Xanthohumol induces apoptosis in cultured 40-16 human colon cancer cells by activation of the death receptor- and mitochondrial pathway

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Xanthohumol (XN) is one of the major prenylflavonoids found in hop cones (*Humulus lupulus* L.). In this study, we investigated the cell growth inhibitory potential of XN on cultured human colon cancer cells. Cell proliferation was measured by sulforhodamine B staining. Poly(ADP-ribose)polymerase (PARP) cleavage, activation of caspases-3, -7, -8, and -9, and Bcl-2 family protein expression were detected by Western blot analyses. XN significantly reduced proliferation of the HCT116-derived colon cancer cell line 40-16. Half-maximal inhibitory concentrations decreased from 4.1 μM after 24 h treatment to 3.6 and 2.6 μM after 48 and 72 h incubation, respectively. Treatment with 15 μM XN for 48 h and with 5 μM for 72 h led to the detection of the cleaved 89 kDa fragment of 116 kDa PARP as an indication of apoptosis induction. Concomitantly, we observed activation and cleavage of the effector caspases-3 and -7, induced by activation of the initiator caspases -8 and -9. Expression of antiapoptotic Bcl-2 was downregulated when the cells were treated with XN for 48 – 72 h. We conclude that induction of apoptosis by downregulation of Bcl-2 and activation of the caspase cascade may contribute to the chemopreventive or therapeutic potential of XN.

Keywords: Apoptosis / Bcl-2 / Caspases / Chalcone / 40-16 Colon cancer cell line / *Humulus lupulus* L. / Xanthohumol

Received: May 6, 2005; revised: May 30, 2005; accepted: May 31, 2005

1 Introduction

The induction of apoptosis in preneoplastic and neoplastic tissue to eliminate cells with severe DNA damage that can not be repaired is a promising mechanism in cancer treatment and prevention [1]. Generally, apoptosis can be induced by two major pathways: the extrinsic, death receptor-mediated pathway and the intrinsic, mitochondrial-mediated activation [2]. Stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily, such as CD95 (APO-1/Fas) or TNF-related apoptosis-inducing ligand (TRAIL) receptors, results in receptor aggregation and recruitment of the adaptor molecule Fas-associated death domain (FADD) and the initiator caspase-8. Consequently, caspase-8 becomes activated and initiates apoptosis by cleavage of downstream effector caspases [3]. Caspases are synthesized as enzymatically inert zymogens and act in a caspase cascade to initiate apoptosis. The zymogens are composed of three domains, an *N*-terminal pro-domain and p20 and p10 domains that are found in the mature enzyme. Initiator caspases-8 and -9 are characterized by longer pro-domains that mediate the transduction of death signals and the assembly of activating complexes, like the death-inducing signalling complex (DISC) or the apoptosome. The major effector caspases-3, -6 and -7 execute apoptosis by cleavage of key cellular proteins that cause the typical morphological changes observed in cells undergoing apoptosis [4]. Cleavage of the DNA repair-associated enzyme poly(ADP-ribose)polymerase (PARP) is accepted as a prominent marker of apoptosis [5].

The mitochondrial pathway of cell death is initiated by Bcl-2 family proteins, which regulate the passage of small molecules like cytochrome *c* through the mitochondrial permeability transition pore (PTM) [6]. The Bcl-2 protein family are a group of anti- (*e.g.*, Bcl-2, Bcl-x_L, Bcl-w) and proapoptotic proteins (*e.g.*, Bax, Bak). Despite of different functions, they are characterized by the presence of con-

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Abbreviations: BH domain, Bcl-2 homology domain; **DISC**, death-inducing signalling complex; **FADD**, Fas-associated death domain; **IC_{50}**, half-maximal inhibitory concentration; **PARP**, poly(ADP-ribose)-polymerase; **SRB**, sulforhodamine B; **XN**, xanthohumol

served sequence motifs, the Bcl-2 homology (BH) domain. Antiapoptotic Bcl-2 family members contain four BH domains BH1-4, whereas proapoptotic members share only BH1-3 domains. In addition, BH-3 only proteins (*e. g.*, Bad, Bim, Bid) have been identified, that act as sensors for cytotoxic signals (oxidants, cytokine deprivation, Ca^{2+} flux, radiation, DNA-damaging agents) and eventually inactivate Bcl-2-type proteins. As an exception, Bid might activate proapoptotic Bax after cleavage by caspase-8 and thus interconnects the death receptor- and the mitochondrial pathway [2, 7]. Intracellular stress signals like a transient raise in pH cause a conformational change and translocation of Bax from the cytosol to the mitochondria, where it can homodimerize and contribute to the permeabilization of the mitochondrial membrane [7]. Release of cytochrome *c* then activates apoptotic protease activating factor 1 (Apaf-1), allowing it to assemble the multiprotein caspase-activating complex apoptosome and to bind to and activate procaspase-9 and the downstream effector caspase cascade [8].

