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Mechanisms of the anti-proliferative and anti-inflammatory effects of the herbal fixed combination STW 5 (Iberogast®) on colon adenocarcinoma (HT29) cells *in vitro*

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ABSTRACT

Introduction: Several conventional pharmaceuticals like non-steroidal anti-inflammatory drugs (NSAIDS) or selective cyclooxygenase-2 (COX-2) inhibitors have been demonstrated to exert anti-proliferative effects and to induce apoptosis in a variety of cell lines, e.g. colon, stomach, or prostate cancer cells. STW 5 (Iberogast®), a combination of nine plant extracts, is widely used in the treatment of gastrointestinal disorders, including functional dyspepsia and irritable bowel syndrome for which the involvement of an inflammatory etiology is discussed. To investigate the possible anti-proliferative effects, STW 5 and its components have been tested by using the colon-carcinoma cell line HT-29. The analyses have been performed in comparison to acetylsalicylic acid (ASA) and diclofenac (Diclo), which are well-known to reduce colon carcinoma risk.

Results: STW 5 showed significant anti-proliferative and pro-apoptotic effects on HT-29 cancer cells, similar to NSAIDs under test. However, using the LDH assay, STW 5 revealed significantly lower cytotoxicity than Diclo at same concentrations. In contrast to NSAIDs, STW 5 induced COX-1/COX-2, caspase-3 and Bax mRNA expressions in HT-29 and blocked LPS mediated translocation of the NF-κB p65 from the cytoplasm into the nucleus in PMA-differentiated THP-1 macrophages. These effects might be relevant, e.g. for prevention of undesirable side effects like gastric erosions.

Conclusion: Our data suggest that the pro-apoptotic effect of STW 5 on HT-29 cells is *involving multiple targets and is* possibly due to an activation of the caspase cascade via mitochondrial destabilization. Active concentrations of STW 5 are, in relation to therapeutic doses, comparable to those of ASA and Diclo, suggesting a similar favorable effect on colon carcinoma risk.

Introduction

Iberogast® (STW 5) is a fixed combination of nine different herbal constituents (Wegener and Wagner 2006; Vinson 2009). Its components are an aqueous-ethanolic fresh plant extract from Iberis amara totalis and drug extracts from peppermint leaves (Menthae piperitae folium), chamomile flower (Matricariae flos), liquorice root (Liquiritiae radix), Angelica root (Angelicae radix), caraway fruit (Carvi fructus), milk thistle fruit (Silybi mariani fructus), lemon balm leaves (Melissae folium), and greater celandine herb (Chelidonii herba) (Kelber et al. 2006; Ammon et al. 2006; Michael et al. 2012) – each of which is reported to have multiple pharmacological properties relevant in gastrointestinal pathophysiology (Wegener and Wagner 2006). STW 5 is indicated in the therapy of motility-related diseases of the gastrointestinal tract (Allescher and Wagner 2007; Raedsch et al. 2007; Rösch et al. 2006), and is widely used in Europe (Gundermann et al. 2003; Storra et al. 2004; Pilichiewicz et al. 2007; von Arnim et al. 2007). Inflammation is a mechanism significantly contributing to the etiology of functional gastrointestinal diseases like inflammatory bowel disease, colitis or infections by Helicobacter pylori (Collins et al. 2001; Germann et al. 2006). In this context, STW 5 has been most recently shown to have anti-inflammatory properties, to influence intestinal motility and to be effective in randomized, double blind clinical studies in functional dyspepsia and inflammatory bowel disease (Michael et al. 2012). Inflammatory bowel disease, Crohn's disease, chronic ulcerative colitis have been associated with the development of colorectal carcinoma (Coussens and Werb 2002). Developing neoplasm containing tumor cells produce various cytokines and chemokines, which lead to attraction of different leukocyte populations such as monocytes and macrophages producing several cytokines as tumor necrosis factor-alpha (TNF-α), interleukins and interferons (IFNs), cytotoxic factors as reactive

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Table 1
Content (mg dry matter/ml and % (v/v) of STW 5 (Iberogast®), a fixed combination of the fresh plant extract from Iberis amara totalis (STW 6) and eight extracts from dried plants.

Components	Name	Binomial name	mg/ml	%
STW 5	Iberogast®		59	100
STW 6	Bitter candytuft	*Iberis amara* (L)	18	15
STW 5-K II	Peppermint leaves	*Menthae piperitae* (L)	88	5
STW 5-K III	Chamomile flower	*Chamomilla recutita* (L)	58	20
STW 5-K IV	Liquorice root	*Glycyrrhiza glabra* (L)	100	10
STW 5-K V	Angelica root	*Angelica archangelica* (L)	99	10
STW 5-K VI	Caraway fruit	*Carum Carvi* (L)	47	10
STW 5-K VII	Milk thistle	*Silybum marianum* (L) Gaertn	20	10
STW 5-K VIII	Lemon balm leaves	*Melissa officinalis* (L.)	71	10
STW 5-K IX	Greater celandine herb	*Chelidonium majus* (L.)	75	10

