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Research Paper

Different Curcuminoids Inhibit T-Lymphocyte Proliferation Independently of Their Radical Scavenging Activities

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Purpose. We investigated the inhibitory effects of curcumin, curcumin derivatives and degradation products on OKT3-induced human peripheral blood mononuclear cell (PBMC) proliferation and the role of their radical scavenging activity.
Methods. OKT3-induced human PBMC proliferation was determined by measuring ^{3}H-thymidine incorporation. Radical scavenging activity was evaluated by using an *in vitro* DPPH assay.
Results. OKT3-induced PBMC proliferation was inhibited by curcumin, isocurcumin, bisdesmethoxy-, diacetyl-, tetrahydro-, hexahydro-, and octahydrocurcumin as well as by vanillin, ferulic acid, and dihydroferulic acid with IC_{50}-values of 2.8, 2.8, 6.4, 1.0, 25, 38, 82, 729, 457, and >1,000 μM, respectively. The investigated substances with the strongest effect on radical scavenging were tetrahydro-, hexahydro-, and octahydrocurcumin with IC_{50} values of 10.0, 11.7, and 12.3 μM, respectively. IC_{50}-values of dihydroferulic acid, ferulic acid, and curcumin were 19.5, 37, and 40 μM. The substances with the lowest radical scavenging activities were vanillin, isocurcumin, diacetylcurcumin, and bisdesmethoxycurcumin with IC_{50} values higher than 100 μM each.
Conclusions. Curcuminoid-induced inhibition of OKT3-induced PBMC proliferation depends on the number of carbon atoms and double bonds of the 1,6-heptadiene-3,5-dione structure as well as on the phenolic ring substitutes of the curcuminoids but is not correlated to their respective radical scavenging activity.

KEY WORDS: antioxidants; curcumin; curcuminoids; immunosuppression; OKT3-induced human PBMC proliferation; radical scavengers.

INTRODUCTION

Curcuminoids are low molecular weight phenolic compounds derived from the rhizomes of turmeric (botanical name: *Curcuma longa*) a member of the ginger family *Zingiberaceae* (1). The major representative of these phytochemicals is curcumin (diferuloylmethane; Fig. 1) which has been used in traditional Indian and Chinese medicine for centuries (2). As a principal ingredient in curry, curcumin is consumed daily by approximately a quarter of the world's population (3). Curcuminoids are now being investigated in more detail for use in Western medicine. Within the last 5 years more than 700 new publications concerning curcumin are listed in the literature database MEDLINE. The most detailed studies using curcumin showed a plethora of beneficial effects including choleretic, anti-inflammatory, antioxidant, anticarcinogenic, antiviral, and anti-infectious activities (4,5) although the underlying molecular mechanism(s) of action remain poorly defined. In experiments with human low density lipoproteins curcumin and some analogues were potent inhibitors of low density lipoprotein oxidation (6). Curcumin has been described to attenuate the proliferation of human lymphocytes and the production of several inflammatory mediators including lipid mediators and cytokines (7–8). Thus, it may be suited as a lead substance for the development of new immunosuppressive and anti-rheumatic drugs.

In vitro, curcumin is described to be an unstable substance that decomposes to other substances like ferulic acid and vanillin (9). The bioavailability of curcumin is low and after resorption it is metabolized to a great extent in the intestine and liver of humans and rats to curcumin glucuronides, sulfates, as well as tetrahydrocurcumin, and hexacurcumin (10). Whether curcumin itself or one of its metabolites or degradation products mediates the numerous pharmacodynamic effects is still under discussion.

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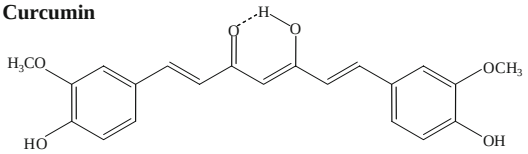
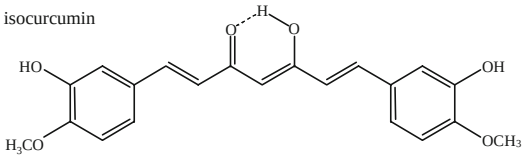
ABBREVIATIONS: ABTS, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; HBC, 2-hydroxypropyl-γ-cyclodextrin; HHC, hexahydrocurcumin; IC_{50}, halfmaximal inhibitory concentration; JNK, junN-terminal kinase; NFκB, nuclear factor κB; OHC, octahydrocurcumin; OKT3, mouse anti-human CD3 antibody; PBMC, peripheral blood mononuclear cells; THC, tetrahydrocurcumin.

