combination of curcumin (1.25–2.5 μM) with sirolimus (5 ng/ml) showed additive effects (Table 1). The effects after combination of curcumin at 5 μM with each sirolimus concentration and sirolimus at 10 ng/ml

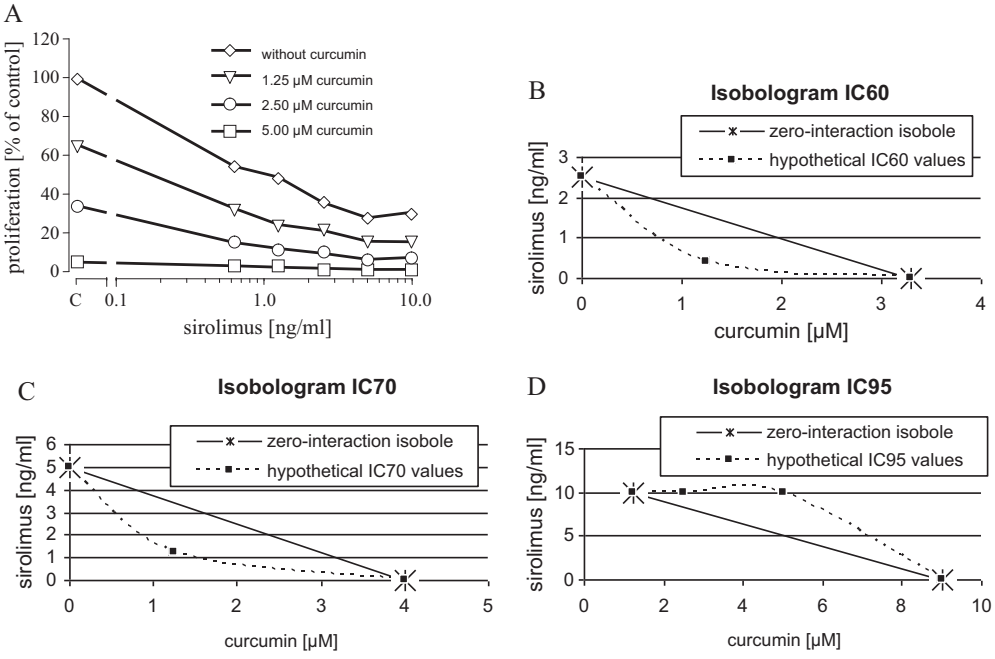

Fig. 2. Influence of the combination of sirolimus and curcumin on proliferation of OKT3-induced PBMC is shown in (A). Means ± S.E.M. of 3 experiments each. S.E.M. was maximal ± 16% of the given values and not shown in the present figure. Isobolograms of the inhibitory concentrations IC_{60}, IC_{70}, and IC_{95} with the zero-interaction isoboles and hypothetical IC values are shown in (B–D).