Xanthohumol (XN) is a prenylated chalcone isolated from hop (*Humulus lupulus* L.), which has been shown to possess a broad spectrum of chemopreventive mechanisms and to inhibit carcinogenesis at the initiation, promotion and progression stage ([9, 10] and literature cited therein). Anti-initiating mechanisms include the modulation of Phase 1 and -2 enzymes involved in carcinogen metabolism. Also, XN is able to scavenge a variety of physiological relevant radicals including peroxyl-, hydroxyl, and superoxide anion radicals. Relevant for the inhibition of tumor promotion, XN was characterized as an effective anti-inflammatory and anti-estrogenic agent. It was found to inhibit both the constitutive and inducible form of cyclooxygenase Cox-1 and Cox-2 and to decrease lipopolysaccharide-mediated iNOS induction in cultured Raw 264.7 murine macrophages. Also, it inhibited human recombinant aromatase (Cyp19) activity and prevented estrogen-induced alkaline phosphatase activity in Ishikawa cell culture. As an indication of antiproliferative activity, XN inhibited human DNA polymerase α activity *in vitro*. Additionally, XN was found to induce terminal cell differentiation in cultured HL-60 cells. Most importantly, XN at nanomolar concentrations prevented carcinogen-induced preneoplastic lesions in mouse mammary gland organ culture (MMOC), providing a first proof for its chemopreventive potential [9].

Cell growth inhibitory activity of XN and related hop compounds was investigated in various cancer cell lines. Miranda *et al.* [11] determined antiproliferative and cytotoxic effects of prenylflavonoids in the human cancer cell lines MCF-7 (estrogen receptor-positive breast cancer), HT-29 (colon cancer) and A-2780 (ovarian cancer). After treatment for 4 days, IC_{50} values for XN were determined as 3.5, > 10 and 5.2 μM, respectively. XN inhibited DNA synthesis in MCF-7 cells, measured by [^{3}H]-thymidine incorporation into DNA, but did not induce apoptosis within 24 h at concentrations up to 100 μM. We could demonstrate that, similar to resveratrol, a cancer chemopreventive agent derived from grapes [12], XN at 10 and 20 μM concentrations led to cell cycle arrest of cultured estrogen receptor-negative MDA-MB-435 breast cancer cells in S-phase [9, 13]. Induction of apoptosis in 14.9% of attached cells after incubation with 25 μM XN for 48 h was observed by flow cytometry, but the mechanism of apoptosis induction was not further elucidated [9]. XN and humulone, a hop-derived bitter acid, induced apoptosis in cultured HL-60 human leukemia cells at low μM concentrations (cited in [14]). Apoptosis-induction by hop bitter acids involved upregulation of CD95/Fas and its ligand FasL, modulation in the expression of various Bcl-2 family proteins, and subsequent activation of the initiator and effector caspases [15].

Recent *in vivo* investigations on bioavailability of XN demonstrated that the chalcone and its metabolites are excreted mainly in feces within 24 h after oral or i.v. administration [16, 17]. Consequently, we were interested whether XN might affect colon cancer cell proliferation. Here, we report that XN dose-dependently inhibits *in vitro* proliferation of 40-16 colon carcinoma cells. Inhibition of cell growth was accompanied by cytotoxic effects and increased with incubation time. Cell death caused by XN was mediated by apoptosis induction, as indicated by PARP cleavage, and involved the death receptor as well as the mitochondrial pathway *via* activation of caspases-3, -7, -8, and -9 and modulation of Bcl-2 protein expression.

2 Materials and methods

2.1 Chemicals

XN (CAS No. 6754-58-1) was isolated as described previously [9]. All cell culture material was obtained from Invitrogen (Eggenstein, Germany). Fetal bovine serum was provided by Pan (Aidenbach, Germany). McCoy's 5A cell culture medium and sulforhodamine B (SRB) were purchased from Sigma (Taufkirchen, Germany). Antibodies against PARP (#9542), caspase-3 (#9662), caspase-7 (#9492), caspase-8 (#9746), caspase-9 (#9502) as well as Bcl-x_L (#2762) and Bax (#2772) were obtained from Cell Signalling Technology (Beverly, MA, USA). Bcl-2 N19 (#sc-492), anti-mouse- and anti-rabbit-secondary antibodies were obtained from Santa Cruz (Heidelberg, Germany). β-Actin antibody was purchased from Sigma. All materials and equipment for gel electrophoresis were purchased from Bio-Rad (München, Germany). All other chemicals were obtained from Sigma.