oxygen species (ROS), or proteases like matrix metalloproteinases (MMPs) (Kuper et al. 2000). Furthermore, upwards of 15% of malignancies worldwide are initiated by infections (Kuper et al. 2000). Moreover, persistent infections within the host induce chronic inflammation involving, migrating leukocytes and phagocytic cells, resulting in the induction of DNA damage in proliferating cells. This is caused by the generation of ROS and reactive nitrogen species that are normally produced by these cells to fight infection and these species react to form peroxynitrite, a well-known mutagenic agent (Maeda and Akaike 1998). In this context, nonsteroidal anti-inflammatory drugs (NSAIDs) seem presently still the most promising drugs for the treatment of inflammation-associated cancer, since they suppress inflammation by inducing apoptosis in a variety of cell lines (Shiff et al. 1995; Yamazaki et al. 2002; Rupnarain et al. 2004). The long-term use of NSAIDs, like intake of aspirin – the oldest and most widely used COX inhibitor – causes the regression of intestinal polyps and reduces the incidence of colorectal cancer development in arthritis sufferers and colorectal cancer patients (Li et al. 2005), but frequently with unwanted side effects. Therefore, the discovery of active natural products with cytocidal (e.g. apoptotic) rather than cytotoxic effects seems to be of great interest for cancer treatment (Bonaterra et al. 2010). Thus, the aim of this study was to determine the anti-proliferative and anti-inflammatory effects of STW 5 on a colon carcinoma cell line (HT29) and to decipher the molecular mechanisms of STW 5 as compared to NSAIDs with well-known effects, like aspirin and diclofenac.

Material and methods

Test substances

STW 5 contains Iberis amara totalis (STW 6) and eight extracts from dried plants (Table 1).

Each single plant extract was HPLC-fingerprinted on its characteristic constituents and standardized. The extracts were obtained from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. Diclofenac sodium salt (Diclo, CAS: 15307-79-6), and acetylsalicylic acid, aspirin (ASA, CAS: 50-78-2) were purchased from Cayman Chemical (Ann Arbor, MI, USA) and were used in our experimental setting as anti-inflammatory reference substances. The lyophilized extracts were dissolved in water to the original volume, to reconstitute the starting concentration.

Cell culture

Human colon adenocarcinoma (HT-29) cells were obtained from German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), additionally, human acute monocytic leukemia cell line (THP-1) was obtained from Cell Lines Service (CLS, Eppelheim, Germany). The cells were cultured in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% inactivated fetal calf serum (FCS), glutamine (4 mM), and penicillin (100 U/ml)/streptomycin (100 g/ml) at 37 °C in humified CO_2 (5%).

Measurement of anti-proliferative effects by the sulforhodamine B (SRB) method

HT-29 cells were seeded in 96-well microtiter plates, and were allowed to attach to the plate surface by growing in RPMI 10% FCS for 18 h. Thereafter, STW 5 samples were added at different concentrations. Diclo, ASA and ethanolic solution (0.1–0.4 mM) served as reference substances. After 72 h of exposure, the cytotoxicity was measured by the SRB method as described by others (Fridrich et al. 2007).

Lactate dehydrogenase (LDH) assay

HT-29 cells were seeded in 96-well microtiter plates; after 48 h the medium was substituted by serum-free RPMI 1640 medium; 30 min later STW 5, components or reference substances were added at various concentrations as indicated and cultured for 5 h. Thereafter 50 μl medium was taken and treated according to the manufacturer's instructions (Roche, Mannheim, Germany). The absorbance was measured at 490–690 nm with an ELISA reader (Tecan Austria GmbH).

RNA-preparation and reverse transcription polymerase chain reaction (RT-PCR)

RNA was extracted from 1×10^6 cells using Tripure reagent (Roche, Mannheim, Germany) according to the manufacturer's specifications. RNA quality/quantity was spectrophotometrically characterized (A280/A260 > 1.8, A260/A230 > 2.4) and an aliquot of 0.5 μg total RNA was treated with 1 unit RNAse (Fermentas, St. Leon-Rot, Germany) for 30 min at 37 °C. Reverse transcription of RNA (0.5 μg) was performed with oligo $(dT)_{12-18}$ primer, 200 units of Superscript II (Life Technologies) and 24 units of Ribo Lock™ RNAse inhibitor (Fermentas) for 1 h at 42 °C. The cDNA was used for quantitative real-time polymerase chain reaction (qRT-PCR) using the QuantiTect/primerAssays from QIAGEN GmbH (Hilden, Germany). The mRNA expression was analyzed for: BCL2-associated X protein, NM_004324 (BAX), amplicon length 111 bp; B-cell CLL/lymphoma 2 NM_000633 (BCL2), amplicon length 80 bp; caspase 3, apoptosis-related cysteine peptidase NM_004346 (CASP3), amplicon length 147 bp; prostaglandin-endoperoxide synthase 1 NM_000962 (PTGS1, cyclooxygenase-1, COX-1), amplicon length 91 bp; prostaglandin-endoperoxide synthase 2 NM_000963 (PTGS2, cyclooxygenase-2, COX-2), amplicon length 68 bp; interleukin 8 NM_000584 (IL8), amplicon length 102 bp; tumor necrosis factor-alpha NM_000594

