Antioxidants inhibit the enhancement of malignant cell transformation induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin

Detlef Wölfe and Hans Marquardt

Department of Toxicology, University of Hamburg Medical School, and Department of Toxicology and Environmental Medicine of the Fraunhofer Society, Grindelallee 117, D-20146 Hamburg, Germany

The mechanisms of the tumor promoting activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were studied using as in vitro model the enhancement (‘promotion’) of malignant transformation of C3H/M2 mouse fibroblasts induced by N-methyl-4'-nitro-3-methylcholanthrene or 3-methylcholanthrene. In this assay, the promoting effect of TCDD was maximal at a very low concentration of 1.5 pM and was comparable to the effect of the reference tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA, 0.25 μg/ml). The role of reactive oxygen species in the promoting action was investigated: mannitol, a scavenger of hydroxyl radicals, or antioxidants, i.e. ascorbic acid plus α-tocopherol, abolished the in vitro promoting effects of TPA and TCDD. Furthermore, the involvement of protein kinase C (PKC) activation was studied: the protein kinase inhibitor H-7 markedly reduced the in vitro promoting activity of TPA but did not affect the promotion by TCDD. In accord with these results, TPA, but not TCDD, enhanced the PKC activity in C3H/M2 fibroblasts. Since the TPA-mediated activation of PKC was not affected by ascorbate plus α-tocopherol, it is concluded that the antioxidants interfere with tumor promotion at a step beyond PKC activation. Thus, the results suggest that the enhancement of malignant cell transformation by TPA and TCDD is dependent on a common mechanism, possibly induced by oxygen radicals, and, in addition, on further mechanisms that may involve agent-specific signalling pathways (e.g. PKC activation by TPA).

Introduction

The multi-step model of carcinogenesis involves initiation, in which supposedly irreversible genetic alterations take place, and promotion, in which the clonal population of initiated cells is expanded and ultimately progresses to malignancy (1). However, the current knowledge concerning the mechanisms of tumor promotion is still scarce. 2.3.7,8-Tetrachlorodibenzo-p-dioxin (TCDD*), a prototype of many halogenated aromatic hydrocarbons, is one of the most powerful tumor promoters in rodent bioassays (2). Nevertheless, only a limited number of in vitro models exist for the study of biochemical events associated with tumor promotion by TCDD at a cellular and molecular level and for characterizing factors that potentiate or inhibit its promoting activity. Such in vitro models used for TCDD include two stage transformation-systems, e.g. the transformation of embryonic fibroblasts such as C3H 10T1/2 (3) and rat tracheal epithelial cells (4) initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Furthermore, human epidermal keratinocytes immortalized by adenovirus 12-SV 40 are transformed by TCDD (5). We used the nontumorigenic mouse fibroblast cell line C3H/M2 which can be malignant transformed to tumorigenic cells by different chemical carcinogens (6).

The involvement of reactive oxygen species (ROS) and free radicals in the multi-step process of carcinogenesis, particularly in tumor promotion, has often been postulated with supportive evidence from in vivo and in vitro studies (7–12). The role of free radicals in tumor promotion is strongly suggested by the following observations: (i) free radical generating organic peroxides and H₂O₂ are known to be mouse skin tumor promoters (12) and (ii) the in vivo and in vitro tumor promoting activity of the mouse skin tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), is associated with oxidative events such as an increased H₂O₂ production and oxidative DNA damage (13–15). Moreover, non phorbol ester-type tumor promoters (16,17), including TCDD (18), have been reported to enhance the formation of ROS.

Indirect evidence supporting a role for free radicals and ROS in carcinogenesis is the inhibitory effect of antioxidants (19,20). Antioxidants, e.g. vitamin C (21) and vitamin E (22), which have been inversely associated with cancer incidence for a variety of sites in humans, have also been shown to inhibit tumor promotion by TPA (23,24) and non-phorbol ester-type tumor promoters (16,25,26). Additionally, the phenolic antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, have been shown to inhibit tumor promotion by both, TPA and benzoyl peroxide (27). The anticarcinogenic effects of many agents, e.g. naturally occurring flavones such as genistein, may also be due to their antioxidant properties (28).