2.2 Inhibition of cell proliferation

The cell line 40-16 was generously provided by B. Vogelstein from the Johns Hopkins Oncology Center (Baltimore, USA). The cells were maintained in McCoy's 5A medium supplemented with 5% fetal bovine serum (FBS) at 37°C in a humidified environment of 5% CO_2 in air. Cultured 40-16 cells (2.5×10^4 cells per mL) were plated in 96-well plates (200 µL/well). After overnight growth, cell culture medium was changed, and cells were treated in duplicates with XN (dissolved in DMSO, final DMSO concentration 0.5%) in a concentration range of 0.5 – 10 µM, or 0.5% DMSO as a solvent control. After an additional 24, 48 and 72 h incubation, respectively, the medium was discarded and cells were fixed with 50 µL 10% TCA for 30 min at 4°C. Sulforhodamin B staining was performed as described by Skehan *et al*. [18]. Calculation of the antiproliferative activity was based on the ratio of absorbance readings at 515 nm of treated cells to those of solvent controls (set as 100%) after correction for the absorbance of cells present at the time of compound treatment (day 0 values). Negative values indicate cytotoxic effects. In these cases, day 0 values of the control were used to calculate the percentage of cytotoxicity. Half-maximal inhibitory concentrations of cell proliferation (IC_{50} values) were computed from the results of six concentrations of test compound from three independent experiments.

2.3 Western blot analysis

40-16 cells were plated in 100 mm tissue culture dishes (5×10^5 cells in 10 mL) and treated after overnight growth with XN or 0.5% DMSO as solvent control, respectively, as indicated in the figure legends. After the respective incubation periods, attached cells were collected after trypsinization, combined with cell culture media containing floating cells, and centrifuged. The cell pellet was washed with PBS, lysed, and homogenized with SDS lysis buffer (62.5 mM Tris HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue). Protein was determined using the bicinchoninic acid (BCA) method [19] after precipitation with cold 10% TCA. Total protein (30 – 50 µg/lane) was electrophoresed on a reducing SDS-polyacrylamide gel (10%T for PARP and 12%T for caspases and Bcl-2 proteins) under standard conditions and electroblotted to PVDF membranes with 15% methanol, 25 mM Tris, and 192 mM glycine. Equal protein loading per lane was ensured by using an anti-β-actin antibody. The membranes were blocked with 5% nonfat dry milk in TBS (10 mM Tris, pH 7.4, 100 mM NaCl) containing 0.01% Tween 20 for 1 h at room temperature and incubated with primary antibody (1 : 500 dilution for caspase-3, 1 : 1000 dilution for antibodies against PARP, caspases-7, -8, -9 and Bcl-2 proteins; 1:10 000 dilution for β-actin in 1% nonfat dry milk in TBS) overnight at 4°C. After thorough washing, the membranes were incubated with secondary antibodies conjugated with horseradish peroxidase (1 : 5000 dilution for anti-rabbit and 1 : 10 000 dilution for anti-mouse antibody in 5% nonfat dry milk in TBS) and streptavidin-horseradish peroxidase (1 : 2500) for 1 h at room temperature. Protein expression was visualized using a standard chemiluminescence system.

3 Results

3.1 Antiproliferative effects and apoptosis induction (PARP cleavage)

The effect of XN on proliferation of human colon cancer cells was investigated using the 40-16 cell line established from a random HCT116 clone, which is deficient in the mismatch repair protein hMLH but expresses wild-type p53 [20]. Incubation with XN in a concentration range of 0.5 – 10 µM for 24 – 72 h led to a dose-dependent inhibition of cell proliferation (Fig. 1). The half-maximal inhibitory concentration (IC_{50}) decreased from 4.1 ± 0.9 µM after 24 h incubation to 3.6 ± 0.6 µM and 2.6 ± 0.1 µM after treatment for 48 and 72 h, respectively. XN at 7.5 and 10 µM concentrations displayed cytotoxic effects, indicated by a reduction in cell numbers (*i.e.*, absorbance values after SRB staining) to values below those present at the time when the treatment was started. Consequently, we were interested whether these cytotoxic effects might be caused by induction of apoptotic mechanisms. As a first indication, we analyzed cleavage of PARP as a well accepted marker of apoptosis induction by Western blotting. The 89 kDa band of cleaved PARP was detectable after treatment with XN at a 15 µM concentration for 24 and 48 h (Fig. 2). Consistent with decreasing IC_{50} values after longer incubation periods, the effect was even more prominent when the cells were incubated with 5 µM XN for 72 h. Since hardly any cells were intact after treatment with 15 µM XN for 72 h, PARP cleavage and the pathways leading to apoptosis induction were not analyzed under these conditions.