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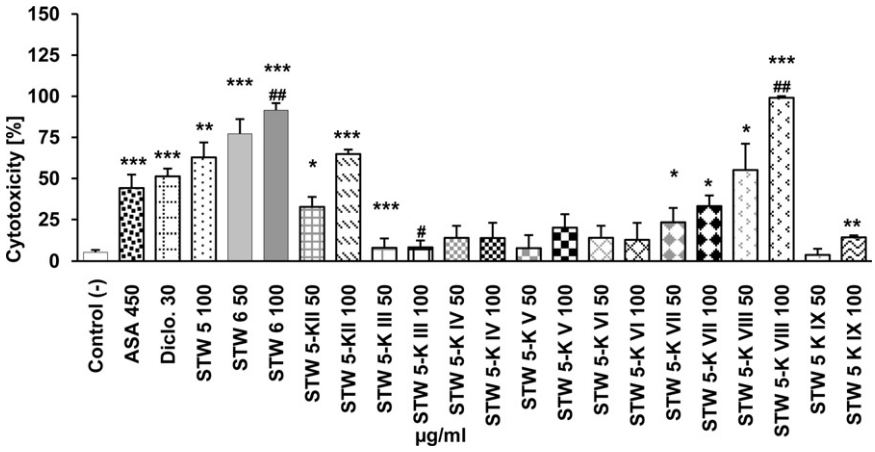

Fig. 1. Effect of STW 5, diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on proliferation of HT-29 carcinoma cells measured with SRB assay. Values (% cytotoxicity) are given as mean + SEM. P, significance vs. control (–), *0.05, **0.01 and ***0.001; vs Diclo 30 µg/ml; vs ASA 450 µg/ml, #0.05, ##0.01 and ###0.001; $n = 3$.

(TNF-α); tumor protein p53 NM_000546 (TP53, p53) amplicon length 112 bp. The following reference genes were used: beta-2-microglobulin NM_004048 (B2M), amplicon length, amplicon length 98 bp; glyceraldehyde-3-phosphate dehydrogenase NM_002046 (GAPDH), amplicon length 119 bp; large ribosomal protein NM_001002 (RPLPO), amplicon length 170 bp and peptidylprolyl isomerase A (cyclophilin A) NM_203430 (PPIA), amplicon length 121 bp. cDNAs were amplified with Brilliant1 II SYBR Green QRT-PCR Master Mix (Stratagene-Agilent Technologies, Waldbronn, Germany). The thermal profile consisted of 1 cycle at 50 °C for 2 min followed by 1 cycle at 95 °C (2 min), 45 cycles at 95 °C (15 s), 60 °C (1 min). Amplification and data analysis were performed using the Mx3005PTM QPCR System (Stratagene). The data were analyzed using the relative standard curve method. For each unknown sample, the relative amount was calculated using linear regression analysis from their respective standard curves. For relative quantification, a standard curve was generated from a pool of cDNA. Specificity of the amplified product was confirmed by melting curve analysis and, additionally, by using a 2% agarose gel electrophoresis to approve the amplicon size in conjunction with melting curve data. The geNorm and NormFinder software programs were used to ascertain the most suitable reference gene to normalize the RNA input as described earlier (Stern-Straeter et al. 2009).

Apoptosis assay

HT-29 cells were seeded in 24-well plates at a density of 5×10^4 cells per well and STW 5 or reference substances were added at various concentrations as indicated. Apoptotic cells were identified by YO-PRO-1 (1 µM) staining (Idziorek et al. 1995) in combination with the Hoechst 33342 dye (5 µg/ml) (Mobitec Company, Goettingen, Germany) as previously described (Deigner et al. 2001). The percentage of apoptotic cells was counted as described earlier, using an inverse fluorescence microscope and a computer-assisted morphometry system developed by our group (VIBAM 0.0-VFG 1 frame grabber) (Kinscherf et al. 1999).

Intracellular location of the NF-κB

THP-1 cells were placed in 12-well culture plates with round glass cover slips and were pre-treated for differentiation with phorbol myristate acetate (PMA; 16 nM; 37 °C; 48 h). Afterward the macrophages were cultured in RPMI 1640 medium without FCS and incubated for 4 h with anti-inflammatory drugs (Diclo 30 µg/ml, ASA 450 µg/ml) or 50 µg/ml STW 5, then exposed to 1 µg/ml LPS (Sigma–Aldrich) for 30 min. After stimulation, the cells were fixed for 10 min with 4% formaldehyde in PBS and incubated with rabbit polyclonal antibody directed against human NF-κB p65 (Santa Cruz Biotechnology, Heidelberg, Germany) (Kim et al. 2006). Thereafter, cells were treated with goat anti-rabbit IgG Cy3-conjugated (Jackson ImmunoResearch, Suffolk, UK) and, finally, nuclei were counterstained with the Hoechst 33342 dye (Life Technologies GmbH, Darmstadt, Germany)

Statistical analysis

The SigmaPlot® 12 software was used to carry out statistical analyses by the unpaired Student's *t* test or Mann–Whitney *U*-test, as well as Pearson's product moment correlation test of gene expression. Gene expression data are shown as mean + SEM. $p < 0.05$ was considered statistically significant.