A possible mechanism of ROS production is the activation of protein kinases (8,29), e.g. protein kinase C (PKC), which is the intracellular receptor for phorbol esters. Interestingly, PKC is also activated by TCDD (30,31) and other tumor promoters, e.g. chlorinated hydrocarbons (32), estrogens (33) and quinones (34). Low concentrations of superoxide have also been implicated in the activation of PKC and this activation may be triggered by an oxidation of critical sulfhydryl groups in the regulatory lipid-binding domain of PKC (35). Thus, it is tempting to speculate that the action of tumor promoters involve similar signal transfer pathways, including the activation of PKC and the formation of ROS.

In the present study, the PKC inhibitor H-7 and the antioxidants, ascorbate and α-tocopherol, were used to investigate the role of PKC activation and ROS formation in the promotion of carcinogen-induced transformation of C3H/M2 mouse fibroblasts by TCDD or TPA.

Materials and methods

Chemicals

TCDD was obtained from Oekometric (Bayreuth, Germany) and was >99% pure. The following compounds were purchased from the indicated companies:

*Abbreviations: H-7, 1-(5-isouquinolinylsulfonyl)-2-methylpiperazine; MCA, 3-methylcholanthrene; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; PKC, protein kinase C; ROS, reactive oxygen species; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TPA, 12-O-tetradecanoylphorbol-13-acetate.

© Oxford University Press 1273
Mannitol, a potent scavenger of the hydroxyl radical, or antioxidants in accord with those previously reported (3). The decreasing promoting effect at higher concentrations of TCDD (0.5-1.5 pM) resulted in a maximal enhancement of the induction of transformed foci, similar to that observed with TPA (Table II). The phorbol ester, TPA, was used as a reference for unpaired observations. A value of $P < 0.05$ was considered significant.

**Results**

Promotion of C3H/M2 malignant transformation by TCDD

The effect of TCDD on the induction of transformation by the initiating agents MNNG or MCA was studied in C3H/M2 fibroblasts (Table I). Although the transformation rate was variable, the promoting effect of TCDD was seen in all experiments; the average enhancement by TCDD was 3.5- and 3.8-fold in MCA- and MNNG-pretreated cultures, respectively. The phorbol ester, TPA, was used as a reference tumor promoter. TPA alone did not induce cell transformation (Tables II, III and IV). However, in MNNG- or MCA-initiated cultures the number of transformed foci was significantly enhanced by subsequent exposure to TPA. In accordance with previously reported findings (3), TCDD was also ineffective at initiating transformation, even at concentrations up to the nanomolar range (data not shown). However, exposure of MNNG- or MCA-pretreated cultures to very low concentrations of TCDD (0.5-1.5 pM) resulted in a maximal enhancement of the induction of transformed foci, similar to that observed with TPA (Table II). The decreasing promoting effect at higher concentrations of TCDD (25 pM) was not associated with a reduction of the plating efficiency. These data on TCDD are in accord with those previously reported (3).

Inhibition of the TPA- or TCDD-mediated promotion by mannitol or antioxidants

Mannitol, a potent scavenger of the hydroxyl radical, or a combination of the antioxidants α-tocopherol and ascorbate were used in non-toxic concentrations to examine the role of reactive oxygen species in the process of malignant transformation. While the mechanisms of both antioxidants, i.e. α-tocopherol as a major chain-breaking agent in the lipidprotein fraction and ascorbate as an electron acceptor in the aqueous medium, are widely accepted, there has been considerable speculation on the role of these antioxidants as pro-oxidants (39,40). However, it has been reported that ascorbate prevents the α-tocopherol-mediated peroxidation of lipids (40). Thus, the protection against radical formation by a single antioxidant (especially at high doses) is not unequivocal; therefore, a mixture of α-tocopherol and ascorbate was used to study their antioxidant effect on tumor promotion. Mannitol or the antioxidants had no effect on the induction of transformation by suboptimal doses of the initiating agents, MNNG or MCA, but abolished the promoting effects of TPA and TCDD on the malignant transformation of MNNG- or MCA-pretreated fibroblasts (Table III).