Curcumin

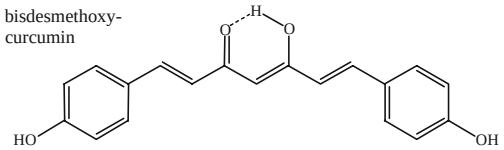


Curcumin analogues

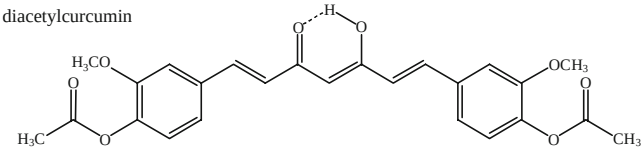
isocurcumin



bisdesmethoxy-curcumin

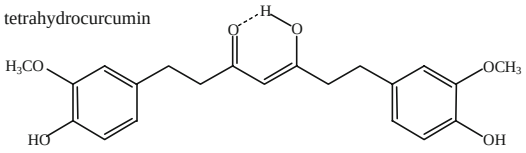


diacetylcurcumin

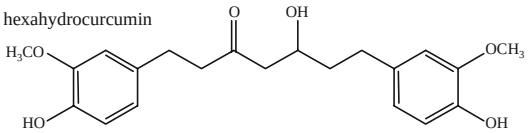


Curcumin biotransformation products

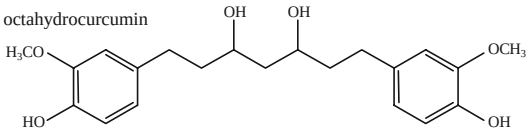
tetrahydrocurcumin



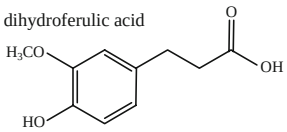
hexahydrocurcumin



octahydrocurcumin

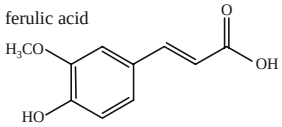


dihydroferulic acid



Curcumin chemical degradation products

ferulic acid



vanillin

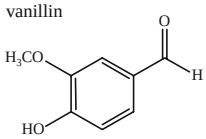


Fig. 1. Chemical structures of different curcuminoids and their degradation products.

Therefore, we investigated the effects of various curcuminoids and curcumin derivatives (curcumin, isocurcumin, bisdesmethoxy-, diacetyl-, tetrahydro-(THC), hexahydro-(HHC), and octahydrocurcumin (OHC) as well as vanillin, ferulic acid and dihydroferulic acid) on OKT3-induced proliferation of human peripheral blood mononuclear cells (PBMC) in order to establish some first structure–activity relationships. Additionally, we determined the radical scavenging activities of these curcuminoids by using a chemical assay in order to proof a possible relationship to their immunosuppressive effects.

MATERIALS AND METHODS

Materials

Curcumin was obtained from Roth, Karlsruhe, Germany. Ferulic acid, vanillin, 2-hydroxypropyl-γ-cyclodextrin, and 1,1-diphenyl-2-picrylhydrazyl radical were purchased from Sigma, Taufkirchen, Germany. Dihydroferulic acid was obtained from Lancaster, Frankfurt, Germany and [^{3}H] thymidine (specific activity 25 Ci/mmol) was from Amersham, Braunschweig, Germany. Stock solutions of the curcuminoids (1 M) were prepared in DMSO except for tetrahydro-hexahydro-, octahydrocurcumin, and dihydroferulic acid, where HBC (0.45%) was used as solvent. All further dilutions were done in RPMI1640 medium/5% fetal calf serum (proliferation assay) or ethanol (radical scavenging).

Synthesis of Curcuminoids

Isocurcumin and bisdesmethoxycurcumin were synthesized at the Pharmaceutical Institute, University Kiel. Briefly, to a solution of 0.04 mol of either isovanillin or 4-hydroxybenzaldehyde in tributyl borate (0.08 mol) the reaction product of acetylacetone (0.02 mol) and boric (0.014 mol) anhydride in dry ethyl acetate was added. Butylamine was then added as a catalyst and the resulting mixture was stirred at 40°C for 18 h. Diluted hydrochloric acid (0.4 N) was used to free isocurcumin or bisdesmethoxycurcumin, respectively, which were purified by recrystallization in ethanol and characterized by combustion analysis, IR, UV and ^{1}H-NMR (data not shown).