3.2 Activation of the death receptor pathway

To identify whether the death receptor pathway is involved in XN-mediated apoptosis induction, we analyzed the activation of the initiator caspase-8 by Western blotting. The signal for pro-caspase-8 decreased after treatment with XN in a time- and concentration-dependent manner (Fig. 3). Cleavage products of 43/45 kDa and 18 kDa were detectable after 24 and 48 h of treatment with XN at a 15 µM concentration, and with 5 µM XN after 72 h. Incubation of 40-16 cells for 72 h led to some extent to an activation of cas-

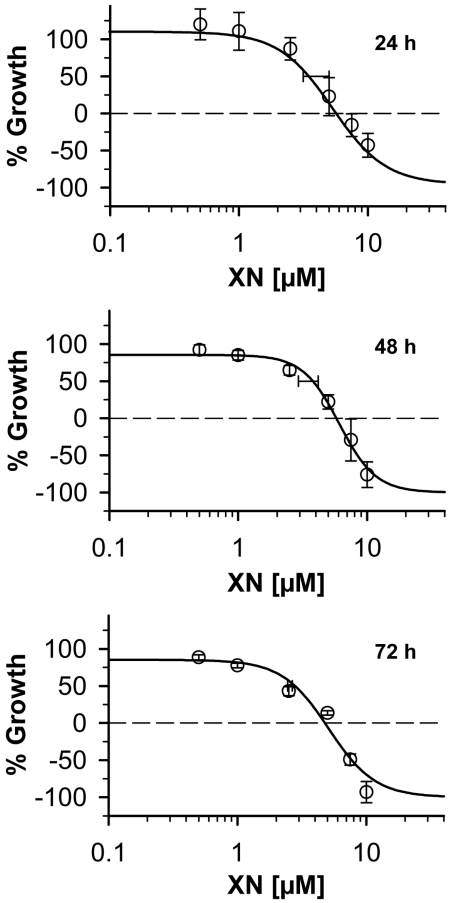


Figure 1. Inhibition of 40-16 cell proliferation. 40-16 cells were cultured in 96-well plates. After overnight growth, cells were treated with increasing concentrations of XN or 0.5% DMSO as a solvent control for further 24 h (upper), 48 h (middle), and 72 h (lower), respectively. Cell growth was determined using SRB staining. Mean values from three independent experiments were computed, and standard deviations were indicated by horizontal (for IC_{50} values) and vertical error bars. Dose-dependent inhibition of cell proliferation is shown by values above zero, wheras values below zero represent cytotoxic effects.

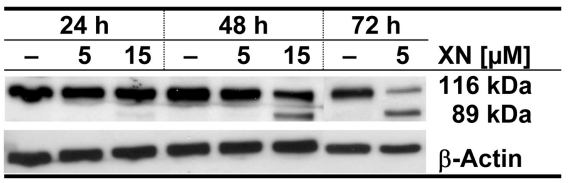


Figure 2. Cleavage of PARP by XN. 40-16 cells were treated with 0.5% DMSO (–) or XN at concentrations as indicated for 24 h, 48 h, and 72 h, respectively. PARP expression was investigated by Western blotting using an antibody against full-length PARP (116 kDa) and cleavage product (89 kDa). Equal loading was confirmed by β-actin staining.

pase-8 even in solvent-treated control cells (suggested by the 43/45 kDa fragment).

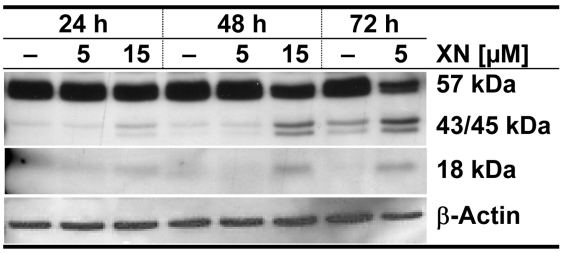


Figure 3. Caspase-8 cleavage by XN. 40-16 cells were treated with 0.5% DMSO (–) or XN as indicated for 24 h, 48 h, and 72 h, respectively. Equal amounts of protein were subjected to Western blot analysis using an antibody directed against pro-caspase-8 (57 kDa) and cleavage products (18, 43 and 45 kDa). Equal loading was confirmed by β-actin staining.