Effect of the PKC inhibitor H-7 on the promotion by TPA or TCDD

The well documented inhibition of the promoting activity of TPA by H-7 and other PKC inhibitors (41,42) was also found using the C3H/M2 fibroblast transformation assay. Treatment of M2 fibroblasts with the PKC inhibitor H-7 in a non-toxic concentration had no significant effect on the transformation by MNNG or MCA, but markedly inhibited the promoting action of TPA (Table IV). In contrast, the promotion by TCDD was not affected by cotreatment with H-7. Preliminary data with the more specific PKC inhibitor bisindolylmaleimide (1 μg/ml) confirmed the findings with H-7 that the promoting action of TPA, but not that of TCDD is inhibited (data not shown).

**Table I. Enhancement of C3H/M2 transformation by TCDD**

<table>
<thead>
<tr>
<th></th>
<th>DMSO (0.5%)</th>
<th>MNNG (0.1 μg/ml)</th>
<th>MCA (1.0 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0/149</td>
<td>12/115</td>
<td>15/150</td>
</tr>
<tr>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.10)</td>
<td>(0.10)</td>
</tr>
<tr>
<td>TCDD</td>
<td>0/163</td>
<td>48/123</td>
<td>58/168</td>
</tr>
<tr>
<td>(1.5 pM)</td>
<td>(0.00)</td>
<td>(0.38)</td>
<td>(0.35)</td>
</tr>
</tbody>
</table>

*Number of transformed foci per dishes treated.*

*Numerical value of the ratio.*
Using C3H/M2 TCDD, on malignant transformation of initiated cells. The present study compares the effects of two chemically unrelated tumor promoters, i.e. TPA and TCDD, on malignant transformation in vitro. Using C3H/M2 mouse fibroblasts initiated with MNNG or MCA, the maximal promoting effect of TCDD—which was equivalent to that of TPA (0.4 mM)—was found at a concentration of 1.5 pM, i.e. at a concentration which was five orders of magnitude lower than that of TPA. This difference between TCDD and TPA might be due to differences in the toxicokinetics and the interactions of TPA and TCDD with different cell types in vivo.

### Table II. Dose-dependent enhancement of C3H/M2 transformation by TCDD

<table>
<thead>
<tr>
<th>Promotor</th>
<th>Concentration</th>
<th>DMSO</th>
<th>MNNG</th>
<th>MCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>Tx</td>
<td>PE</td>
<td>Tx</td>
</tr>
<tr>
<td>DMSO (0.5%)</td>
<td>26</td>
<td>0/13</td>
<td>28</td>
<td>1/15 (0.07)</td>
</tr>
<tr>
<td>TPA 0.25 µg/ml</td>
<td>27</td>
<td>0/16</td>
<td>31</td>
<td>3/14 (0.21)</td>
</tr>
<tr>
<td>TCDD 0.1 pM</td>
<td>27</td>
<td>0/7</td>
<td>31</td>
<td>0/7 (0.00)</td>
</tr>
<tr>
<td>0.5 pM</td>
<td>37</td>
<td>0/6</td>
<td>31</td>
<td>2/5 (0.40)</td>
</tr>
<tr>
<td>1.5 pM</td>
<td>29</td>
<td>0/14</td>
<td>26</td>
<td>4/14 (0.29)</td>
</tr>
<tr>
<td>6.0 pM</td>
<td>28</td>
<td>0/14</td>
<td>25</td>
<td>4/15 (0.27)</td>
</tr>
<tr>
<td>25.0 pM</td>
<td>29</td>
<td>0/13</td>
<td>25</td>
<td>(1/14 (0.07)</td>
</tr>
</tbody>
</table>

Cultures were pretreated for 24 h with DMSO or the initiating agents, MNNG (0.1 µg/mL) or MCA (1.0 µg/mL). Results are a summary of two separate experiments.