The curcumin derivative diacetylcurcumin, as well as the hydrocurcumins THC, HHC, and OHC used in this study were synthesized at the Institute of Organic Chemistry, University Hannover. Diacetylcurcumin was synthesized from curcumin using acetanhydride and potassium carbonate in waterfree acetone. Briefly, curcumin (0.30 mmol), acetanhydride (0.75 mmol), and potassium carbonate (1.00 mmol) were given to 3 ml waterfree acetone and stirred for 48 h at room temperature. The reaction mixture was taken up in dichlormethane and water. After addition of diluted HCl the organic phase was removed and extracted twice with dichlormethane. The extracted organic phase was dried by sodium sulfate and evaporated. The product was purified chromatographically on a silica gel column (2.4×5.0 cm). As eluents hexane/methyl-*t*-butylether, 1/2 (*v*/*v*), and pure methyl-*t*-butylether were used. In the initial fraction diacetylcurcumin (light-yellow crystalloid substance) with a melting point of 172°C was gained. The spectroscopic data were as described in the literature (11).

The hydrocurcumins were synthesized according to previously described methods (10,12–14) with variations of the reaction conditions regarding the hydrogenation of curcumin by palladium/carbon in 10% acetic acid with the addition of 3% acetic acid and control of hydrogen uptake. Hydrogenation was stopped immediately when the yellow colouring of the educt disappeared. Because the velocity of hydrogenation of THC is in the same range as the hydrogenation of curcumin always HHC and OHC (as diastereomere) can be found as by-products. The hydrogenation of curcumin should be finished totally to avoid problems of preparative chromatography. Briefly, curcumin (5 mmol, purified by repeated crystallization) in ethyl acetate/acetic acid, 60:1.6 ml (*v*/*v*), was hydrogenated until the yellow colour disappeared. The volume of the hydrogen taken up was 14.5 mmol. The product was filtrated, evaporated and purified chromatographically on a silica gel column (3.8× 12 cm). As eluents hexane/methyl-*t*-butylether mixtures and pure methyl-*t*-butylether were used. The gain was as follows: THC, Fp=98°C (46% gain), HHC, Fp=80°C (32% gain), OHC, Fp=23°C (23% gain). All three substances showed the same spectroscopic characteristics as described in the literature (10,12–14).

Proliferation Assay

Peripheral blood mononuclear cells were isolated from heparinized blood drawn from healthy adults by Ficoll/Hypaque gradient centrifugation. After overnight culture non-adherent lymphocytes were resuspended in RPMI1640 medium containing 5% fetal calf serum. Proliferation assays were performed in 96-well microtiter plates (15). The cells (10^5 per well) were preincubated with the various curcuminoids for 1 h and then activated by addition of the monoclonal anti human T-cell receptor/CD3 complex antibody OKT3 (1 ng/ml). After an incubation time of 44 h [^{3}H]thymidine (0.5 mCi/well) was added and the culture was carried on for further 4 h prior to cell harvest by an automatic 12-well harvester. Incorporated radioactivity was determined by liquid scintillation counting.

Determination of Radical Scavenging Activity of Curcuminoids

The radical scavenging activity of the various curcuminoids was investigated similar as described previously (16–17). The curcuminoids were dissolved at concentrations of 0.63–200 μM in ethanol and given 1:1 (*v*/*v*) to a 100 μM radical solution stabilized by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The reaction time was 20 min at room temperature. The decrease in the optical density was measured photometrically at 517 nm.

RESULTS

Effect of Curcuminoids on Proliferation of OKT3-induced Human PBMC

Curcumin concentrations up to 0.63 μM showed no or only a little effect on PBMC proliferation (Fig. 2). The proliferation of PBMC was inhibited by 50% (IC_{50}) at a curcumin concentration of 2.8 μM (Table I). Dissolving the stock solution of curcumin in either NaOH or DMSO revealed no difference in the IC_{50} value (data not shown). The commercial curcumin (Roth, Karlsruhe, Germany) contains 77% curcumin, 19% desmethoxy- and 4% bisdesmethoxycurcumin. We compared the influence of purified curcumin to commercial curcumin on proliferation of OKT3-induced human PBMC. The IC_{50} values of purified and commercial curcumin were 2.8 and 3.7 μM, respectively. Because this difference was statistically not significant we used the commercial curcumin for further experiments.