Results

The anti-proliferative activities of STW 5, its components, as well as Diclo and ASA were assessed in HT-29 carcinoma cells. Drug effects of all substances under test on HT-29 cells after 72 h are shown in Fig. 1. A significant (dose-dependent) increase of the cytotoxicity of STW 5, STW 6, STW 5 KII, -VII and -VIII was observed at 50–100 µg/ml. STW 5 was already effective at 25 µg/ml (not shown), and revealed at 100 µg/ml a cytotoxicity of 60% ($p < 0.01$), which is comparable to ASA at 450 µg/ml 45% (p = n.s.) and Diclo at 30 µg/ml 52% ($p < 0.001$) (Fig. 1). In detail, in comparison with the control, the following substances (50 or 100 µg/ml) showed a significant increase in cytotoxicity: STW 6 fifteen- and seventeen-fold ($p < 0.05$, $p < 0.001$), STW 5-KII six- and twelve-fold ($p < 0.05$, $p < 0.001$), STW-KVII four- and six-fold ($p < 0.05$, $p < 0.001$), as well as STW 5-KVIII 10- and 19-fold ($p < 0.05$, $p < 0.001$) (Fig. 1). On the other hand, cytotoxicity of STW 5-KIII, -KIV, -KV, -KVI or -KIX at concentrations under test (50 µg/ml, 100 µg/ml) was similar to that of the control (Fig. 1). Moreover, STW 6 (50 µg/ml) in comparison with Diclo (30 µg/ml) or ASA (450 µg/ml), showed a significant ($p < 0.01$, $p < 0.01$) 1.5- and 2-fold higher cytotoxicity. The cytotoxic effect of STW 5-K VIII (50 µg/ml) is not different to Diclo (30 µg/ml), however, significant 2.2 fold increase of the cytotoxic effect of STW 5-K VIII (100 µg/ml) compared with ASA (450 µg/ml) was found (Fig. 1).

In order to investigate the potential mechanisms involved in the anti-proliferative effect of STW 5 on the induction of apoptosis in HT-29 cells in comparison to ASA or Diclo was evaluated. At concentrations of 3, 30 or 300 µg/ml STW 5 significantly induced

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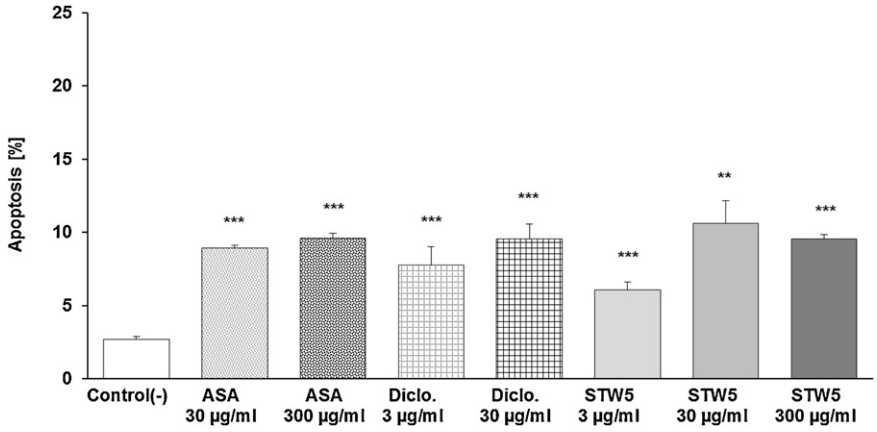

Fig. 2. Effect of STW 5 diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on the apoptosis rate in HT-29. Values (in % of apoptosis) are given as mean + SEM. P, significance vs. control (−), **0.01 and ***0.001; $n = 3$.

a 0.5- to 4-fold increase in the apoptosis rate of HT-29 cells, in comparison to the control (Fig. 2). Significant 3-fold and 4-fold increases in percentage of apoptotic HT-29 cells were also found after treatment with Diclo (3 µg/ml, 30 µg/ml) or ASA (30 µg/ml, 300 µg/ml) in comparison to the control (Fig. 2). However, in comparison to the control, the increase of percentage of apoptotic cells by STW 5 (3–300 µg/ml) was similar to ASA (30–300 µg/ml) or Diclo (3–30 µg/ml).