### Table III. Inhibition of TCDD-induced enhancement of C3H/M2 transformation by mannitol or antioxidants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DMSO</th>
<th>MNNG</th>
<th>MCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>Tx</td>
<td>PE</td>
</tr>
<tr>
<td>DMSO (0.5%)</td>
<td>20</td>
<td>0/21</td>
<td>18</td>
</tr>
<tr>
<td>DMSO + Mannitol</td>
<td>20</td>
<td>0/20</td>
<td>15</td>
</tr>
<tr>
<td>DMSO + Toc/Asc</td>
<td>20</td>
<td>0/20</td>
<td>15</td>
</tr>
<tr>
<td>TPA (0.25 µg/ml)</td>
<td>21</td>
<td>0/15</td>
<td>20</td>
</tr>
<tr>
<td>TPA + Mannitol</td>
<td>25</td>
<td>0/19</td>
<td>26</td>
</tr>
<tr>
<td>TPA + Toc/Asc</td>
<td>25</td>
<td>0/19</td>
<td>26</td>
</tr>
<tr>
<td>TCDD (1.5 pM)</td>
<td>25</td>
<td>0/20</td>
<td>16</td>
</tr>
<tr>
<td>TCDD + Mannitol</td>
<td>25</td>
<td>0/17</td>
<td>20</td>
</tr>
<tr>
<td>TCDD + Toc/Asc</td>
<td>29</td>
<td>0/11</td>
<td>26</td>
</tr>
</tbody>
</table>

Cultures were pretreated for 24 h with DMSO or the initiating agents, MNNG (0.1 µg/ml) or MCA (1.0 µg/ml). Thereafter, cultures were treated for 6 weeks with mannitol (10 mg/ml) or α-tocopherol (10^{-4} M) (Toc/Asc) in the presence or absence of TPA and TCDD, respectively. Results are a summary of two separate experiments.

### Table IV. Effects of the protein kinase inhibitor H-7 on the enhancement of C3H/M2 transformation by TPA and TCDD

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DMSO</th>
<th>MNNG</th>
<th>MCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>Tx</td>
<td>PE</td>
</tr>
<tr>
<td>DMSO (0.5%)</td>
<td>27</td>
<td>0/26</td>
<td>27</td>
</tr>
<tr>
<td>DMSO + H-7 (1 µg/ml)</td>
<td>31</td>
<td>0/27</td>
<td>25</td>
</tr>
<tr>
<td>TPA (0.25 µg/ml)</td>
<td>22</td>
<td>0/24</td>
<td>21</td>
</tr>
<tr>
<td>TPA + H-7 (1 µg/ml)</td>
<td>23</td>
<td>0/22</td>
<td>27</td>
</tr>
<tr>
<td>TCDD (1.5 pM)</td>
<td>28</td>
<td>0/27</td>
<td>25</td>
</tr>
<tr>
<td>TCDD + H-7 (1 µg/ml)</td>
<td>23</td>
<td>0/28</td>
<td>25</td>
</tr>
</tbody>
</table>

Cultures were pretreated for 24 h with DMSO or the initiating agents, MNNG (0.1 µg/ml) or MCA (1.0 µg/ml). Results are a summary of three separate experiments.

### Discussion

A major unresolved problem in tumor promotion is to define the critical cellular targets which lead to the clonal expansion of initiated cells. The present study compares the effects of two chemically unrelated tumor promoters, i.e. TPA and TCDD, on malignant transformation in vitro. Using C3H/M2 mouse fibroblasts initiated with MNNG or MCA, the maximal which TCDD (1 pM) significantly activated PKC activity in the membrane fraction.

Numbers in parentheses, numerical value of the ratio.

Numbers in parentheses, numerical value of the ratio.

Numbers in parentheses, numerical value of the ratio.
biphenyls (44) are investigated. In the promotion/ transformation assay with C3H10T1/2 cells initiated with MNNG, the maximal effect of TCDD had been observed at 40 pM (3) and in a transformation assay with rat tracheal cells the promoting effect of TCDD was maximal at 300 pM (4). Neoplastic transformation of immortal human keratinocytes was achieved with 10 nM or higher concentrations of TCDD (5). Moreover, in primary rat hepatocytes TCDD at 1 pM maximally stimulated replicative DNA synthesis and mitosis (45,46). Thus, mouse fibroblasts and rat hepatocytes appear to be very sensitive with respect to TCDD-mediated effects associated with tumor promotion.