The inhibitory effect on OKT3-induced PBMC cell proliferation of the chemical derivative diacetylcurcumin was three times higher and that of bisdesmethoxycurcumin 2.5 times lower than of curcumin (Fig. 2) with IC_{50} values of 1.0 and 6.4 μM, respectively, whereas the curcumin analogue isocurcumin showed the same inhibitory effect (IC_{50} 2.8 μM) as curcumin itself (Table I).

The physiological metabolites of curcumin THC, HHC, and OHC inhibited the proliferation of OKT3-stimulated human PBMC less than 4.4 to 14.4 times compared to curcumin (Fig. 2) with IC_{50} values of 25, 38, and 82 μM, respectively (Table I).

Compared to curcumin, the effects of the curcumin degradation products vanillin and ferulic acid on PBMC proliferation were weak (Fig. 2). The IC_{50} of curcumin was 163 times lower than the IC_{50} of ferulic acid (IC_{50} 457 μM) and even 260 times lower than the IC_{50} of vanillin (729 μM;

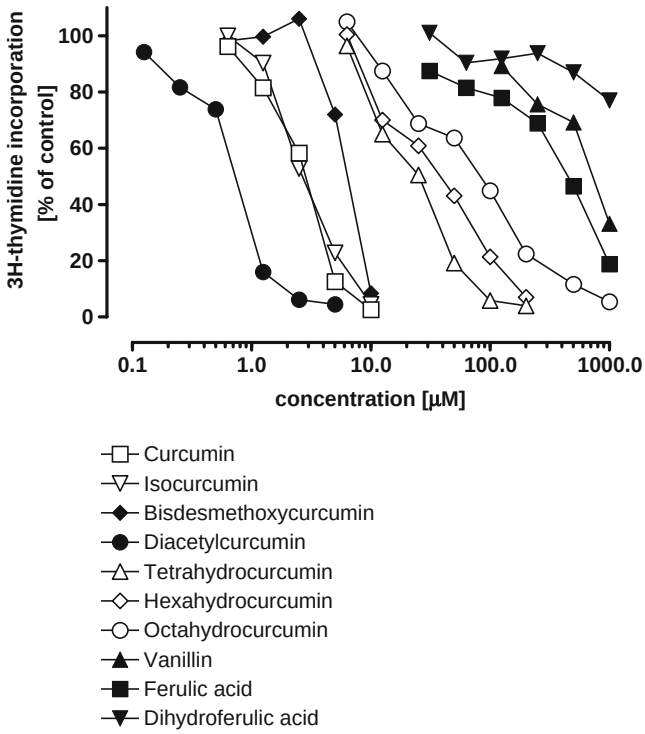


Fig. 2. Influence of curcuminoids and their degradation products on proliferation of OKT3-stimulated human PBMC. Human PBMC of different donors were stimulated by 1 ng/ml OKT3 and incubated with different concentrations of the test substances for 48 h. Means of at least three independent experiments each. Standard error of the means (S.E.M.) was ±11% in maximum and is not shown in the figure.

Table I. IC_{50} Values [μM] of Various Curcuminoids and their Degradation Products Concerning Inhibition of PBMC Cell Proliferation and Scavenging DPPH Stabilized Radicals

	IC_{50} [μM]	
	Inhibition of proliferation	Binding of radicals
Curcumin	2.8	40
Isocurcumin	2.8	>100
Bisdesmethoxycurcumin	6.4	>100
Diacetylcurcumin	1.0	>100
Tetrahydrocurcumin	25	10.0
Hexahydrocurcumin	38	11.7
Octahydrocurcumin	82	12.3
Vanillin	729	>100
Ferulic acid	457	37
Dihydroferulic acid	>1,000	19.5

Table I). Dihydroferulic acid showed no effect on proliferation of OKT3-stimulated human PBMC up to a concentration of 1,000 μM (Table I).