In order to determine, whether the growth inhibitory effects of STW 5 in HT-29 colon carcinoma cells were due to cytotoxicity, we measured LDH release into the medium. At concentrations of 3, 30 or 300 µg/ml STW 5 showed dose-dependently (6-, 10-, 14-fold) increased LDH release of HT-29 cells, in comparison to the control (Fig. 3); however, induction of LDH release by Diclo was significantly ($p < 0.01$, $p < 0.001$), 3-fold higher than that of STW 5 at concentrations of 3 µg/ml or 30 µg/ml (Fig. 3). In fact, Diclo significantly ($p < 0.001$) increased LDH release about 21- or 29-fold at 3 µg/ml or 30 µg/ml in comparison to the control (Fig. 3). STW5 (3, 30 or 300 µg/ml) showed a much lower LDH release than Diclo at corresponding concentrations (Fig. 3).

qRT-PCR revealed that STW 5 (25, 50 or 100 µg/ml) increased 2.4- and 1.9-fold the expression of COX-1 – as also did Diclo at a concentration of 7.5 µg/ml – in comparison to the control, whereas ASA (100 µg/ml, 200 µg/ml) or Diclo (15 µg/ml) down-regulated COX-1 expression in HT-29 cells (Fig. 4). Furthermore, qRT-PCR showed that STW 5 (25–100 µg/ml) – as also did Diclo only at a concentration of 7.5 µg/ml - marginally stimulated the COX-2 expression in comparison with the control, whereas ASA (100 µg/ml, 200 µg/ml) and Diclo (15 µg/ml) down-regulated COX-2 expression in HT-29 cells (Fig. 4).

Moreover, qRT-PCR showed that STW 5 (50 µg/ml, 100 µg/ml), as well as ASA (100 µg/ml, 200 µg/ml) or Diclo (15 µg/ml) down-regulated TNF-α and IL-8 expression in HT-29 cells, whereas Diclo (7.5 µg/ml) or STW 5 (25 µg/ml) up-regulated TNF-α and IL-8 expression, in comparison to the control (Fig. 5).

Additionally, qRT-PCR showed that STW 5 (25 µg/ml, 50 µg/ml, 100 µg/ml) increased the expression of caspase-3 (Casp3) – in comparison to the control – whereas ASA (100 µg/ml, 200 µg/ml) or Diclo (7.5 µg/ml, 15 µg/ml) down-regulated Casp3 expression in HT-29 cells (Fig. 6). Furthermore, STW 5 (25 µg/ml, 50 µg/ml) as well as ASA (100 µg/ml, 200 µg/ml) or Diclo (7.5 µg/ml, 15 µg/ml) up-regulated p53 expression in comparison to the control (Fig. 6), whereas at highest concentration (100 µg/ml) STW 5 down-regulated p53 expression in HT-29 cells (Fig. 6).

Moreover, in HT-29 cells, qRT-PCR showed that STW 5 (25 µg/ml, 50 µg/ml, 100 µg/ml), ASA (100 µg/ml, 200 µg/ml) or Diclo (15 µg/ml) increased the BAX expression (1.3- to 4.3-fold) in comparison with the control (Fig. 7), whereas STW 5 (25 µg/ml), ASA (100 µg/ml, 200 µg/ml) or Diclo (7.5 µg/ml, 15 µg/ml) inhibited the BCL-2 expression in comparison with the control (Fig. 7).

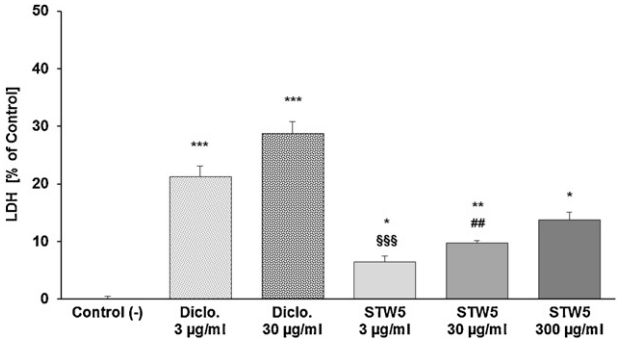

Fig. 3. Effect of STW 5 or diclofenac sodium salt (Diclo) treatment on the LDH release in HT-29 cells. Values (in % of control) are given as mean + SEM. P, significance vs. control (−), *0.05, **0.01 and ***0.001; Diclo 3 µg/ml vs STW 5 3 µg/ml, §§§0.001; Diclo 30 µg/ml vs STW 5 30 µg/ml, ##0.01; $n = 3$.

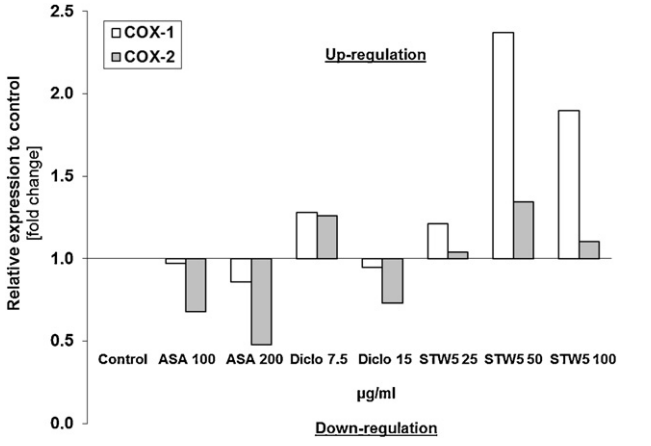

Fig. 4. Effect of STW 5, diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on COX-1 and COX-2 mRNA expression in HT-29 cells. The COX-1 and -2 expressions were normalized with a normalization factor. The relative expression was calculated using the control without treatment as calibrator (control). Representative values of two experiments are shown.