Furthermore, the results of this study suggest that the production of ROS may be a common mechanism in the promotion induced by TPA and TCDD. It is known from in vivo studies that antioxidants inhibit tumor promotion by TPA (23,24) and other promoters (25). In in vitro studies, the addition of the hydroxyl radical scavenger mannitol resulted in a dose-dependent inhibition of the promoting effect of TPA on JB6 mouse epidermal cell transformation (47) and on the transformation of Balb 3T3 cells initiated with MCA (48). The results of our study with mannitol (at the maximally effective concentration reported by Saito et al.; 48) in the transformation assay with MCA- and MNNG-initiated C3H/M2 fibroblasts are in accord with these observations and show, in addition, that the in vitro promoting effect of TPA is also abolished by the antioxidants ascorbate plus α-tocopherol. Thus, our findings are consistent with the hypothesis that tumor promotion by TPA is mediated by the production of reactive oxygen species. For phosphol ester-type tumor promoters, a correlation has been found between their in vitro promoting potencies and the formation of H2O2 and oxidized DNA bases by these agents (14). TPA is known to decrease the activities of superoxide dismutase, catalase and glutathione peroxidase in vivo and the ratio of reduced glutathione/oxidized glutathione in mouse skin (49,50). Treatment in vitro of epidermal cells with tumor promoters rapidly increases the steady state levels of hydroperoxides by stimulation of the prooxidant activities of various endogenous enzymic and non-enzymatic sources of ROS (51). ROS are able to induce DNA strand-breaks, chromosomal damages and enhanced expression of the protooncogenes c-myc and c-fos (52). Highly persistent c-myc levels correlate with anchorage-independent growth and focus formation in chemically transformed C3H 10T1/2 mouse embryo fibroblasts and with an increased susceptibility to spontaneous transformation of rat fibroblasts (53,54). Furthermore, TPA activates the transcription factors AP-1 and NF-kB; the latter is known as an oxidative stress-responsive transcription factor which is strongly activated by H2O2. The activation of NF-kB by phorbol esters is potentiated by H2O2 and suppressed by antioxidants (55). Thus, tumor promoter-mediated formation of ROS results in an altered regulation of transcription factors which may be associated with hyperplasia and cell transformation. The production of free radicals and ROS is not restricted to phorbol ester-type tumor promoters, but is a common feature of tumor promoters, e.g. peroxisome proliferators (56), endogenous and synthetic steroidal estrogens (57), phenobarbital (17), chlordane (58) and Aroclor (59). Treatment with high doses of TCDD has also been reported to stimulate ROS production in vivo (18). In the present study, we demonstrate that mannitol or antioxidants inhibit the promoting effect of very low TCDD concentrations (1.5 pM) on the malignant transformation of C3H/M2 cells induced by MNNG or MCA. Since the synergism between ascorbate and α-tocopherol in the inhibition of lipid peroxidation is well established (39,40), we tentatively conclude from our results with these antioxidants and mannitol that tumor promotion by TPA and TCDD involves lipid peroxidation possibly via the formation of hydroxyl radicals.

Many biochemical effects of TCDD, especially TCDD-induced early events, e.g. the activation of PKC and the elevation of AP-1 transcription factor activity (30), resemble the TPA-induced effects. Recently we have reported that in primary rat hepatocytes after treatment (3–48 h) with 1 pM TCDD the particulate PKC activity is significantly enhanced (31). In the present study, therefore, the involvement of PKC in the promoting effect of TPA and TCDD was investigated. The inhibition of the promoting activity of TPA by various inhibitors of PKC in vitro (41) and in vivo (42) is well established and our in vitro data on the inhibition of the TPA-mediated enhancement of transformation by H-7 are in accord with these observations. In contrast to TPA-treated M2-fibroblasts, the enhanced transformation rate of TCDD-treated cells was not affected by H-7 or the more specific PKC inhibitor bisindolylmaleimide (preliminary data, not shown). In addition, TCDD failed to enhance PKC activity in M2 mouse fibroblasts. Our previous observation in primary rat hepatocytes that antioxidants inhibit TCDD-enhanced PKC activity (unpublished results) suggest that this TCDD effect is rather an indirect than a direct activation of PKC. In contrast, the PKC activation by short-term treatment of M2-fibroblasts with TPA was not affected by antioxidants. In conclusion, it appears that PKC activation by TPA is an agent-specific pathway which is not involved in the promoting activity of TCDD. There is further evidence that PKC activation is not generally associated with enhanced cell transformation: (i) the