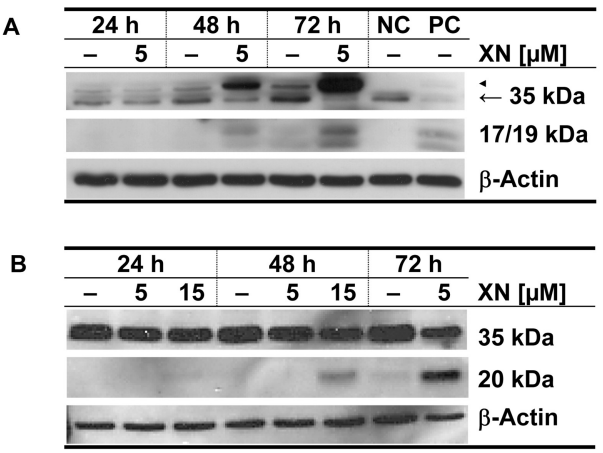


Figure 4. Effect of XN on executioner caspases. 40-16 cells were treated with 5 μM and 15 μM XN (as indicated) or 0.5% DMSO (–) as a solvent control. Cell lysates were collected after 24, 48, and 72 h, and equal amounts of protein were subjected to Western blotting. (A) Detection of caspase-3 using an antibody recognizing pro-caspase-3 and the cleavage products (17 and 19 kDa). The 35 kDa band of pro-caspase-3 is marked by an arrow; the arrowhead points to an unidentified protein which is upregulated by XN. A lysate of untreated Jurkat cells (BioVision) was used as a negative control (NC) to facilitate detection of uncleaved caspase-3. Cleavage products were detected by using a lysate of camptothecin-treated Jurkat cells (BioVision) as a positive control (PC). (B) Detection of caspase-7 using an antibody binding to pro-caspase-7 (35 kDa) and its cleavage product (20 kDa). Equal loading was confirmed by β-actin staining.

Under *in vitro* conditions, PARP is cleaved by almost all caspases, while *in vivo* it is the target of the effector caspases-3 and -7 [5]. In 40-16 cell culture, pro-caspase-3 expression in control cells treated with 0.5% DMSO increased with prolonged incubation times up to 72 h. Treatment with 5 μM XN for 48 h and especially for 72 h led to the activation and cleavage of pro-caspase-3, indicated by the detection of 17 and 19 kDa cleavage products

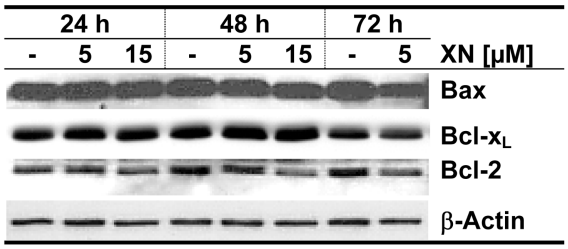


Figure 5. Effect of XN on Bcl-2 family protein expression. 40-16 cells were treated with 0.5% DMSO (–) or XN as indicated for 24 h, 48 h, and 72 h, respectively. Equal amounts of whole cell lysates were loaded to investigate protein expression by Western blotting. Antibodies were directed against proapoptotic protein Bax (20 kDa) as well as antiapoptotic proteins Bcl-x_L (30 kDa) and Bcl-2 (26 kDa). Equal loading was confirmed by β-actin staining.

(Fig. 4A). Concomitantly, incubation with 15 μM XN for 48 h and with 5 μM XN for 72 h lead to a strong activation of caspase-7 and detection of the 20 kDa fragment (Fig. 4B). These observations were consistent with the cleavage of PARP (Fig. 2).

3.3 Involvement of the mitochondrial pathway

To further investigate whether XN exclusively activated the death receptor pathway or also affects the intrinsic apoptosis induction pathway, we evaluated the expression of pro- and antiapoptotic Bcl-2 family proteins by Western blotting (Fig. 5). Proapoptotic Bax expression slightly decreased after prolonged treatment of 40-16 cells with 5 μM XN for 72 h. Concomitantly, the expression of antiapoptotic Bcl-x_L decreased in both the control and in XN-treated cells after 72 h. Importantly, levels of antiapoptotic Bcl-2 protein were significantly reduced after 24 and 48 h incubation with 15 μM XN and after 72 h treatment with 5 μM XN. Consequently, activation of the mitochondrial apoptosis pathway was detectable by pro-caspase-9 cleavage after incubation with 15 μM XN for 48 h and especially after a 72 h incubation with XN at a 5 μM concentration (Fig. 6). Similar to pro-caspase-3, pro-caspase-9 expression increased in solvent-treated control cells with prolonged incubation time.

4 Discussion

XN has been described as an antiproliferative and apoptosis-inducing agent before. However, little is know regarding the mechanisms involved in the induction of programmed cell death by XN. Here, we investigated the influence of XN on the proliferation of cultured colon cancer cells *in vitro* and focused our mechanistic investigations on caspases activation as well as Bcl-2 family protein expression.