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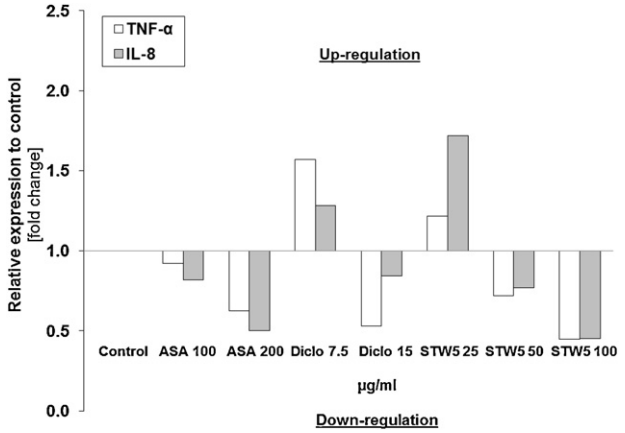

Fig. 5. Effect of STW 5, diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on TNF-α and IL-8 mRNA expression in HT-29 cells. The TNF-α and IL-8 expressions were normalized with a normalization factor. The relative expression was calculated using the control without treatment as calibrator (control). Representative values of two experiments are shown.

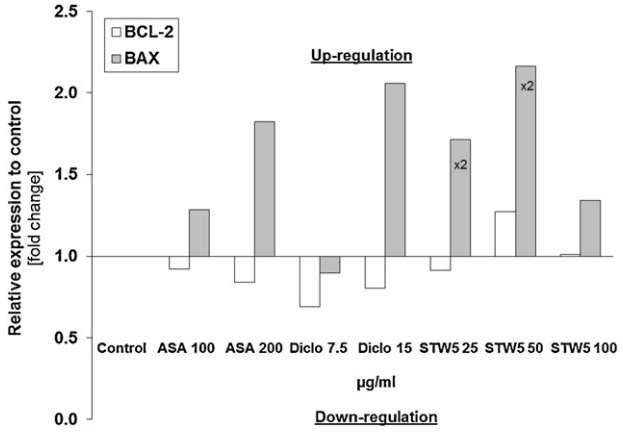

Fig. 7. Effect of STW 5, diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on bcl-2 and bax mRNA expression in HT-29 cells. The bcl-2 and bax expressions were normalized with a normalization factor. The relative expression was calculated using the control without treatment as calibrator (control). Representative values of two experiments are shown.

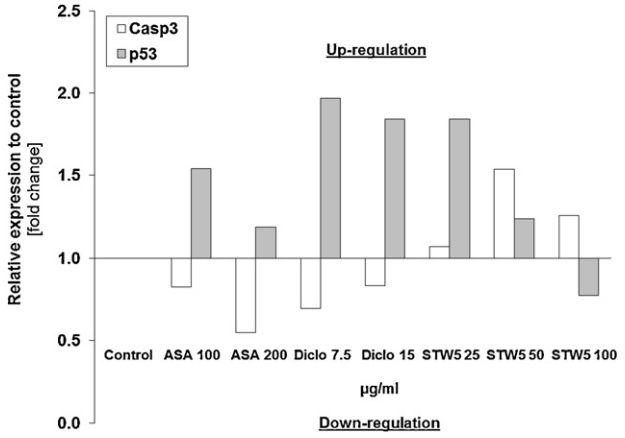

Fig. 6. Effect of STW 5, diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA) treatment on caspase-3 (Casp3) and p53 mRNA expression in HT-29 cells. The Casp3 and p53 expressions were normalized with a normalization factor. The relative expression was calculated using the control without treatment as calibrator (control). Representative values of two experiments are shown.

Furthermore, we investigated the effect of STW 5 on the nuclear translocation of the transcription factor NF-κB in PMA-differentiated THP-1 macrophages activated with LPS. LPS stimulation [LPS(+)] caused a translocation of the NF-κB p65 from the cytoplasm into the nucleus (Fig. 8). The presence of 50 µg/ml of STW 5 blocked this nuclear translocation. Similar effects were observed for ASA (450 µg/ml) or Diclo (30 µg/ml) (Fig. 8).