Radical Scavenging Activity of Curcuminoids

To get further information about the mechanism of the curcuminoid-mediated inhibition of PBMC proliferation we investigated the effect on curcuminoids and their degradation products on binding radicals stabilized by 1,1-diphenyl-2-picryl-hydrazol (DPPH; Fig. 3). The investigated substances with the strongest effect on scavenging radicals were THC, HHC, and OHC with IC_{50} values of 10.0, 11.7, and 12.3 μM, respectively (Table I). The radical scavenging activity of dihydroferulic acid, ferulic acid and curcumin were in the middle range with IC_{50}-values of 19.5, 37 and 40 μM each. The lowest ability as radical scavengers were observed in experiments with vanillin, isocurcumin, diacetylcurcumin, and bisdesmethoxycurcumin with all IC_{50} values higher than 100 μM (Fig. 3; Table I).

DISCUSSION

In our experiments it was shown that curcumin inhibited OKT3-induced proliferation of human PBMC in a dose-dependent manner with an IC_{50} of 2.8 μM. 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 and 7.7 μM, respectively (8). 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, it was possible to use the commercial powder for all further experiments.

The main degradation products of curcumin are vanillin and ferulic acid. We studied the inhibitory effect of these products on OKT3-induced cell proliferation of PBMC, as well. The inhibitory effect of curcumin (IC_{50} 2.8 μM) was 163 times stronger than of ferulic acid (IC_{50} 457 μM) and 260 times stronger than of vanillin (IC_{50} 729 μM). Because cleavage of the 1,6-heptadiene-3,5-dione moiety resulted in a significant loss of the inhibitory effect on OKT3-induced PBMC proliferation the number of carbon atoms of this structure and the presence of the aromatic ring structure are obviously important for the immunosuppressive effect of curcumin.

In vivo, curcumin is not only degraded but also metabolized rapidly, as well. The main metabolites are THC and HHC as well as dihydroferulic acid. Curcumin and these metabolites are conjugated to glucuronic acid (10,18). Because of the short halftime of curcumin (less than 1 h) the biological effects could be mediated by the metabolites rather than by curcumin itself. We investigated the inhibitory effects of tetra-, hexa-, and octahydrocurcumin, and dihydroferulic acid on OKT3-induced proliferation of human PBMC. Because of the low solubility, these substances had to be dissolved in HBC. Dissolution of curcumin in HBC reduced its inhibitory effect on cell proliferation statistically not significant by the half (IC_{50} 5.7 μM). However, the inhibitory effects of THC (IC_{50} 25 μM), HHC (IC_{50} 38 μM), and OHC (IC_{50} 82 μM) were lower by a factor of 4.4, 6.7, and 14.4, respectively. For the curcumin metabolite dihydroferulic acid no IC_{50} value could be determined (Table I). The reduction of curcumin to THC, HHC, and OHC was accompanied by a loss of double bonds of the 1,6-heptadiene structure and a loss of inhibitory effect on OKT3-induced PBMC proliferation. From these results we conclude that the intact 1, 6-heptadiene structure is an important feature of the immunosuppressive effect of curcumin. The remaining immunosuppressive effect of THC, HHC, and OHC was much higher than that of ferulic acid, dihydroferulic acid, and vanillin

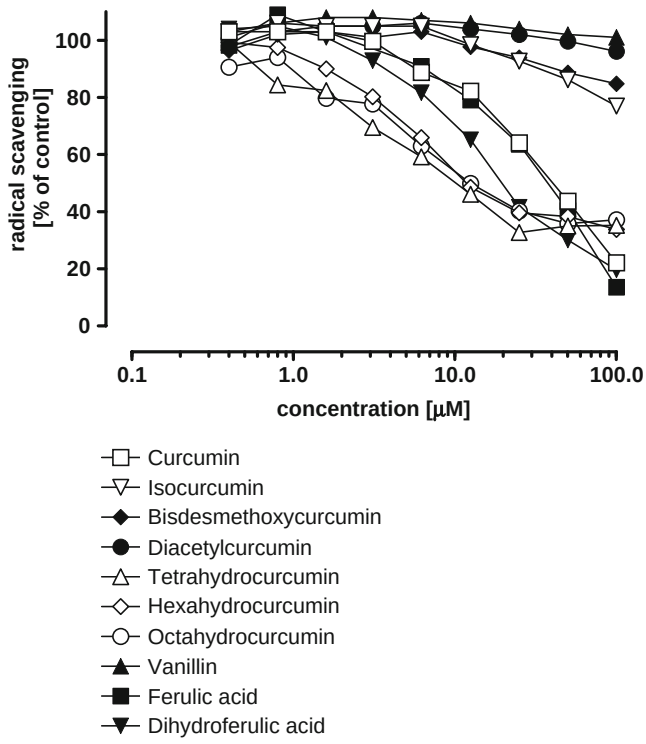


Fig. 3. Radical scavenging activities of different curcuminoids and their degradation products. The radical scavenging activities were determined in the established DPPH assay. Means of at least three independent experiments each. Standard error of the means (S.E.M.) was ±7% in maximum and is not shown in the figure.