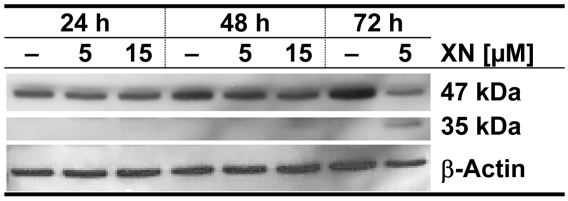


Figure 6. Activation of capsase-9 by XN. 40-16 cells were treated with 0.5% DMSO (–) or XN as indicated for 24 h, 48 h, and 72 h, respectively. Equal amounts of protein were subjected to Western blot analysis using an antibody directed against pro-caspase-9 (47 kDa) and cleaved caspase-9 (35 kDa). Equal loading was confirmed by β-actin staining.

Half-maximal inhibition of cell proliferation was observed in the low μM range, and IC_{50} values decreased with increasing incubation times. Concentrations that caused cytotoxic effects and induced apoptosis detected by PARP cleavage were about two- to threefold higher than the observed IC_{50} values for cell growth inhibition. This might be due to an initial inhibition of DNA synthesis and arrest of cells in S-phase, similar to the effects observed in breast cancer cell lines, before apoptosis is induced [9, 11]. Incubation with 5–15 μM concentrations of XN for 48–72 h, however, resulted in an activation of both the death receptor as well as the mitochondrial pathway of apoptosis induction. Although so far we have not investigated the extrinsic signal triggering the Fas receptor pathway, we could demonstrate cleavage of pro-caspase-8 and downstream activation of caspases-3 and -7. Simultaneously, the influence of XN on the mitochondrial pathway was indicated by downregulation of antiapoptotic Bcl-2 expression and activation of caspase-9. The activation of the cellular machinery of programmed cell death by XN is summarized in Fig. 7.

Several lines of evidence suggest that antiproliferative activity is a common feature of chalcones. A recent review of Go *et al*. [21] thoroughly summarizes the current literature and potential mechanisms that lead to cytotoxic effects of chalcones. These include interference at the transcription level by inhibiting p53-MDM2 interaction, induction of mitochondrial uncoupling and membrane collapse, and induction of apoptosis. In addition, antimitotic properties of chalcones have been described. Activities strongly depended on the substituents and the substitution pattern. Mechanisms related to the toxicity of flavonoids and related dietary phenolics were also compiled by Galati and O'Brien [22]. These authors stress the potential prooxidant activity of phenolics in the presence of transition metals or peroxidases as an important factor contributing to mitochondrial toxicity and apoptosis. More specifically, molecular cytotoxic mechanisms of anticancer hydroxychalcones were investigated in normal hepatocytes using 'accelerated cytotoxicity mechanism screening' techniques (utilizing high doses and short incubation times) [23]. Phloretin (from

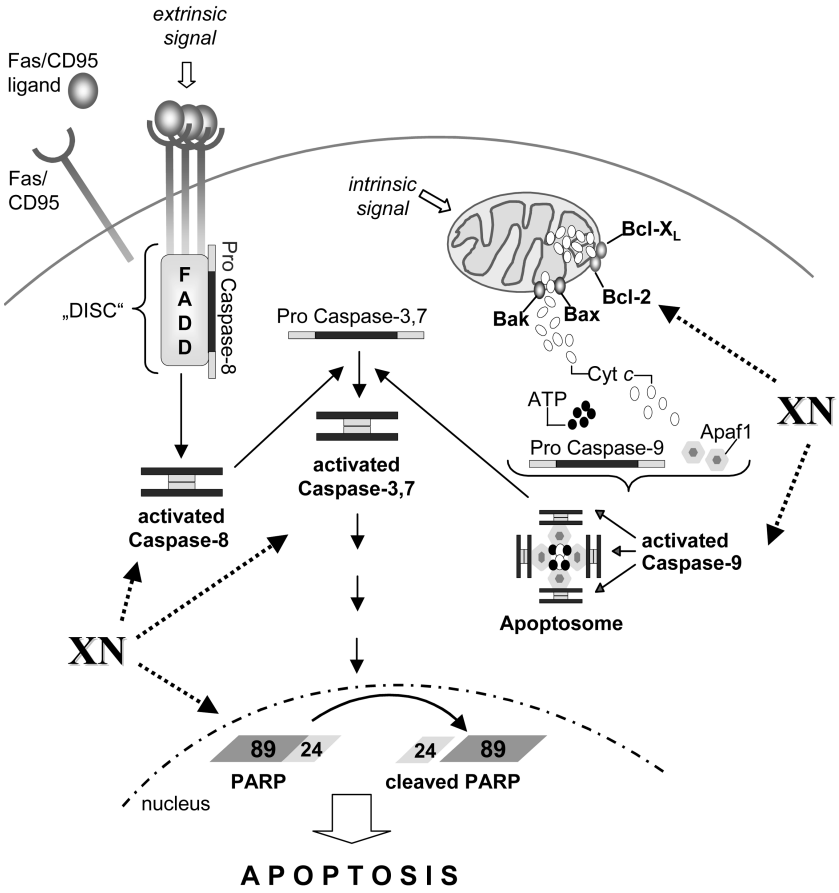
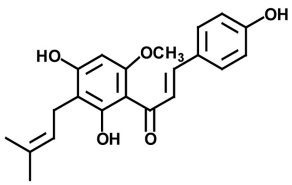