Discussion

The discovery of active natural products with anti-proliferative rather than cytotoxic effects is of great interest for cancer treatment. In our study, we found that STW 5 induced cytotoxicity related apoptosis in HT-29 carcinoma cells. The pro-apoptotic action of STW 5 was similar to that of the two NSAIDs under test, i.e. ASA or Diclo, also in terms of comparability to clinically used doses. These observations confirm and extend data of others showing that relatively high concentrations of NSAIDs induce apoptosis in human colon cancer cell lines, including HT-29 cells (Elder et al. 1997; Calatayud et al. 2001; Chell et al. 2005). In this context, STW 5 may exert similar effects on colorectal cancer like NSAIDs, but STW 5 has the advantage to act without the well-known side effects that limits the use of NSAIDs.

The cytotoxic effect indicated by LDH release may originate from the apoptotic effect which has been detected by the YOPRO-1 assay. Activation of apoptotic pathways is a mechanism by which drugs with anti-cancer effect kill tumor cells (Shishodia et al. 2007), by signaling via two major pathways: the mitochondrial or intrinsic or extrinsic (death-receptor) pathway (Reyes-Zurita et al. 2011). Moreover, apoptosis can also be induced by p53 via transcriptional up-regulation of the pro-apoptotic BAX and by inhibition of the anti-apoptotic BCL-2 gene (Yee and Vousden 2005). Thus, we determined potential molecular mechanisms via which STW 5 induces its cytotoxic effects in HT-29 cells. In this context, using qRT-PCR, we found that STW 5 (25 µg/ml) activates p53 mRNA expression and may trigger apoptosis via the induction of BAX, inhibition of BCL-2 and the activation of caspase-3. According to our data, Diclo

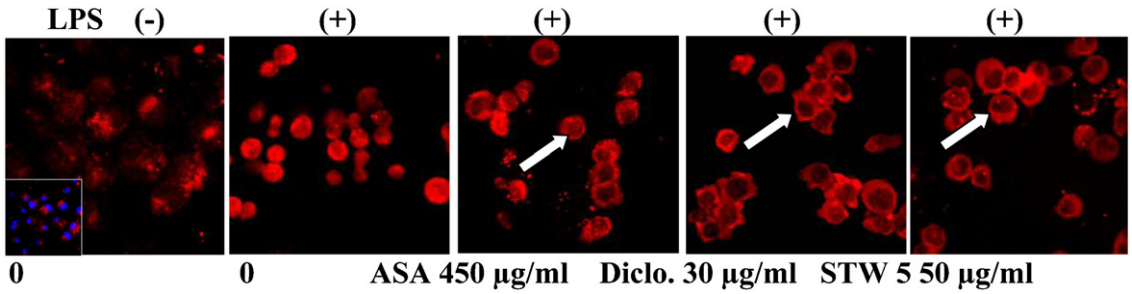

Fig. 8. Inhibition of nuclear translocation of the NF-κB p65 subunits in LPS-activated macrophages after pre-treatment with STW 5 diclofenac sodium salt (Diclo) or acetylsalicylic acid (ASA).