(Table I). Therefore, the number of carbon atoms of the 1,6-heptadiene structure seemed to be even more important for the inhibitory effect than the loss its double bounds. After reduction of the 3,5-dione structure (THC, HHC, OHC) a little decrease of the inhibitory effect on cell proliferation was observed, as well (Table I) but the decrease of the immunosuppressive effect was quite lower than after the loss of the 1,6-heptadiene structure.

To get further information about the role of different substitutes and their position in the aromatic ring structure, we investigated the inhibitory effect of the curcumin analogues iso-, diacetyl-, and bisdesmethoxycurcumin on OKT3-induced proliferation of human PBMC. The inhibitory effect of isocurcumin was nearly the same as of curcumin (Table I). The inhibitory effect of diacetylcurcumin, however, was three times higher (IC_{50} 1.0 μM) and that of bisdesmethoxycurcumin 2.5 times lower than of curcumin (Table I). According to these observations, we hypothesize that the location of the 4-hydroxy-3-methoxyphenyl group (Fig. 1) is not important for the immunosuppressive effect. The loss of the 3-methoxy group (bisdesmethoxycurcumin), however, reduces and acetylation (diacetylcurcumin) of the 4-hydroxyl group (Fig. 1) increases the inhibitory effect of curcumin on OKT3-induced proliferation of human PBMC. The role of the *in vivo* observed glucuronidation of curcumin has to be investigated in further experiments.

The underlying molecular mechanism(s) of the immunosuppressive effects of curcumin remain poorly defined. Curcumin and some synthetic symmetrical curcumin analogues have been described to show activity for scavenging free radicals (16–17,19–23) and in experiments with human low density lipoproteins curcumin and some analogues were potent inhibitors of low density lipoprotein oxidation (6). Curcumin is known to exert radical scavenging activities via several mechanisms (21), due to its various functional groups, i.e. the ß-diketone group, carbon–carbon double bonds, and phenyl rings containing hydroxyl and methoxy substituents (Fig. 1). Therefore, we determined the radical scavenging activity of the curcuminoids by using an established DPPH assay and proofed a possible relationship to their immunosuppressive effects. Venkatesan and Rao (16) found in their experiments with inhibition of lipid peroxidation, scavenging of DPPH and ABTS+ that the phenolic analogues of curcumin were more active than the non-phenolic analogues, some of which were inactive. The highest antioxidant activity was obtained when the phenolic group was sterically hindered by the introduction of two methyl groups at the ortho position. In our experiments we investigated only phenolic curcuminoids. The substances with the strongest effect on scavenging radicals were THC, HHC, and OHC with IC_{50} values of 10.0, 11.7, and 12.3 μM, respectively (Table I). Recently, Somparn *et al.* (17) also studied the effect of curcumin, THC, HHC, OHC, and bisdesmethoxycurcumin on radical scavenging determined by the DPPH assay. In their experiments the scavenging activity decreased in the order THC > HHC = OHC > curcumin > bisdesmethoxycurcumin. In our present study, the radical scavenging activities of dihydroferulic acid, ferulic acid and curcumin were in the middle range with IC_{50}-values of 19.5, 37 and 40 μM each. The lowest effects as radical scavengers were observed in experiments with vanillin, isocurcumin, diacetylcurcumin (23), and bisdesmethoxycurcumin with IC_{50} values higher than 100 μM (Table I). From these results we conclude that reduction of the 1,6-heptadiene structure (THC, HHC, and OHC) increases the radical scavenging effects of curcumin. Because the IC_{50} values of THC, HHC, and OHC radical scavenging activity were in the same range the loss of the 3,5-dione structure seemed to have no influence on the antioxidative effect of curcumin. Sugiyama *et al.* (19) examined the inhibitory effects of curcumin and THC on the lipid peroxidation of erythrocyte membrane ghosts induced by tertbutylhydroperoxide. Their results demonstrated that THC showed a greater inhibitory effect than curcumin, as well. On the other side, however, their results suggested that the beta-diketone moiety of THC must exhibit antioxidative activity by cleavage of the C–C bond at the active methylene carbon between two carbonyls in the beta-diketone moiety. In our present study, changes of the 4-hydroxy-3-methoxyphenyl group position (isocurcumin), loss of the 3-methoxy group (bisdesmethoxycurcumin), and derivatisation of the 4-hydroxy group (diacetylcurcumin) significantly reduced the curcumin radical scavenging effect. This observation is in accordance with the results of Chen *et al.* (6) who also found that the 4-hydroxy-3-methoxyphenyl group is important for the antioxidant activity of curcumin. In another study the antioxidant activity of curcumin and dimethoxycurcumin was tested by following radiation-induced lipid peroxidation in rat liver microsomes. It was concluded that the phenolic OH group plays a major role in the activity of curcumin (20). Venkateswarlu *et al.* (21) evaluated the antioxidant activity of polyhydroxycurcuminoids by superoxide radical and DPPH free radical scavenging methods. In addition, they proofed their cytotoxicity in lymphocytes from normal or patients with leukemia and antitumor activity on Dalton's lymphoma ascites tumor cells. In their experiments the inhibitory effect of curcumin on scavenging DPPH free radicals was comparable with our results (IC_{50} 21 μM). As in our experiments no correlation between the cytotoxicity and the antitumor activity of the curcuminoids on the one hand and their radical scavenging effect on the other was observed. Venkateswarlu *et al.* (21) found in their experiments that the superoxide as well as the DPPH radical scavenging activity of the curcuminoids increased with the number of hydroxyl groups of their benzene ring. Furthermore, the substitution pattern of hydroxyls played an important role. In our experiments, no radical scavenging activity of polyhydroxycurcuminoids was investigated. The acetylation of the hydroxylated benzene rings of curcumin to diacetylcurcumin was accompanied by a significant loss of DPPH free radicals scavenging activity (Table I). This observation is in accordance with the conclusion of Venkateswarlu *et al.* (21) that hydroxyl groups of the benzene rings are important in radical scavenging activity of curcuminoids.