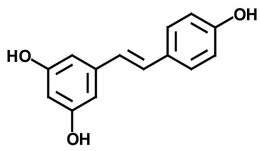
Figure 7. Potential targets of XN on apoptosis-inducing pathways in human colon carcinoma cells. Death receptor pathway activation is indicated by cleavage of the initiator caspase pro-caspase-8 and release of activated caspase-8 from the FADD in the DISC. Cleaved caspase-8 then activates the downstream caspase cascade of effector caspases-3 and -7 and consequently, PARP is cleaved as a hallmark of apoptosis. Furthermore, XN affects the mitochondrial pathway. Downregulation of antiapoptotic Bcl-2 protein, which facilitates the release of cytochrome *c* (Cyt *c*) from the mitochondria, is followed by the formation of the apoptosome (Apaf 1: apoptotic protease activating factor 1) and activation of caspase-9. Caspase-9 then likewise activates effector caspases, which leads to induction of apoptosis.

apples), isoliquiritigenin (from licorice) and ten other hydroxylated chalcones all partly depleted hepatocyte glutathione (GSH), owing to their α,β-unsaturated carbonyl moiety, or oxidized GSH to GSSG. GSH oxidation was attributed to phenoxyl radicals formed when chalcones undergo peroxidase-catalyzed oxidation. GSH depletion preceded cytotoxicity, and GSH-depleted hepatocytes were much more susceptible to chalcone-mediated toxic effects. All chalcones tested caused a collapse of the mitochondrial membrane potential, measured by uptake of the cationic fluorescent dye rhodamine 123. Also, all hydroxylated chalcones caused an increase in hepatocyte respiration, indicating uncoupling of mitochondrial oxidative phosphorylation. These effects correlated with cytotoxicity. Overall, cytotoxicity decreased with an increase in the number of hydroxyl groups on the chalcone A-ring (adjacent to the carbonyl group) [23]. For comparison, primary hepatocytes were not affected by XN at 10 μM concentrations and treatment for 24 or 48 h [11], but its effects might be different under the experimental conditions of 'accelerated cytotoxicity mechanism screening' utilized in [23].

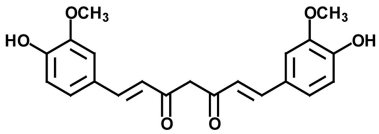
Recent reports on the structurally related molecule curcumin (diferuloylmethane), a chemopreventive agent derived from *Curcuma longa* L., and synthetic curcumin derivatives also link mitochondrial uncouplers to induction of apoptosis, detected by release of cytochrome *c*, activation of caspase-3 and PARP cleavage [24, 25]. Curcumin itself did not act as an uncoupling agent but promoted opening of the mitochondrial permeability transition pore by a mechanism involving reduction of Fe^{3+} to Fe^{2+}, formation of hydroxyl radicals under conditions of excess H_2O_2 production and oxidation of critical protein thiol groups by these hydroxyl radicals, leading to pore opening in the presence of Ca^{2+} [24, 26].



Xanthohumol



Resveratrol



Curcumin

Further studies have to demonstrate whether a direct influence on the mitochondrial membrane potential or an uncoupling effect as described for hydroxylated chalcones and other phenolic compounds also account for the effects observed with XN. We have demonstrated that XN activates both death-receptor as well as mitochondrial-mediated pathways of programmed cell death by downregulation of Bcl-2 and activation of the caspase cascade. In conclusion, this cytotoxic potential and induction of apoptosis may contribute to the chemopreventive activity of XN.

Financial support of the Wissenschaftsförderung der Deutschen Brauwirtschaft e.V. for our work on beer and hop constituents is highly acknowledged.

5 References

[1] Kim, R., Recent advances in understanding the cell death pathways activated by anticancer therapy. *Cancer* 2005, *103*, 1551 – 1560.

[2] Khosravi-Far, R., Esposti, M. D., Death receptor signals to mitochondria. *Cancer Biol. Ther.* 2004, *3*, 1051 – 1057.