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apparently exhibits other apoptosis induction mechanisms than STW 5. However, the molecular mechanisms of action of NSAIDs, such as ASA and Diclo, are not known in detail. Nevertheless, it is generally accepted that NSAIDs *in vitro* and *in vivo* inhibit a variety of enzymes including the cyclooxygenases COX-1 and COX-2 (Shiff and Rigas 1997; Tegeder et al. 2001; Mahdi et al. 2006). Because the cell growth of HT-29 is regulated by the inhibition of COX-1 and -2 (Shiff and Rigas 1997), we investigated the effects of STW 5 on the expression of these two genes. Using qRT-PCR, we surprisingly found a relative high expression of the "constitutive" COX-1 (but marginally also COX-2) gene in HT-29 cells after treatment with STW 5, indicating a non-constitutive regulation, as described by others (Church et al. 2004; Zidar et al. 2008), whereas inhibition of both, COX-1 and COX-2, was observed for ASA and Diclo. Concerning NSAIDs, it is generally accepted that they inhibit COX-1 and COX-2 activity and expression *in vitro* as well as *in vivo* (Shiff et al. 1995; Mahdi et al. 2006; Fogli et al. 2010), and that regular intake of ASA reduces the incidence of colorectal cancer in arthritis sufferers and colorectal cancer patients (Li et al. 2005). On the one hand a reduction of prostaglandin synthesis has been proven in HT-29 cells to be a key in the chemopreventive and anti-neoplastic effect of NSAIDs (Chell et al. 2005). On the other hand, extensive side effects limit the clinical usefulness of NSAIDs, because they can induce direct injury of the gastrointestinal tract and lead to, e.g. ulcerous colitis (Calatayud et al. 2001). Moreover, it can be suggested that activation of prostaglandins production could maintain the beneficial role known for COX-1, and reducing the risk of developing colon cancer (Bonaterra et al. 2010), as well reduces the side effect on the gastrointestinal tract. In pharmacological doses prostaglandins increase gastrointestinal blood flow, mucus and bicarbonate secretion and generally help maintaining the gastric mucosal barrier (Gana et al. 1988), whereas inhibition of COX-1 decreased mucosal blood flow in the stomach (Wallace et al. 2000) and increased bacterial numbers in the intestine (Tanaka et al. 2002; Hotz-Behofsits et al. 2003). According to our results, STW 5 may be gastroprotective, in contrast to ASA or Diclo, which inhibit COX-1 expression with well-known side effects. STW 5 has been shown to exert anti-inflammatory, anti-oxidative as well as gastroprotective properties (Khayyal et al. 2004), and in contrast to NSAIDs no gastric side effects have been described (Vinson 2009). In this context, our experimental observations may explain why no side effects on the gastrointestinal tract such as gastric erosion were observed after treatment with STW 5. In fact, our *in vitro* results support that gastric prostaglandins, mainly derived from COX-1, seem to be important for gastrointestinal health (Hanif et al. 1996). Thus, a promising new therapeutic strategy may be the manipulation of the prostaglandin profile (Wright et al. 2004) where STW 5 may participate as preventive or protective pharmaceutical. Because it has been estimated that 15% of all malignancies are initiated by inflammation (Coussens and Werb 2002), our findings may have implications for the development of anti-cancer therapies and offer an opportunity to develop anti-inflammatory strategies without disrupting the immune system (Li et al. 2005). In this context tumor cells produce various cytokines and chemokines attracting different leukocytes as monocytes/macrophages (Coussens and Werb 2002). Hence, we here show that STW 5 inhibits the expression of the pro-inflammatory cytokine TNF-α and the chemokine IL-8 in HT-29, which is partly in accordance with findings of others, showing that HT-29 cells produce IL-8 following stimulation with TNF-α (Kolios et al. 1996). However, these data may be of interest, because it has been shown that the severity of inflammation in the colon is congruent with the IL-8 expression (Daig et al. 1996), whereas on the one side in normal tissues, IL-8 expression is low or undetectable, and on the other side IL-8 expression can be induced upon stimulation by various stimuli including TNF-α (Baggiolini et al. 1994). In contrast to the constitutively released chemokines that sustain leukocyte trafficking, induced IL-8 production attracts leukocytes to local sites and aggravates inflammation (Laing and Secombes 2004). Moreover, during tumor formation and growth, IL-8 also acts as an angiogenic factor (Heidemann et al. 2003). Hence, we suggest that our data in HT-29 cells indicate a possible control of the tumor inflammation via inhibition of leukocytes migration into the tumor by inhibition of TNF-α production as well as inhibition of angiogenesis by inhibition of IL-8 production. In this context, a developing neoplasm may include a diverse leukocyte population capable of producing several cytokines as TNF-α, interleukins and interferons (IFNs), cytotoxic factors like reactive oxygen species or matrix metalloproteinases (MMPs) (Coussens and Werb 2002). In this context tumor-associated macrophages (TAMs) play a key role in the tumor growth (Pollard 2004; Forssell et al. 2007), e.g. by secretion of a variety of factors that directly stimulate the growth and migration of tumor cells, such as PDGF, EGF or TGF-β (Leek and Harris 2002) and angiogenesis-promoting factors like VEGF and TNF-α (Leek et al. 2000). Several studies have characterized new molecules that can prevent the expression of pro-inflammatory genes by targeting transcription factor NF-κB pathways (Kim et al. 2006). Using LPS-activated macrophages, we found that STW 5 blocked the translocation of subunit p65 into the nucleus; a similar effect was found by the use of ASA or Diclo. NF-κB regulates a large number of genes involved in inflammation and is a key regulatory molecule in the transcriptional activation of the genes associated with e.g. proinflammatory cytokines, inducible nitric oxide synthase or apoptosis (Baeuerle and Baichwall 1997). The control of the pro- anti-inflammatory and pro- anti-apoptotic status of TAMs may play a key role in tumor growth progession or regression, and might be used as treatment strategy associated with the cytotoxic effect of STW 5 direct on the cancer cells. In summary, we have shown that STW 5 have anti-proliferative/cytotoxic and anti-inflammatory effects on human colon carcinoma cell lines *in vitro*. These effects are mediated at least in part through the induction of apoptosis and may be independent from an inhibition of COX-2 expression. The different influence of STW 5 on the COX mRNA expressions in comparison to NSAIDs might be further relevant, e.g. for prevention of undesirable gastrointestinal side effects. Furthermore, it has been demonstrated that multitude of phytochemical components from STW 5 possess a good bioavailability in concentration in the range utilized by others (Kelber et al. 2006) using Caco-2 colorectal adenocarcinoma cells and the everted intestinal sac -an ex vivo absorption model- (Kelber et al. 2006), which can explain its known pharmacological effects and clinical efficacy in terms of a multidrug and multi-target therapy (Wagner 2005, 2006; Vinson 2009).

Finally, the mechanisms of action of STW 5 seem to be different from ASA or Diclo. Further investigations are required to find out the molecular target(s) of STW 5 in NF-κB pathways and to determine, whether STW 5 can suppress other inflammatory reactions mediated by macrophages.

Conflict of interest

None declared.

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