![Fig. 1. PKC activity in the particulate fractions of TPA- or TCDD-treated C3H/M2 fibroblasts. Fibroblasts were treated with 1 μM TPA (dark bars) or 1 pM TCDD (hatched bars) for the indicated times (d, day). Particulate fractions were prepared and assayed for PKC as described. Data represent the mean ± SD (duplicate cultures from two representative experiments).](image-url)
PKC inhibitors staurosporine (60), H-7 and HA1004 (61) were reported to be tumor promoters; (ii) triphenylmethane, a chemopreventive agent, stimulates PKC activity in C3H10T1/2 cells (62); (iii) the high incidence of cell transformation in a cell variant of Balb/c3T3 was not associated with PKC activation (63); (iv) a C3H10T1/2 cell line stably overexpressing PKC$_{\beta_1}$ was found to have an untransformed phenotype and to be dependent on TPA for focus formation suggesting that other TPA-mediated events in addition to PKC$_{\beta_1}$ activation may be necessary (64); (v) no differences between normal and SV40-transformed rat embryo fibroblasts were found with respect to quantity or distribution of total PKC or PKC$_{\beta_2}$ (65); (vi) PKC activities in the epidermis of a TPA promotion-sensitive mouse strain (SEN CAR) and that of a TPA-resistant strain (C57BL/6J) were roughly the same, whereas the activity of 8-lipoxygenase and the levels of oxidants were higher in epidermal cells of the sensitive mouse strain as compared to the resistant strain (13,15). Thus, sensitivity to TPA-mediated promotion may not be exclusively determined by PKC activation but also by additional mechanisms. Many investigations on PKC are now focused on the role of PKC isoenzymes and their subcellular location (65): PKC isoenzymes exert many biological effects and are differentially regulated during carcinogenesis (66,67) and in cell transformation (68), probably depending also on the cell type under investigation.

In summary, different classes of tumor promoters, e.g. phorbol esters and polychlorinated hydrocarbons, share a number of common biological and pathological responses, including stimulation of cellular growth (43,44,67), malignant cell transformation and tumor promotion. Inhibitors of the cellular metabolism, including antioxidants, are valuable tools to elucidate essential mechanisms involved in tumor promotion, possibly the formation of ROS. A further characterization of the modulation of ROS pathways by different promoting agents should provide deeper insight into the mechanisms of tumor promotion.

Acknowledgements

The authors are grateful to E.Becker, A.Piascki and A.Ruge for excellent technical assistance. The work was funded by grants of the Bundesministerium für Bildung und Forschung (07 DIX12).

References

18. Slaga,T.J., Solanki,V. and Logani,M. (1983) Studies on the mechanism of chemopreventive agent, stimulates PKC activity in C3H10T1/2 SV40-transformed rat embryo fibroblasts were found with respect to quantity or distribution of total PKC or PKC$_{\beta_2}$ (65); (vi) PKC activities in the epidermis of a TPA promotion-sensitive mouse strain (SEN CAR) and that of a TPA-resistant strain (C57BL/6J) were roughly the same, whereas the activity of 8-lipoxygenase and the levels of oxidants were higher in epidermal cells of the sensitive mouse strain as compared to the resistant strain (13,15). Thus, sensitivity to TPA-mediated promotion may not be exclusively determined by PKC activation but also by additional mechanisms. Many investigations on PKC are now focused on the role of PKC isoenzymes and their subcellular location (65): PKC isoenzymes exert many biological effects and are differentially regulated during carcinogenesis (66,67) and in cell transformation (68), probably depending also on the cell type under investigation.

In summary, different classes of tumor promoters, e.g. phorbol esters and polychlorinated hydrocarbons, share a number of common biological and pathological responses, including stimulation of cellular growth (43,44,67), malignant cell transformation and tumor promotion. Inhibitors of the cellular metabolism, including antioxidants, are valuable tools to elucidate essential mechanisms involved in tumor promotion, possibly the formation of ROS. A further characterization of the modulation of ROS pathways by different promoting agents should provide deeper insight into the mechanisms of tumor promotion.

Acknowledgements

The authors are grateful to E.Becker, A.Piascki and A.Ruge for excellent technical assistance. The work was funded by grants of the Bundesministerium für Bildung und Forschung (07 DIX12).

References