[3] Debatin, K. M., Krammer, P. H., Death receptors in chemotherapy and cancer. *Oncogene* 2004, *23*, 2950 – 2966.

[4] Rupnarain, C., Dlamini, Z., Naicker, S., Bhoola, K., Colon cancer: genomics and apoptotic events. *Biol. Chem.* 2004, *385*, 449 – 464.

[5] Soldani, C., Scovassi, A. I., Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. *Apoptosis* 2002, *7*, 321 – 328.

[6] Cory, S., Adams, J. M., The Bcl-2 family: regulators of the cellular life-or-death switch. *Nat. Rev. Cancer* 2002, *2*, 647 – 656.

[7] Adams, J. M., Cory, S., Life-or-death decisions by the Bcl-2 protein family. *Trends Biochem. Sci.* 2001, *26*, 61 – 66.

[8] Reed, J. C., Apoptosis mechanisms: implications for cancer drug discovery. *Oncology (Huntingt.)* 2004, *18*, 11 – 20.

[9] Gerhauser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., *et al.*, Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. *Mol. Cancer Ther.* 2002, *1*, 959 – 969.

[10] Gerhauser, C., Beer constituents as potential cancer chemopreventive agents, *Eur. J. Cancer* 2005, in press.

[11] Miranda, C. L., Stevens, J. F., Helmrich, A., Henderson, M. C., *et al.*, Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines. *Food Chem. Toxicol*. 1999, *37*, 271 – 285.

[12] Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., *et al*., Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997, *275*, 218 – 220.

[13] Hsieh, T. C., Burfeind, P., Laud, K., Backer, J. M., *et al*., Cell cycle effects and control of gene expression by resveratrol in human breast carcinoma cell lines with different metastatic potentials. *Int. J. Oncol*. 1999, *15*, 245 – 252.

[14] Botta, B., Vitali, A., Menendez, P., Misiti, D., *et al.*, Prenylated flavonoids: Pharmacology and Biotechnology. *Curr. Med. Chem.* 2005, *12*, 713 – 739.

[15] Chen, W. J., Lin, J. K., Mechanisms of cancer chemoprevention by hop bitter acids (beer aroma) through induction of apoptosis mediated by Fas and caspase cascades. *J. Agric. Food Chem.* 2004, *52*, 55 – 64.

[16] Avula, B., Ganzera, M., Warnick, J. E., Feltenstein, M. W., *et al*., High-performance liquid chromatographic determination of xanthohumol in rat plasma, urine, and fecal samples. *J. Chromatogr. Sci.* 2004, *42*, 378 – 382.

[17] Nookandeh, A., Frank, N., Steiner, F., Ellinger, R., *et al*., Xanthohumol metabolites in faeces of rats. *Phytochemistry* 2004, *65*, 561 – 570.

[18] Skehan, P., Storeng, R., Scudiero, D., Monks, A., *et al.*, New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 1990, *82*, 1107 – 1112.

[19] Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., *et al.*, Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 1985, *150*, 76 – 85.

[20] Kennedy, A. S., Harrison, G. H., Mansfield, C. M., Zhou, X. J., *et al*., Survival of colorectal cancer cell lines treated with paclitaxel, radiation, and 5-FU: effect of TP53 or hMLH1 deficiency. *Int. J. Cancer* 2000, *90*, 175 – 185.

[21] Go, M. L., Wu, X., Liu, X. L., Chalcones: an update on cytotoxic and chemoprotective properties. *Curr. Med. Chem*. 2005, *12*, 481 – 499.

[22] Galati, G., O'Brien, P. J., Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Radic. Biol. Med.* 2004, *37*, 287 – 303.

[23] Sabzevari, O., Galati, G., Moridani, M. Y., Siraki, A., O'Brien, P. J., Molecular cytotoxic mechanisms of anticancer hydroxychalcones. *Chem. Biol. Interact.* 2004, *148*, 57 – 67.

[24] Stoetzer, O. J., Pogrebniak, A., Pelka-Fleischer, R., Hasmann, M., Hiddemann, W., Nuessler, V., Modulation of apoptosis by mitochondrial uncouplers: apoptosis-delaying features despite intrinsic cytotoxicity. *Biochem. Pharmacol.* 2002, *63*, 471 – 483.

[25] Ligeret, H., Barthelemy, S., Bouchard Doulakas, G., Carrupt, P. A., *et al.*, Fluoride curcumin derivatives: new mitochondrial uncoupling agents. *FEBS Lett.* 2004, *569*, 37 – 42.

[26] Kowaltowski, A. J., Castilho, R. F., Vercesi, A. E., Mitochondrial permeability transition and oxidative stress. *FEBS Lett.* 2001, *495*, 12 – 15.