Comparing the influences of changes of the curcumin molecule on its inhibitory effect on OKT3-induced proliferation of human PBMC and on its radical scavenging activity the results can be summarized as follows: Decreasing the number of carbon atoms of the 1,6-heptadiene-3,5-dione structure (vanillin, ferulic acid, and dihydroferulic acid; Fig. 1) significantly reduces the inhibitory effect of curcumin on OKT3-induced proliferation of human PBMC but for example in the case of dihydroferulic acid the radical

scavenging activity was even increased (Table I). Reduction of curcumin to THC, HHC, and OHC is accompanied by a loss of double bounds of the 1,6-heptadiene structure and a loss of inhibitory effect on OKT3-induced PBMC proliferation but by an increase of radical scavenging activity. After reduction of the 3,5-dione structure (THC, HHC, OHC; Fig. 1) a little decrease of the inhibitory effect on cell proliferation but no significant change of the antioxidant activity was observed (Table I). The position of the 4-hydroxy-3-methoxyphenyl group (Fig. 1) is not important for the inhibitory effect but changes in this position (isocurcumin) resulted in a distinct loss of curcumin radical scavenging activity (Table I). The loss of the 3-methoxy group (bisdesmethoxycurcumin) only slightly decreases the immunosuppressive effect of curcumin but significantly reduces its antioxidant activity (Table I). Acetylation of the 4-hydroxy group increases the immunosuppressive activity of curcumin but significantly diminishes its antioxidant activity (Table I).

Taken together, the correlation of the structural relationships of curcuminoids being responsible for the immunosuppressive effect on the one hand and for the antioxidant activity on the other was low in our experiments. Therefore, it can be stated that the curcuminoid-induced inhibition of OKT3-induced PBMC proliferation is not mediated by the radical scavenging activity of the curcuminoids. The role of other mechanisms responsible for the immunosuppressive effect of the curcuminoids like inhibition of CD28 costimulatory pathway (8) or of the transcription factors JNK and NFκB, as described in literature (24,25) has to be examined in further experiments.

CONCLUSIONS

Curcuminoid-induced inhibition of OKT3-induced PBMC proliferation depends on the number of carbon atoms and double bonds of the 1,6-heptadiene-3,5-dione structure as well as on the phenolic ring substitutes of the curcuminoids but is not correlated to their scavenging activity.

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